Scientists untangle Barr body of inactive X chromosome

July 21 2016

Scientists at UMass Medical School, the Institut Curie in Paris and Stanford University, have taken a detailed look inside the small, densely packed structure of the inactive X chromosome found in female mammals called the Barr body and developed a model system that may be an important tool for understanding chromosome structure and gene expression.

The inactive X chromosome has long been thought to be a rather amorphous compacted structure, but the new study, published in *Nature*, reveals a highly organized chromosome consisting of two distinct lobes of condensed inactive DNA with smaller structured domains of active DNA embedded in them. These smaller domains, referred to as topologically associating domains, are highly defined genetic "neighborhoods" and are found on other chromosomes as well. These domains have a significant role in gene expression, and their presence within the otherwise inactive Barr body was surprising.

"This is the most detailed molecular view we've been able to obtain of the DNA inside the Barr body," said Job Dekker, PhD, Howard Hughes Medical Institute Investigator, professor of biochemistry & molecular pharmacology and co-director of the Program in Systems Biology. "Under a microscope, the inactive X chromosome is very different than other chromosomes; it looks like a condensed, undefined, inactive 'blob.' Our study, using a range of experimental approaches including imaging and genomic methods, describes something else entirely: a highly organized and elaborate structure, rich in features that may silence or

activate genes all along the chromosome."

Although DNA is composed of a linear sequence of bases, it doesn't exist inside the cell nucleus in a simple, straight form. Instead, the genome folds and loops back on itself so it can fit inside the tight confines of the nucleus. The shape it takes has a profound influence on which genes in a cell are turned on or off. To properly understand how the genome works to coordinate gene expression, it's necessary to understand how the genome is organized in space inside cells.

In the case of the inactive X chromosome, scientists know that female mammals contain two X chromosomes, one of which is "turned off" to avoid overexpression of genes. This inactive X chromosome can be clearly seen with a microscope as a dense, shapeless, dark stain, called a Barr body. It is thought that the Barr body's dense shape is a result of it being mostly inactive. But the precise structure of the Barr body, how it is condensed and why some pieces of the DNA remain active have been very difficult to explore using even the most advanced imaging technologies, and more recently with genomic approaches based on chromosome conformation capture.

A pioneer in the study of the three-dimensional structure of the genome, Dr. Dekker has developed a suite of chromosome conformation capture technologies—biochemical techniques for determining how DNA segments interact and are linked to one another, which are the heart of the "3C," "5C," and "Hi-C" tools used by researchers worldwide to map the three-dimensional organization of chromosomes inside cells. Using the Hi-C technology, Dekker and colleagues were able to construct a detailed view of the shape and architecture of the inactive X chromosome.

To unravel the structure of the inactive X chromosome, Dekker and colleagues first had to address some major obstacles. One problem,

according to Bryan R. Lajoie, a bioinformatician in the Dekker lab, is that it's all but impossible to tell the inactive X chromosome apart from active X chromosome given that they have virtually the same sequence. "The mice models we use in the lab lack the diversity we need genetically to be able to make this sort of distinction," he said.

"In order to properly determine the three-dimensional structure of the chromosomes using the Hi-C sequencing technology, we crossed two different mouse strains and built a new computational approach using naturally occurring mutations in one of the X chromosomes as guideposts," said Lajoie. "By filling in the gaps computationally, we were able to connect enough dots to begin building a three-dimensional model of the inactive X inside the Barr body."

What they found was that the Barr body wasn't a single dense mass of DNA but instead is composed of two separately packed lobes separated by a highly repetitive segment of DNA called a macrosatellite repeat, found only in a few places in the genome. Dekker speculates that these macrosatellite repeats are responsible for packing and organizing DNA inside the Barr body. When the team used CRISPR technology to surgically remove the macrosatellite repeat from the chromosome, they found that the bi-lobed structure disappeared.

"It's remarkable, that a single element can have such a global impact on shape and function of a chromosome," said Edith Heard, PhD, chair of epigenetics and cellular memory at the Institut Curie in Paris.

Though mostly inactive, there are still clusters of genes inside the lobes that are being expressed. These genes reside inside topologically associating domains (TADs), which organize the genome into neighborhoods separated by boundaries rich in CTCF proteins, which repress transcription. This finding suggests that TADs play a role in making organizing gene expression within the otherwise inactive lobes of

the silent X chromosome.

"In order for a gene to be expressed inside the inactive Barr body it had to be located inside a TAD. It's possible that TADs may be playing a kind of protective role, allowing genes to be accessible for expression even when they are located inside the condensed and inactive chromosome," said Dekker.

This breakthrough establishes the inactive X chromosome as a powerful and unique model system for studying the relationship between the spatial organization of the genome and gene expression and will help scientists learn more about how the genome works inside living cells.

Provided by University of Massachusetts Medical School