New study helps solve a great mystery in the organization of our DNA

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Findings by Benoit Bruneau (left) and Elphège Nora (right) from the Gladstone Institutes reshape our understanding of how DNA is organized in cells. Credit: Chris Goodfellow, Gladstone Institutes

After decades of research aiming to understand how DNA is organized in human cells, scientists at the Gladstone Institutes have shed new light on this mysterious field by discovering how a key protein helps control gene organization.

Humans have nearly 30,000 genes that determine traits from eye color to risk for hereditary diseases. Those genes sit along six feet of DNA, which are carefully organized into chromosomes and stuffed into each and every microscopic human cell.

"The extreme compacting of DNA into chromosomes is like taking a telephone cord that stretches from San Francisco to New York, and stuffing it into a backpack," described Benoit Bruneau, PhD, a senior investigator at Gladstone and lead author of a new study. "The organization of chromosomes is not random, but rather very complex, and it is critical for normal development. When this process goes wrong, it can contribute to various diseases."

How is our DNA organized?

Chromosomes are coiled into loops and then organized into many large domains called topologically associating domains, or TADs. Within each TAD, several genes and the elements that regulate them are packaged together, and they are insulated from those in neighboring TADs.

"Imagine TADs are like adjoining rooms: like the genes in each TAD, people in each room can talk to one another, but not to people in the next room," explained Elphège Nora, PhD, postdoctoral scholar in Bruneau's laboratory and first author of the study. "In previous work, we showed that TADs package genes together and insulate them from neighboring genes. The burning question then became: what controls this TAD organization?"

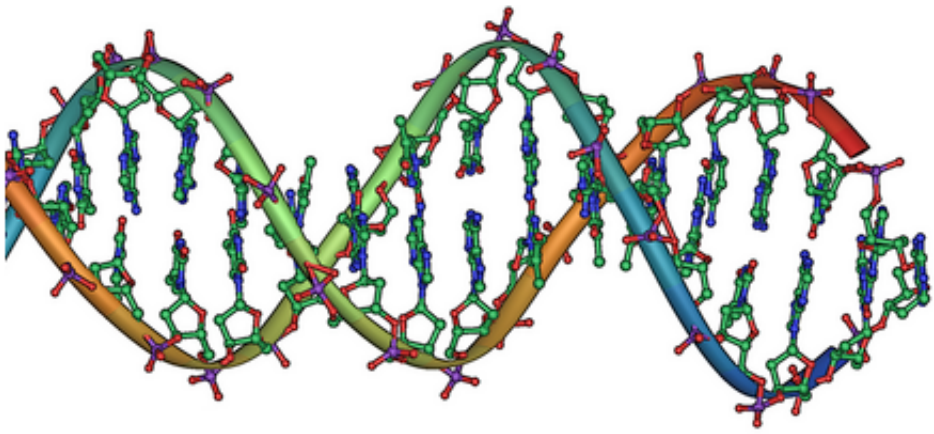
In the new study, published in the renowned scientific journal *Cell*, the scientists discovered that the key to organizing these TADs is a protein called CTCF.

"CTCF is a fascinating protein," said Bruneau, who is also a professor at

the University of California, San Francisco. "It can be found at the boundaries of TAD domains, and was previously thought to be involved in many aspects of chromosome organization. We wanted to see what would happen to the structure of chromosomes if we removed all the CTCF from cells."

CTCF: observing a protein that is impossible to study

Researchers have struggled with studying the role of CTCF in the past, because it is absolutely essential to cells' survival. Therefore, completely removing CTCF would cause cells to die, making them impossible to study.



DNA double helix. Credit: public domain

"We used a new genetic method to completely eliminate CTCF in mammalian cells," said Nora. "Using this technique, we destroyed the

protein very quickly so that we could study the cells before they died. This allowed us to look at the entire genome in the absence of CTCF and observe the effects."

In collaboration with a team of computational biologists led by Leonid A. Mirny at the Massachusetts Institute of Technology, and a team of biochemists led by Job Dekker at the University of Massachusetts Medical School, the Gladstone scientists demonstrated the importance of CTCF for the insulation of TADs.

"We noticed that, in the absence of the CTCF protein, the insulating boundaries of TAD domains had almost fully disappeared, so that genes and regulatory elements could now interact with those in adjacent TADs," added Nora. "This would be like removing the wall between adjoining rooms, so that people could now freely interact with others in the neighboring room."

However, the absence of CTCF had little effect on how genes connect within a single TAD. This indicates that CTCF is required for insulating TADs from one another, but not for packaging genes within these domains. This represents the first conclusive study to show that the two mechanisms are separate and controlled by different proteins.

Redefining our understanding of CTCF

Now that the scientists finally had a way of removing CTCF from cells, and disrupting the organization of TADs, they could start studying its impact on various aspects of the genome. They leveraged this new ability to examine other levels of chromosome organization.

"We looked at a level of organization called compartmentalization, which separates active and inactive genes within a cell nucleus," said Nora. "This helps the cell identify which genes to use. For example,

skins cells don't need eye-related genes, so these genes would be tightly packaged in a compartment and put away, because the cell will never use them. We used to think that boundaries of TAD domains were a prerequisite for the organization of these compartments."

"To our surprise, we found that is not the case," said Bruneau. "When we deleted the CTCF protein, which caused TAD boundaries to disappear, we saw no effect on the organization of the larger compartments. This interesting finding revealed that CTCF and TAD structure are not required for compartmentalization but, rather, that an independent mechanism is responsible for this chromosome organization."

"Our findings redefine the role of CTCF in gene regulation and provide new insights about the fundamental processes that govern genome organization" added Bruneau. "With this knowledge, we can now start reevaluating the cause of several diseases, as chromosome organization-including TADs-is often disrupted in many cancers and involved in significant developmental defects, such as congenital heart disease."

Prior to its publication in the peer-reviewed journal *Cell*, a preliminary version of the study was posted on bioRxiv, an open-access distribution service for unpublished preprints in the life sciences, and downloaded nearly 5,000 times over the past few months.

"Websites like these offer new, exciting, and more direct ways to share research results," said Bruneau. "It also provided us with valuable input that helped shape our final manuscript and future research."

Provided by Gladstone Institutes