The not so inactive X chromosome

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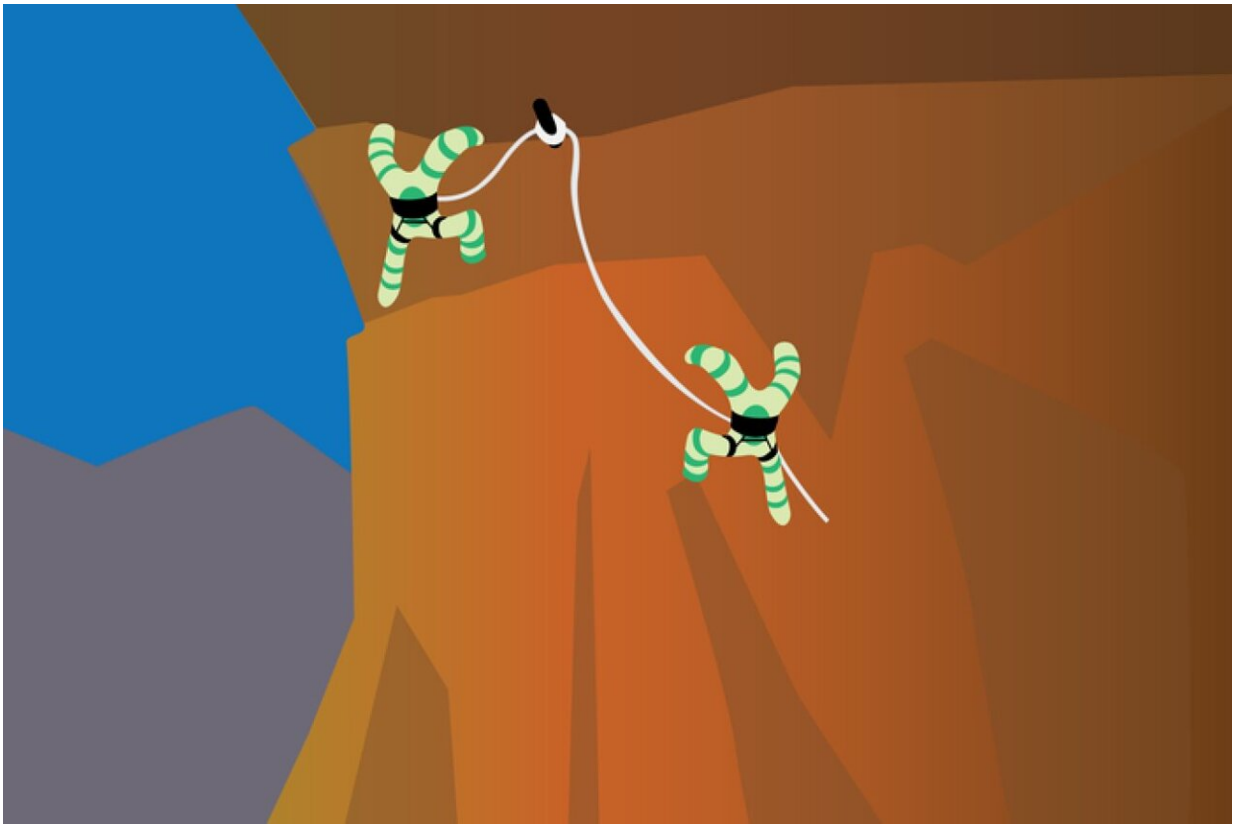

Credit: Jennifer Cook-Chrysos/ Whitehead Institute

Nearly every cell in our body contains pairs of each of our chromosomes, and these pairs are identical in all but one case: that of our sex chromosomes. Males typically have one X and one Y sex chromosome, while females typically have two X chromosomes.

In recent years, research has suggested that these different chromosomes can influence far more than sex determination. Gene expression from the sex chromosomes appears to contribute to sex differences in health and disease, which males and females experience in everything from the incidence of getting certain diseases, to the symptoms of diseases, to responses to drugs, and more. For example, women are more likely to develop autoimmune disorders, while men are more likely to develop heart conditions.

Whitehead Institute Member David Page has spent his career understanding how the differences between X and Y contribute to these sex differences, but a recent project is taking his lab in a new direction: understanding how the differences between X chromosomes contribute to sex differences. Although females' pair of X chromosomes contain the same genes, they have different patterns of gene expression.

New research from Page and postdoc Adrianna San Roman reveals just how different the two types of X chromosomes are. The findings, published in the journal *Cell Genomics* on February 8, show that one type of X chromosome, known as the inactive X chromosome, can modulate the gene expression of the other type of X chromosome, known as the active X chromosome. Their work indicates that inactive X chromosomes have underappreciated roles in gene regulation and, most likely, in sex differences in health and disease.

Difference rooted in history

Females' two X chromosomes have different gene expression activity because of the sex chromosomes' evolutionary history. The X and Y sex chromosomes evolved from a pair of identical non-sex chromosomes. Because of this ancestry, the sex chromosomes still contain genes that are important outside of regulating sex differences, such as genes that contribute to our immune system or regulate gene expression throughout

the body. However, over time the Y chromosome shrank and lost most of its genes.

Researchers think that in order to make up for the loss of necessary genes on the Y, expression of the corresponding genes on the X chromosome increased. This ensured that males still had the necessary levels of gene expression from their sex chromosomes, but now females, with two copies of X both working overtime, had levels of gene expression that were too high. To solve this problem, our bodies developed a process called X chromosome inactivation, by which the majority of genes on all but one copy of the X chromosome in each cell are silenced, or turned off.

This means that everyone, male and female alike, has one copy of the X chromosome working at full strength–the active X chromosome. In males, the active X chromosome is paired with a Y chromosome, and in females, it is paired with a so-called inactive X chromosome, on which most of the genes are turned off.

In spite of the evolution of X chromosome inactivation, some percentage of genes on the inactive X chromosome are still expressed, such as genes that have an active counterpart on the Y chromosome. Previous research has indicated that about a quarter of the genes on the inactive X are, in fact, active, so researchers have long been aware that the chromosome is not completely silent. However, it's still often painted as a passive copy playing backup for its more active partner. San Roman's work shows that the inactive X chromosome's gene expression is much more potent and complex than that.

A spectrum of sex chromosomes

In order to understand the inactive X chromosome's contributions to gene expression, San Roman and colleagues in the Page lab collected

blood and skin samples from people born with unusual combinations of sex chromosomes—everything from X0 (one X chromosome) to XXXXY. People with these different sets of chromosomes often have health issues; for example, X0 females have Turner syndrome, which can cause heart defects, hearing impairment, and more; and XXY males have Klinefelter syndrome, which can cause infertility, weak muscles, and more. Page and San Roman hope their research could provide useful insights into these health issues as well as into sex differences between XY males and XX females.

In people with more than one X chromosome, every X but one is an inactive X. The researchers graphed sex chromosome gene expression, measuring the change in expression level of each gene with the addition of each inactive X, for people with anything from zero to three inactive X chromosomes, as well as different numbers of Y chromosomes. They also looked at the relative contribution to overall expression from the active versus inactive X chromosomes.

One might expect the graphs they made to be relatively straightforward: for genes that are turned off on the inactive X chromosome, the gene expression level would not change at all as the number of copies of the inactive X increased. For genes that are turned on, the gene expression level would double with two X chromosomes, triple with three X chromosomes, and so on. When the researchers looked at chromosomes other than X with extra copies—namely, Y and chromosome 21—this is essentially the pattern they observed. Gene expression from additional X chromosomes, however, was not so straightforward.

Each additional inactive X chromosome changes gene expression by the same amount. However, the researchers found a surprising diversity in expression levels across X chromosome genes. The presence of each additional inactive X might increase one gene's expression by 20% and another's by 70%. Then the results grew more surprising: for some

genes, the addition of an inactive X decreased their expression. For some genes that are only expressed on the active X chromosome, and completely silent on the inactive X, additional inactive X chromosomes nonetheless changed their expression level.

These discrepancies led the researchers to a startling finding. The X chromosomes do not function independently of each other. Instead, the inactive X chromosome can modulate expression of genes on the active X chromosome. In other words, some genes on the inactive X chromosome regulate genes on the active X chromosome, dialing their expression up or down. Altogether, the researchers found that 38% of the X chromosome genes in the two cell types that they tested are affected by the presence of inactive X chromosomes, either because the genes are expressed on the inactive X, or because the inactive X regulates their expression on the active X, or through some combination of the two mechanisms.

These findings show that the inactive X plays a much more active role in gene expression and regulation than was previously appreciated. Rather than just playing second fiddle to the active X chromosome, the inactive X is sometimes harmonizing with and sometimes even conducting its partner.

Rethinking the role of the inactive X in health and disease

Page and San Roman hope that their findings will help refocus research into sex differences. Previous research into the mechanisms behind these differences has focused on the effects of having X versus Y chromosomes. Page and San Roman's work show that researchers also need to consider how the presence (in females) or absence (in males) of an inactive X chromosome contributes to sex differences.

"Everybody on the planet carries one active X chromosome, so that first X chromosome really does not contribute, we think, to differences between males and females," says Page, who is also a professor of biology at the Massachusetts Institute of Technology and Investigator with the Howard Hughes Medical Institute. "If we transition from saying that females are XX and males are XY, to saying that females are Xi [have an inactive X] and males are Y, that really focuses the question."

Page lab researchers have already begun using their findings to identify X chromosome genes that are likely to be important for sex differences in health and disease. From their list of genes that change in expression based on the presence of an inactive X, the researchers narrowed in on a top ten list of genes that need to maintain a specific expression level or else there will be severe negative consequences. These genes are also likely to be responsible for causing the health issues associated with different atypical sex chromosome compositions, because changes in their expression level are most likely to have strong effects on cells.

"This is a new way of thinking about how the X chromosome is expressed and how it might be impacting our biology," San Roman says. "This top ten list will be really interesting to consider in the future in terms of how the level of expression of these genes affects cells and tissues in very fundamental ways."

More information: Adrianna K. San Roman et al, The human inactive X chromosome modulates expression of the active X chromosome, *Cell Genomics* (2023). DOI: 10.1016/j.xgen.2023.100259

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https://phys.org/news/2023-02-inactive-chromosome.html