Scientists unveil sex-linked control of genes

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A genetic phenomenon called X inactivation is the reason that most calico cats with both orange and black fur are female. Stanford researchers have identified the molecule that triggers X inactivation, which may shed light on some sex-specific diseases. Credit: Wikipedia

Many proteins interact with an RNA molecule called Xist to coat and silence one X chromosome in every female cell. Learning how genes are targeted and silenced may help researchers studying sex-specific diseases.

Garfield has a dark secret. The cartoon cat is a genetic anomaly, not because of his insatiable lasagna cravings, but because of his coat color.

Outside the world of the Sunday comics, orange and black cats are almost invariably female.

This truism is due to a curious biological phenomenon called X inactivation, which ensures that females of all species have only one active X chromosome in every cell. Early in development, when embryos have just few cells, one X chromosome is shut down or silenced in each cell. This chromosome remains inactive in all of that cell's progeny throughout the life of the animal. In cats and many other species, the selection of the chromosome to be inactive is random; in some other species, the X chromosome inherited from the father is always chosen.

X inactivation is necessary to ensure that females, who have two X chromosomes, and males, who have only one, end up with roughly the same dosage of genes that occur on that chromosome.

The "orange or black fur" gene is on the X chromosome, so a female cat can have a calico coat with both colors if "orange" and "black" remain active in different cells, but because a male cat has just one X chromosome it can be only one of these colors.

Scientists have known about X inactivation for decades. Recently they learned that an RNA molecule called Xist is responsible. But it's not been at clear exactly how Xist works to silence genes on the X chromosome.

Now researchers at the Stanford University School of Medicine have outlined the molecular steps of inactivation, showing that it occurs in an orderly and directed fashion as early embryonic cells begin to differentiate into more specialized tissues. They've identified more than 80 proteins in mouse cells that bind to Xist to help it do its job. They hope their findings will shed light on conditions in humans that are typically more severe in one gender than the other.

Gender differences in diseases

"We see some very interesting phenomena with X-linked diseases in humans," said Howard Chang, MD, PhD, professor of dermatology. "Often, when the faulty gene is on the X chromosome, the condition is more severe in boys. This happens in hemophilia, for example. In contrast, women are far more likely than men to suffer from autoimmune diseases, for reasons we don't yet understand. This research opens the door to possibly understanding the biological basis for these differences."

A paper describing the research findings will be published in the April 9 issue of *Cell*. Chang is the senior author, and former graduate student Ci Chu, PhD, is the study's lead author. The research required an entirely new technique, which was developed by Chu, to identify proteins interacting with Xist.

"Usually people start with a protein of interest and look for RNA molecules that might associate with that protein. We wanted to start with the RNA and find out what proteins Xist is talking to," said Chang. "Ci's new technique allowed us to discover the RNA and protein complexes responsible for X chromosome silencing."

The genes for orange and black fur are on the X chromosome in cats. Therefore, a female cat can have a patchwork of both orange and black fur depending on which chromosome was randomly inactivated in each ancestor cells. A male, however, can be orange or black, but not both. (An exception would be a male cat that had somehow inherited an extra X chromosome along the way, making it an XXY animal likely to have other significant genetic problems.)

Chu's technique, which the researchers call CHIRP-MS for "comprehensive identification of RNA-binding proteins by mass

spectrometry," allowed the researchers to identify the sequential interaction of over 80 proteins with Xist during X inactivation. Many of these proteins have never before been associated with that process. It's thought that they may help target and anchor Xist to active genes along the length of the X chromosome like burrs on a shoelace after a hike in the woods.

Elaborate genetic machinery

"If you lay all the copies of Xist in a cell end to end, they are not long enough to coat the entire X chromosome," said Chang. "Instead, Xist spreads judiciously, finding active genes and shutting them down. It also must stay anchored to the chromosome and not float over to any other chromosomes in the nucleus. This requires an elaborate set of machinery that we believe acts in a sequential fashion."

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Specifically, the researchers suspect that some proteins help Xist locate and silence active genes, while others work to maintain that silencing once it has been established.

"We're interested in really understanding this process, including how the inactivation of a specific X chromosome is maintained during DNA replication and cell division," said Chang. "We and others are really excited by this research."

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