

XISTIX

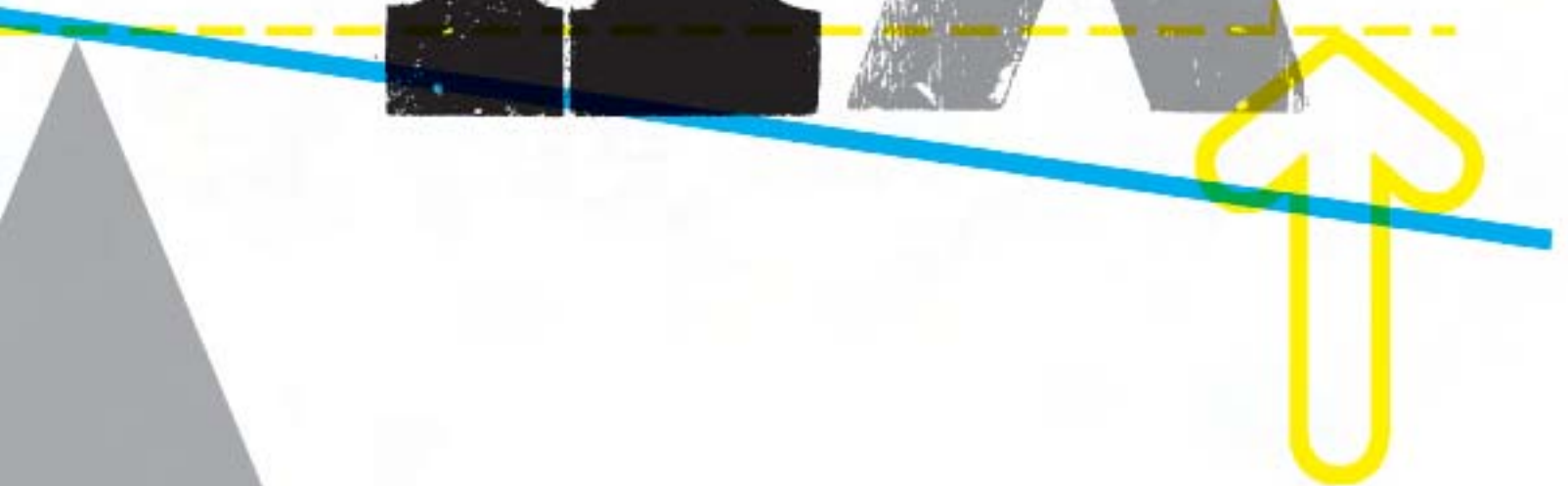


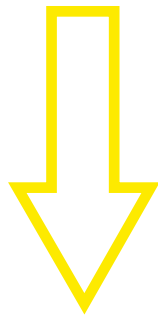
**GENETIC**  
**BALANCING**  
**ACT**

**BY  
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ILLUSTRATION  
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When it comes to chromosomes, men get short-changed. Among their 23 pairs of chromosomes, women have two strapping X chromosomes, while men sport an X chromosome and a Y. It might be the ultimate source of maleness, but the Y chromosome is downright diminutive, with only about one-third as many DNA bases as the X chromosome and fewer than one-tenth as many genes.

This disparity leaves our cells in a jam. A woman's cells carry twice as many copies of most X chromosome genes as a man's cells and—if the genes work at full throttle—can crank out correspondingly more of their protein products. Although genes on the X chromosome are essential for everything from filtering blood to repairing DNA (most of them have nothing to do with sex differences), a double dose of their proteins is disastrous for cells.

And that's not only true in humans. Animals as different as roundworms, fruit flies, and opossums face the same problem. "It's essential to have gene balance" between the sexes, says HHMI investigator Barbara J. Meyer of the University of California, Berkeley. If the numbers are out of whack, "the animals are dead, it's that simple," she says.

Necessity, however, is the mother of adaptation. Animals have evolved myriad mechanisms to keep gene activity the same in the two sexes. Known as dosage compensation, this type of gender equality can work in several ways. For example, female mammals shut down one X chromosome, preventing most of its genes from yielding any proteins—what's termed X chromosome inactivation. This process is complete by the early stages of embryonic development, and the inactivated chromosome remains off for life. Fruit flies use the opposite approach: responsibility for dosage compensation falls on the male, where the output of the genes on its single X chromosome doubles, thereby ensuring the same gene expression as in XX female flies.

Two HHMI investigators, Meyer and Jeannie T. Lee of Harvard Medical School and Massachusetts General Hospital, are at the forefront of research on the intricacies of dosage compensation. In more than 20 years of experiments on nematodes, or roundworms, Meyer has shown how cells count the number of X chromosomes they contain and revealed the workings of a protein cluster that adjusts gene activity on the X chromosome. Studying mice, Lee has deciphered details of the molecules that regulate X inactivation. Both scientists say their results have surprised them at every turn.

Now their work is having an impact on biologists in other fields. Researchers are looking to dosage compensation for clues about how cells orchestrate changes to large tracts of DNA or entire chromosomes. And Lee's recent findings of peculiarities in X inactivation among embryonic stem cell lines have heightened concerns about the safety of these stem cells when used to develop replacement tissues and organs.

### CELLULAR ABACUS

To pull off dosage compensation, cells need basic math skills. They have to be able to count the number of X chromosomes and non-sex chromosomes (called autosomes) they harbor. Meyer uncovered the basis for this arithmetic ability in roundworms.

The discovery began with an Alexander Fleming moment. Fleming, a Scottish bacteriologist, stumbled on penicillin in 1928 when he took a close look at some moldy bacterial cultures, rather than tossing them out as he usually did. The Meyer lab version of this event occurred 60 years later and involved a flask of misbehaving worms.

In the late 1980s, the lab was studying animals with a genetic mutation called *dumpy* that disables dosage compensation

and sickens or kills the worms. But the nematodes in one container were hale, and their population was booming. Thinking that the culture had been contaminated, Meyer's lab technician started to throw the animals away. "He was literally pouring the culture down the sink," recalls Meyer, "when I said, 'Stop!'" Under the microscope, any nematodes that survived the mutation should have appeared short and squat, but she noticed that they were long and sleek. Investigating the animals' mysterious survival led Meyer and colleagues to a gene called *xol-1*. "That broke it open for us," she says.

As she and her co-workers have revealed over the last 20 years, *xol-1* serves as a cellular abacus, with its tallies dictating not only whether dosage compensation kicks in, but also the animal's sex. Worm sexuality differs from our own. Male worms carry one X chromosome, but no Y. The other sex, with two Xs, is a hermaphrodite (an organism with male and female reproductive systems).

By hunting down mutated genes that turn *xol-1* on or off at the wrong time, Meyer's team identified the "counters" that tell worm cells how many sex chromosomes they have. Several genes on the X chromosome, the researchers found in a series of studies, code for proteins called X signal elements that can shut down *xol-1*. The researchers also found genes on the autosomes whose proteins, known as autosomal signal elements, can activate *xol-1*. In other words, worm cells simultaneously fashion proteins that can turn *xol-1* off and proteins that can turn it on.

The fate of an X chromosome depends on a molecular scuffle between the X signal elements and the autosomal signal elements. The battle occurs at *xol-1*'s promoter, its on-off switch. "They're all duking it out for *xol-1*'s promoter," says Meyer. In the free-for-all, numbers prevail. Male worms have one X chromosome but two copies of each autosome. So their "on" signals from the autosomes overwhelm their "off" signals from the solitary X. Thus, *xol-1* is active and thwarts dosage

compensation. This allows cells to turn on another gene that triggers the animal to develop male characteristics.

With a pair of X chromosomes, a hermaphrodite produces twice as many X signal elements as does a male. In this case, the "off" proteins from the X chromosomes win out over the "on" proteins from the autosomes, quieting *xol-1* and permitting dosage compensation.

Meyer and colleagues continue to refine this story. In a November 2007 paper, for instance, they reported identifying another X signal element, the fifth found so far.

They have also tracked down the molecular "dimmer switch" responsible for dosage compensation in roundworms.

Meyer's lab first learned that the process operates differently in worms than in mice and humans. Instead of turning off one of its two X chromosomes, a hermaphroditic worm dials down the activity of all genes on both Xs by half, on average. Meyer's group went on to discover that worms rely on a cluster of proteins called the dosage compensation complex (DCC) to achieve this feat. The complex attaches to the X chromosome and turns gene activity down. But the DCC's proteins don't unite and start working until the cell inactivates *xol-1*.

Some of the DCC's proteins are similar to proteins that help chromosomes compact and then separate during cell division. That finding suggests that the DCC is derived, evolutionarily, from a protein combination that performs a completely different task. In other words, when cells in some ancestral worm needed



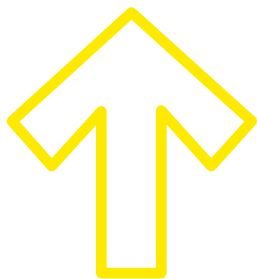
THROUGH HER WORK IN ROUNDWORMS, BARBARA MEYER HAS REVEALED HOW A PROTEIN CLUSTER ADJUSTS GENE ACTIVITY ON THE X CHROMOSOME, A PROCESS CALLED DOSAGE COMPENSATION.



JEANNIE LEE HAS IDENTIFIED KEY GENES INVOLVED IN X CHROMOSOME INACTIVATION AND HAS UNCOVERED DETAILS OF HOW MOLECULES REGULATE THAT INACTIVATION IN FRUIT FLIES.

a dosage compensation system, “they stole it” from another cellular mechanism, Meyer says, thus avoiding the need to evolve entirely new molecular machinery to do the job.

Her lab has answered the key question of how the DCC distinguishes X chromosomes from autosomes. To find out, the researchers attached different chunks of an X chromosome to an autosome and determined which ones attract the DCC. In 2004, the team first revealed that the DCC homes in on several DNA stretches along the X chromosome. One stretch was about 800 nucleotide bases long. In a



2006 follow-up study, Meyer and colleagues whittled that segment down and dissected others, demonstrating that the DCC recognizes two specific DNA sequences, or motifs, each about 8 nucleotide bases long.

The surprising fact, Meyer says, is that these motifs appear on autosomes as well as on the X chromosome. The arrangement of motifs, not just their presence, might dictate whether the DCC latches on, a possibility Meyer’s lab is now exploring.

### TWISTS, TURNS, AND MORE TWISTS

Once a cell has finished counting chromosomes, it’s ready to take action. In female mammals, that means muffling one of the X chromosomes. How cells turn on dosage compensation when it’s needed—and keep it off when it’s not—has occupied Jeannie Lee for 13 years. When she began her experiments as a post-doc in Rudolph Jaenisch’s lab at the Massachusetts Institute of Technology, she didn’t envision that the work would take

this long. “I thought it would be solved by now,” she says. Yet scientists are far from having all the answers, in part because their studies keep throwing them curve balls, including odd molecular pathways, backward DNA silencing, and unexpected chromosomal liaisons.

British geneticist Mary Lyon galvanized research into dosage compensation in 1961, when she determined that the Barr body, a shadowy structure lurking at the edge of the nucleus in mammalian cells, was an inert X chromosome. On the basis of that finding, researchers posited that cells must have an “X inactivation center” that closed down the chromosome. But the responsible genes were elusive. It wasn’t until 1991 that Huntington Willard, now an HHMI professor at Duke University in Durham, North Carolina, and colleagues identified a gene, named *Xist*, that instigates dosage compensation (see sidebar). Lee cites that finding as one of her motivations for switching to X inactivation research in 1995.

Her first big discovery came four years later, when she and her colleagues pinpointed the gene *Tsix*, which blocks *Xist*. *Tsix*’s existence makes sense—cells need to keep *Xist* under control to prevent it from shutting down both X chromosomes in females or the only X in males. *Xist* and *Tsix* aren’t just opposites in function. Their nucleotide sequences are mirror images, what researchers call complementary. The name Lee’s team chose for their new gene reflects this inverse relationship: *Tsix* is *Xist* backward.

How *Tsix* and *Xist* work caught researchers off guard. Unlike the genes that control the color of our eyes or allow our cells to break down sugars, *Tsix* and *Xist* don’t encode proteins. When a cell needs to perform a task, it typically makes an RNA copy of a gene, which in turn codes for a protein that takes care of business. But for *Xist* and *Tsix*, the RNA molecule itself is all that’s needed to carry out functions in

the cells. Willard's lab demonstrated this unusual behavior for *Xist*, whereas Lee's group showed it for *Tsix*.

That wasn't the last surprise that awaited Lee and colleagues as they delved into *Tsix*'s actions. Because strands of *Tsix* and *Xist* RNA are complementary, if they meet they'll stick together. A reasonable hypothesis is that *Tsix* RNA prevents X chromosome inactivation by grabbing and disabling the *Xist* RNA.

Reasonable but wrong, Lee and colleagues concluded in a 2006 *Molecular Cell* paper. Inside the cell, a chromosome's DNA wraps around spool-like proteins called histones. To shut down a specific gene, cells typically coil the DNA strands tighter, denying access to essential DNA-reading proteins. But to silence the *Xist* gene in fruit flies, Lee and colleagues found, *Tsix* appears to do the opposite; it loosens the DNA. Cinching up the DNA, by contrast, turns on *Xist*. Only a few fruit fly genes are known to operate in this way, she says.

In retrospect, the seemingly backward mechanism makes sense, she adds. To close down an entire X chromosome, a cell might compress all the chromosome's DNA. But if *Xist* were a conventional gene, that tightening would also shut it down—and once *Xist* stopped working, other X chromosome genes might start up again. So the unusual method for switching off *Xist* might permit the gene to remain active even when the rest of the chromosome is silenced, Lee says.

Thanks to her team's report two years ago in *Science*, researchers have another oddity to ponder that involves *Tsix*. Lee's group discovered that before one X is inactivated, the two X chromosomes in female mouse cells line up and briefly touch at the X-inactivation center. Such

pairing doesn't normally occur once X inactivation is complete, and it requires at least three genes, including *Tsix*. Without the contact, the cell can't figure out how many X chromosomes it contains or which of them to inactivate, so it might shut down both or neither. During their brief dalliance, the two chromosomes appear to be communicating. What information passes between them "is something we're vigorously pursuing," Lee says.

### A FLAG FOR STEM CELLS

Findings by Lee and others also raise concerns about the safety of embryonic stem cells. Researchers have high hopes that these flexible cells, which can specialize into heart cells, liver cells, or any of the body's other cell types, can be

directed to repair or replace damaged tissue and organs.

But only if X inactivation proceeds normally. Lee's group assessed the amount of *Xist* RNA, viewed as an indicator of chromosome shutdown, in 11 stem cell lines being maintained in the lab. The samples included "approved" lines that scientists can study with federal government money and other lines provided by HHMI investigator Douglas A. Melton, co-director of Harvard's Stem Cell Institute, that had been developed without federal funds.

"When we looked at their X inactivation state, they were all over the map," Lee says.

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### A MYSTERY THAT GRABS HOLD

"This is a subject that people fall in love with," says Huntington Willard, an HHMI professor at Duke University in Durham, North Carolina. → Willard, who describes himself as "the old guy" in the field, says that his attraction to dosage compensation and X chromosome inactivation began in the early 1970s, when he was a Harvard undergraduate working on a lab project with the late HHMI investigator Sam Latt. During mitosis, cells copy their chromosomes and then divide, and Latt and Willard wanted to determine whether silenced and active X chromosomes behaved differently during this process. They did—the inactive X was slower to duplicate and copied its sections in a different order than did its active partner. → When he moved to Yale University for grad school, Willard kept up his work on X inactivation—but only by moonlighting. By day, he worked on his official dissertation project, studying the genetics of an inherited metabolic disorder called methylmalonic acidemia. Willard has divided his effort between X inactivation and other genetic projects ever since. He was part of the team that nailed down the DNA sequence of the X chromosome in 2005, for instance, and in 1997 his group designed the first artificial human chromosome. In 1991, he and his co-workers jump-started molecular investigation into X inactivation by identifying the first mammalian gene that controlled the process. They called it *Xist*.

→ Three years ago, Willard and his colleague Laura Carrel of Pennsylvania State University gave the field something else to explore. They measured the activity of 95 percent of the X chromosome genes—the most comprehensive survey so far. Ten to 15 percent of the genes escaped inactivation entirely, and another 10 percent sometimes shut down. → How cells manage to inactivate most X chromosome genes but leave some working is a mystery. Scientists had thought that *Xist* RNA spurs shutdown by enveloping the X chromosome. In fact, if researchers tag the RNA with a fluorescent marker, these *Xist* molecules show up as a luminous cloud swaddling the chromosome. The question is how this cloud clears over the genes that remain active. One of the many seductive mysteries, Willard says, that captivate and keep researchers coming back for more. —M.L.

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(GENETIC BALANCING ACT)

As her team revealed this March in the *Proceedings of the National Academy of Sciences*, two of the stem cell lines carried out X inactivation just fine. But in six lines, after one X was inactivated the cells stopped producing *Xist* RNA. Although the team found no evidence that an entire X chromosome reawakened in these cell lines, it's possible that some—perhaps many—genes on the X could fire up again. The scientists have already found evidence that this happens in mouse stem cells.

Reactivation might just kill the cells, but it could spell trouble for another reason. Some tumor cells carry an extra X chromosome, so it's not unreasonable to wonder whether a partially reactivated X might prompt similar abnormal growth. "It's extremely disconcerting," says Lee. "There's nothing we can do to restore X inactivation once reactivation occurs." The findings, she says, indicate that researchers need to do more experiments to determine

whether stem cells induce tumors if they are transplanted into patients.

Other stem cell experts praise this work. Although researchers have previously pinpointed X inactivation mishaps in stem cells, "this is the most thorough study" to date, says Renee Reijo Pera, director of the Center for Human Embryonic Stem Cell Research at Stanford University. "It definitely raises a red flag," though we need more information about X inactivation in the early embryo to judge how serious the problem is, she says.

#### Expect the Unexpected

What intrigues Meyer these days is the connection between dosage compensation and other cellular events that involve large-scale alterations to chromosomes. One example is crossing over, which occurs during meiosis, the type of cell division that leads to sperm and eggs. During crossing over, chromosomes pair up and swap DNA. The exchange is important from an evolutionary standpoint because it boosts the genetic diver-

sity of offspring. But it's also important to get the chromosomes in position for meiosis.

Meyer and colleagues revealed this January that a protein that's part of the all-important dosage compensation complex has another job—helping govern the number of times crossing over happens. According to Meyer, this link is "completely unexpected" and suggests that crossing over and dosage compensation in worms use a similar molecular mechanism to make big changes to the chromosomes.

As they've investigated the details of dosage compensation, Lee, Meyer, and other researchers have wandered into strange territory. They've come across molecular battles, take-charge RNA molecules, and furtive liaisons between chromosomes. And that's just the beginning. Plenty of unknowns remain. Mammal cells, for example, count their X chromosomes and randomly pick one for inactivation. Nobody knows how they manage either task. Whatever the answers turn out to be, Lee and Meyer say they're expecting more surprises. ■

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(SOURCES OF RENEWAL)

before making a broader point. "It's worthwhile to bear in mind," he says, "that we would not have iPS cells except for the ability to study embryonic stem cells. The same people who are now crowing that we don't need embryonic stem cell research tend to forget that we would never have gotten to this point without it."

Advances are coming so quickly that it's difficult to get top scientists to speculate about where the field will be a year from now. Orkin expects that Hochedlinger's

work comparing both types of cells will raise a "cautionary note" for researchers. And Orkin hopes his research will provide the tools needed to create iPS cells that more closely mimic ES cell lines.

Daley is making more iPS cells, creating lines of cells with various blood diseases. In the near term, he hopes that, by transferring diseases from patients into Petri dishes, he'll be able to learn more about disease progression and possibly identify therapies, as he can conduct experiments in cell cultures that he wouldn't do with patients. Looking further

ahead, he remains committed to the possibility of doing for people what he's already done for mice.

"We think that these disease-specific lines will ... help lay the foundation for using genetically repaired cells to replace disease tissues," he says.

Of course, before he can do that, scientists will have to learn to reprogram cells without using viral vectors, a challenge that everyone seems to be pursuing but that no one wants to discuss in any detail. Daley will only say, "That's the hottest area of research in the lab right now." ■



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