Study shows how female immune cells keep their second X chromosome shut off

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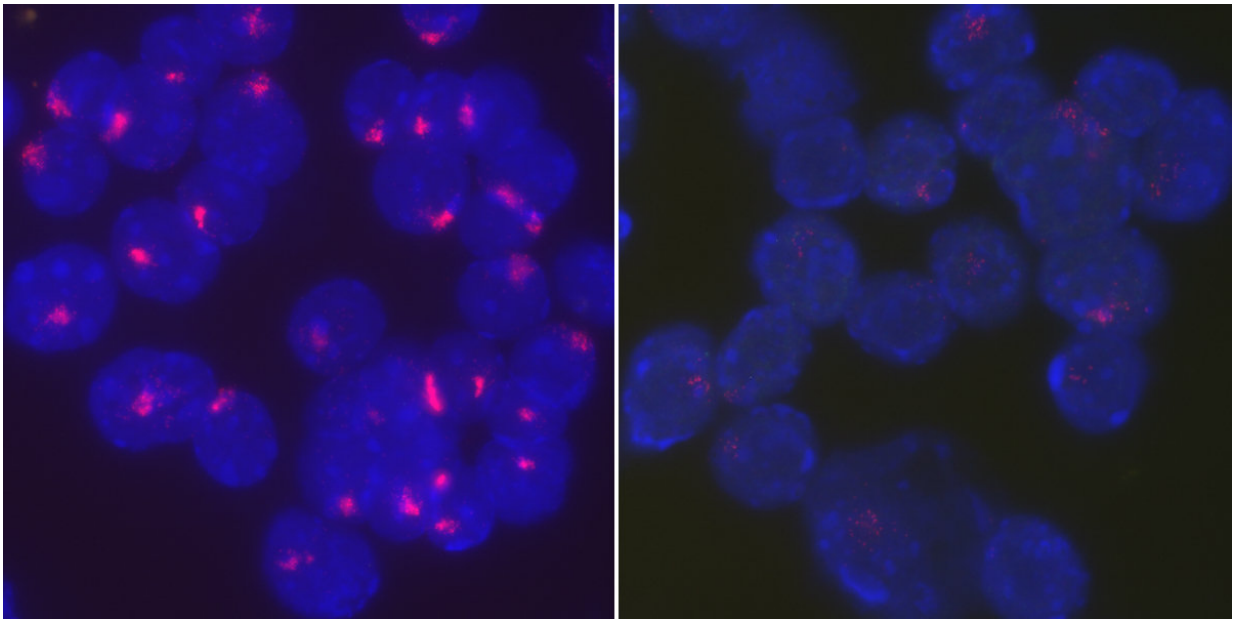

Researchers found that the protein YY1 brings Xist RNA back to the inactive X chromosome to maintain X chromosome inactivation in stimulated B cells. Activated, wild type B cells from female mice tightly localize Xist RNA at their inactive X chromosomes (left), whereas Xist RNA becomes dispersed throughout the entire nucleus when YY1 is deleted (right). Credit: University of Pennsylvania

Autoimmune diseases tend to strike women more than men and having multiple X chromosomes could be the main reason why. While a process called X chromosome inactivation serves to balance out gene dosage

between males and females, some genes on the "inactive X" chromosome in immune cells can sometimes escape this process, giving women an extra dose of immunity-related gene expression.

In a new study, a team from the University of Pennsylvania describes how X chromosome inactivation is regulated in the immune system's B cells as they develop in bone marrow and when they encounter antigens. Their work elucidates a dynamic, two-step mechanism in females whereby B cells lacking the markers of X chromosome inactivation regain these modifications during B cell activation, in a process involving the transcription factor YY1.

"What's remarkable is that B cells are the ones making antibodies and autoantibodies, so they're really crucial in both protective immune responses and autoimmunity," said Montserrat C. Anguera, assistant professor in the Department of Biomedical Sciences in Penn's School of Veterinary Medicine and the senior author on the study. "A big question that remains is, Why are these immune cells priming for this chromosome to be regulated differently and also, If these proceses go awry, how does that lead to autoimmunity and loss of self-tolerance?"

The study, published in *PLOS Genetics*, was led by Camille M. Syrett, a doctoral student in Anguera's lab. Coauthors, representing both Penn Vet and Penn's Perelman School of Medicine, were Vishal Sinhava, Suchita Hodawadekar, Arpita Myles, Guanxiang Liang, Yue Zhang, Satabdi Nandi, Michael Cancro and Michael Atchison.

In a study last year in the journal *Proceedings of the National Academy of Sciences*, Anguera and colleagues found that naïve, unstimulated female lymphocytes, the B and T cells of the immune system, failed to completely inactivate an X chromosome. The research showed that this was because Xist, a long non-coding RNA transcript well known to initiate and maintain the process of X chromosome inactivation, failed to

localize to the inactive X chromosome. When the immune cells were activated, or primed to respond to infection, Xist RNA reappeared in the proper location.

"We saw that the inactivated B cells don't have this RNA cloud, but it comes back upon stimulation," said Syrett. "We were really interested in determining what is bringing the Xist RNA back."

Using a fluorescent tracker molecule, the team began by tracking the location of Xist in developing B cells in female mice. They found that the precursors of B cells, such as hematopoeietic stem cells and common lymphoid progenitors, had clear patterns of Xist RNA on the inactive X chromosome. But, as these B-cell precursors went down a developmental path toward becoming B cells, Xist RNA first seemed to disappear, then reappear, but only as diffuse pinpricks across the nucleus instead of being localized to the inactive X.

In addition, small-molecule tags called heterochromatin modificiations, which are known to maintain gene repression during X chrosomsome inactivation, disappeared during B cell development.

"We could see these really unusual changes at the chromatin level happening in female B cell development," Anguera said.

To find out how the Xist returned to the inactive X upon B cell activation, as they had shown in last year's PNAS paper, the researchers tracked Xist RNA in B cells in culture. They found the reappearance took place in two distinct phases: Between four and 16 hours after the cells were stimulated, the speckles of Xist RNA began to appear. And between 16 and 30 hours after stimulation, Xist RNA concentrated exclusively at the inactive X chromosome. The heterochromatin modifications appeared to increase and localize on the inactive X in this second phase as well.

The team's earlier study had pointed to the protein YY1 as playing a role in the return of Xist in activated B cells, so they began to look more closely at it in this work. Atchison, a leader in studies of YY1 in B cell development, was able to lend his expertise to investigate the protein's role in X chromosome inactivation.

When the researchers examined B cells from mice that lacked YY1, they saw greatly reduced levels of heterochromatin marks as well as less localization of Xist RNA to the inactive X.

The team also observed evidence that YY1 was influencing the expression of X chromosome genes. When they deleted YY1 from male and female cells and compared genes that were differentially expressed on the X chromosome, they found 68 that were specific to females, one that was specific to males and 11 that were shared.

Of the 68 genes specific to females, many were related to immunity, and at least two are known to be overexpressed in human female B cells compared to male B cells.

A further set of experiments identified the area of YY1 with DNA binding activity as the key domain of this protein involved in bringing Xist RNA to the inactive X.

"It seems to be acting as a tether, bringing the Xist RNA together with the DNA of the inactive X chromosome," Anguera said.

She and her colleagues will be exploring the role of YY1 further, using clinical samples as well as mouse models, to look at the protein in diseases like lupus to deepen their understanding of how autoimmunity could result from the "escape" of immune genes from X chromosome inactivation.

"If you want to develop a therapy for autoimmune diseases, the idea is, How do we get Xist to the inactive X chromosome and keep it there so we maintain dosage compensation in these B cells." said Anguera. "Certainly YY1 is looking like a really promising target."

More information: Camille M. Syrett et al, Loss of Xist RNA from the inactive X during B cell development is restored in a dynamic YY1-dependent two-step process in activated B cells, *PLOS Genetics* (2017). DOI: 10.1371/journal.pgen.1007050

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