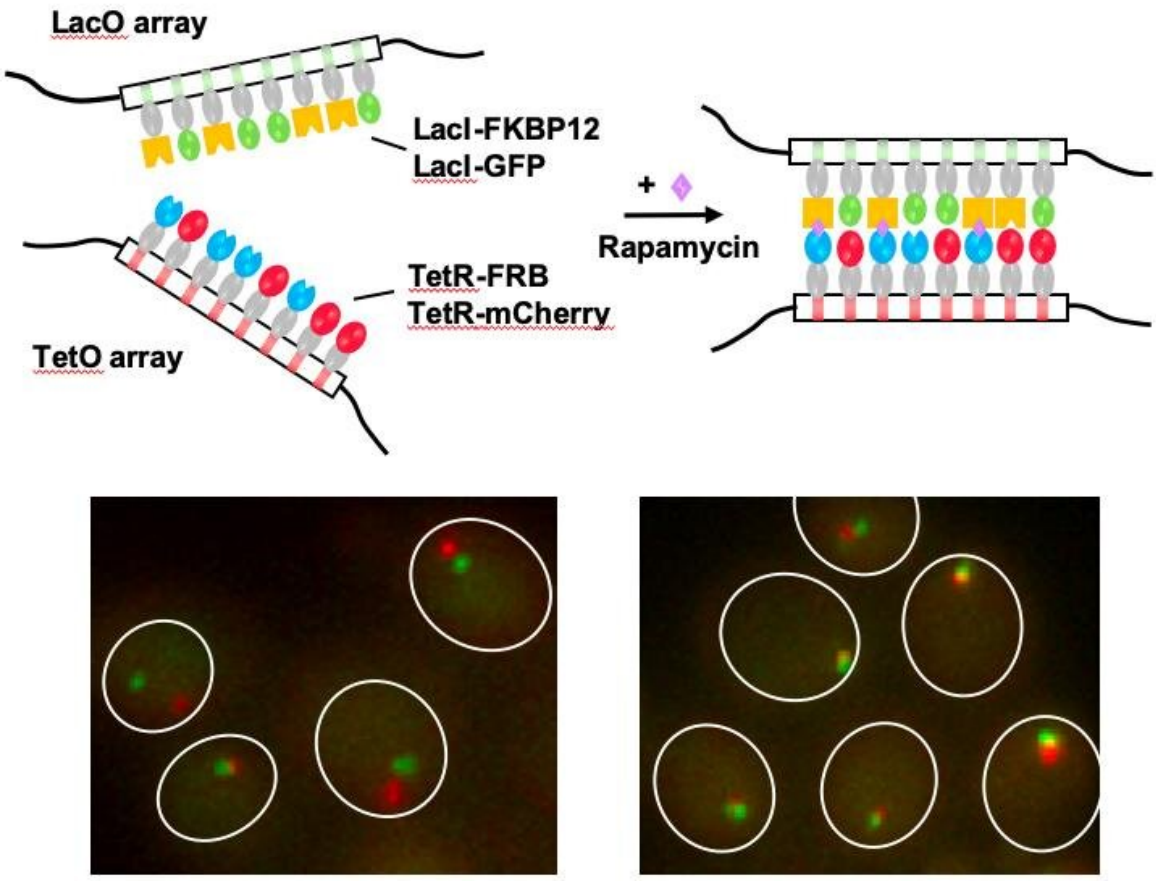
New, targeted method for probing the function of 3D chromosomal structure

February 18 2022, by Sam Sholtis



A new method—chemically induced chromosomal interaction (CICI)—can induce interactions between any two regions of the genome to test relationships between genome structure and function. The illustration (top) shows the scheme of the method. Researchers insert long arrays of binding sites into two genomic locations. These arrays associate with a large amount of two transcription factor proteins, LacI and TetR. LacI and TetR then fuse with two additional proteins,

FKBP12 and FRB, that bind in the presence of the compound rapamycin. Thus, researchers can induce the two genomic loci to strongly associate with each other by adding rapamycin to the cells and compare cellular function before and after the induced interaction. Typical data are shown below. The two loci (labeled by the red and green fluorescent dots) are spatially separated prior to the addition of rapamycin, but become co-localized after adding rapamycin. Credit: Bai Laboratory, Penn State

A new method that can induce interactions between specifically chosen locations on the genome allows researchers to begin to identify the causal relationship between three-dimensional chromosome structure and genome function. A paper by researchers at Penn State describing the method, called "chemically induced chromosomal interaction (CICI)," and two functional tests of the method appears Feb. 9 in the journal *Nature Communications*.

The genomes of eukaryotes—organisms ranging from yeast to humans whose cells have a distinct nucleus—are made up of chromosomes. Inside the nucleus, the chromosomes, which are long, linear strands of DNA packaged with numerous proteins that carry genetic information, are arranged in a three-dimensional conformation that, depending on the cell type, can bring genomic regions that are linearly distant from one another into close enough contact to functionally interact. These interactions are thought to be important for things like gene regulation, which controls when and where certain genes are used by the cell.

"It is now fairly straightforward to sequence the DNA and identify functional units like genes and regulatory regions, but understanding how the genome actually works is more complicated," said Lu Bai, associate professor of biochemistry and molecular biology and of physics at Penn State and leader of the research team. "We know that the three-dimensional structure of the genome, including the interactions between

different genomic regions on a chromosome or between two chromosomes, can be important for genome function, so we wanted to develop a generalizable method to causally link this structure to function."

The three-dimensional structure of the genome has been the focus of numerous recent studies using a variety of "chromosome conformation capture" techniques. Generally, these techniques work by chemically crosslinking the DNA and proteins in chromosomes forming bonds that lock together any areas where the chromosomes are close together in three-dimensional space and may be interacting. The crosslinked genomes can then be broken up, manipulated, and sequenced to identify which regions of the genome have been locked together.

"These methods have been hugely successful for identifying regions of the genome that are linearly distant on chromosomes but are close together in three-dimensional space," said Bai. "But we want to know if this chromosomal conformation matters. Comparisons of chromosomal conformation and functional measures, like gene expression, between different cell types can allow us to correlate structure with function but don't directly test for causation. Methods that can directly test causality that have been developed often have other issues, such as off-target impacts that disturb cellular function more generally making the results more difficult to interpret."

The research team developed a method—CICI—to address these issues. Briefly, they can insert short sequences of DNA into any two locations in the genome, express artificially engineered proteins that bind to these inserted sequences, then chemically induce the proteins to bind to each other when they are in close proximity. They can then compare cellular functions before and after inducing the structural change. Importantly, the system doesn't disrupt any normal cellular function beyond the regions being tested.

"Essentially, we can put two sticky pads anywhere we want in the genome, and the genome behaves normally until we 'activate' the sticky pads with a chemical signal," said Bai. "We can therefore directly compare the cell's function before and after we make the change."

The team tested their method in yeast cells and showed that the two selected regions did not interact prior to chemical induction and strongly interacted after induction. They also performed two functional experiments showing that three-dimensional structure is important for DNA repair in the yeast mating-type switching system and that it does not seem to impact the timing of DNA replication.

"Our method allows us to target specific locations in the genome and directly test the functional impact of artificially forcing the regions to interact without any off-target effects," said Bai. "We can also use this method to look at chromosome dynamics by tracking the two regions over the course of the cell cycle to see how long it takes for the regions to encounter each other. Although we developed the method using yeast as a model system, there's no reason that it shouldn't be able to be expanded into mammalian cells. We hope to be able to demonstrate that in the future."

More information: Manyu Du et al, Chemically Induced Chromosomal Interaction (CICI) method to study chromosome dynamics and its biological roles, *Nature Communications* (2022). DOI: 10.1038/s41467-022-28416-3

Provided by Pennsylvania State University

function-3d-chromosomal.html