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Protein May Play Role in Sex Chromosome Inactivation

The decision to inactivate one of the two X chromosomes, which occurs early in development, is a life-or-death decision that is made in the female eggs of all mammals, including humans. For reasons that are still not completely understood, failure to choose and completely shut down one X chromosome means immediate death for the developing embryo, due to a genetic “dosage” imbalance.

Researchers studying the X-inactivation process have now identified a molecule, called CTCF, which appears to be central to regulating X-chromosome inactivation. The research team, which was led by Jeannie T. Lee, a Howard Hughes Medical Institute investigator at Massachusetts General Hospital, published its findings in the December 7, 2001, issue of *Science*.

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— Jeannie T. Lee

Researchers had long known that *Xist*, a gene on the X chromosome, triggers the inactivation of a single X chromosome by generating RNA that “paints” the entire chromosome and renders it inoperable. In previous studies, Lee and her colleagues had identified an antagonizing gene, dubbed *Tsix*, that produces an “antisense” RNA whose complementary structure might cause it to adhere to the *Xist* gene and block its expression. Blocking *Xist* prevents inactivation of the X chromosome. Both *Tsix* and *Xist* are situated on the X chromosome in a region known as the X-inactivation center.

Knowing about the two genes, however, was not sufficient to explain how inactivation is triggered, said Lee. Experiments by Lee and her colleagues on X-inactivation suggested that there is likely to be a mark at one end of the *Tsix* gene to which an unknown external protein would bind and work with *Tsix* to cause the X chromosome to remain activated. Their earlier experiments revealed a candidate region of the genome adjacent to *Tsix*

where the protein would likely bind; and Lee and her colleagues reasoned that the genetic sequence of this critical region must be highly conserved throughout all mammals.

“We then took a computational strategy, in which we compared the human and mouse genome sequences in this region and asked what elements are conserved,” said Lee. Another important piece of information came from the work of other researchers who showed that the transcription factor CTCF appeared to be a regulator of imprinted genes that are selectively switched off during development. “CTCF sites are known to exist in a number of other genes, in human and mouse genomes,” said Lee. “And when we simply asked whether the conserved regions we discovered matched those CTCF binding sites, we found that they did.”

In additional studies, Lee and her colleagues demonstrated that the CTCF protein did, indeed, bind to the sites they had discovered in the X-inactivation center. While the researchers have not yet determined the precise nature of CTCF’s interaction with *Tsix*, their experiments hint that it serves as an activator of the gene. Alternately, said Lee, CTCF binding sites might act as an “insulator,” blocking an unidentified enhancer from attaching to the *Xist* gene, preventing it from inactivating the chromosome. According to Lee, discovery of CTCF’s involvement opens the way to understanding how the developing embryo chooses which X chromosome to inactivate.

“The next step is to figure out how this CTCF protein is binding to one X chromosome allele and not the other,” she said. “In other words, what’s recruiting CTCF to the antisense *Tsix* gene?” Another central mystery, said Lee, is what the control signals are that interact with the CTCF protein to make it so precisely choose to bind at the X-inactivation center.

“The problem is that the CTCF protein is ubiquitous,” said Lee. “It’s expressed early in development and late in development; it’s present in males and in females. So, how does CTCF know to go only onto one X chromosome? And how does it know to only attach at the onset of X-inactivation and not before or after? We need to understand how all these developmental decisions are made that are specific to the *Tsix* gene and specific to the future active X chromosome. While we currently have no idea what those factors are, now that we’ve identified one protein, CTCF, we believe it’s only a matter of time before we or our colleagues identify additional proteins in this decision mechanism,” she said.