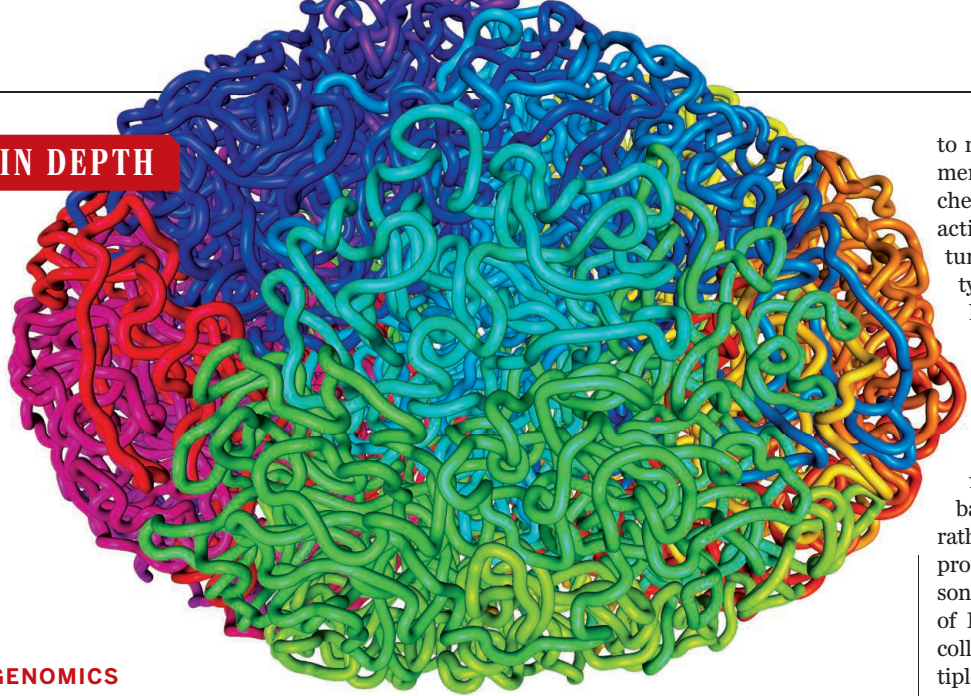


GENOMICS

Inching toward the 3D genome

Maps of DNA's loops and folds advance—but may disagree

By **Elizabeth Pennisi**

The metaphors for DNA keep multiplying. It is a string of code, a spiral staircase, and, now, something very like origami. Just as folding a flat sheet of paper can transform it into a crane or lotus flower, researchers have come to realize that a complex pattern of loops and folds helps transform our genome into something meaningful. The twists and turns bring particular genes into close contact with distant stretches of DNA that regulate those genes' activity, spurring the gene expression that makes a bone, muscle, or brain cell—or fuels a cancer.

The elegance and potential of this idea has fascinated many biologists, but they've struggled to get good enough data to reliably understand these sinuous patterns. An 11 December report online in *Cell* revealed the most elaborate maps yet of how the 2 meters of DNA crammed into the cell nucleus fold up—the so-called nucleome. "[L]andmark work," said Francis Collins, the director of the National Institutes of Health (NIH), in a blog post.

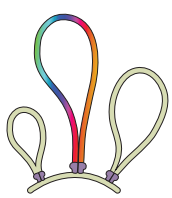
Four days later, however, a paper in *Genes & Development* reported that various DNA mapping techniques sometimes yield patterns that are radically different, raising questions about just what these maps really show. Now, through a newly funded NIH effort called the 4D Nucleome, researchers will set out to develop more reliable, accurate, and affordable ways to map and interpret the genome's elaborate folds. The program "is inviting people to come up with different and better ideas," says Job Dekker, a biologist at the University of Massachusetts Medical School in Worcester.

Over the past 2 decades, he; Erez Lieberman Aiden at Baylor College of Medicine in Houston, Texas; and others have used techniques such as chromosome conformation capture to look at genomes in 3D. The researchers chemically "freeze" any spots along the DNA chain where disparate stretches are in contact. Then they cut up all the DNA and glue the contacting DNA together. Based on the number of times two regions are in contact, the researchers estimate how far apart the regions are in 3D.

At first, researchers could look at one gene and a partner at a time, but ever more comprehensive methods soon appeared. A 2009 version, Hi-C, reveals how every piece of a genome's DNA interacts with every other piece. By figuring out how to process the DNA while it's still in a nucleus and then revamping the analysis techniques, Baylor's Suhas Rao and Harvard University graduate student Miriam Huntley, both in Aiden's lab, and colleagues got the resolution down from a million bases—way bigger than a gene—to 1000 bases—smaller than a gene.

The $3 million experiments reported in *Cell* generated more than 5 trillion sequenced bases and analyzed millions of human cells from eight cell lines, including cancer, and from one mouse cell line. The data revealed 10,000 loops, thousands to millions of bases long, and six compartments, regions where DNA with similar chemical modifications and levels of gene activity come together. Some of the structures are common to all the tested cell types, whereas others are unique to each kind of cell. "It opens up a new way of looking at biology," says Vishy Iyer, a molecular biologist at the University of Texas, Austin.

There's one wrinkle, as the *Genes & Development* paper showed: A different nucleome mapping technique based on direct observation of the DNA rather than on computational models can produce conflicting results. Iain Williamson and Wendy Bickmore of the University of Edinburgh in the United Kingdom and colleagues applied fluorescent labels to multiple pieces of DNA, using a different fluorescent probe for each one so that they could easily identify pieces that were close to each other. The researchers looked at a 1-million-base-long region of mouse chromosome 2, which contains a cluster of *Hox* genes that are key in development. For comparison, they analyzed the same DNA region using a computational technique similar to Hi-C. The two techniques agreed sometimes, for some parts of the *Hox* cluster, but in other cases, one indicated the DNA was stretched out whereas the other indicated it was tangled into a tight ball. "We don't know why and we don't know what method is right," says Ana Pombo, a cell biologist at the Berlin Institute for Medical Systems Biology. "We need to look carefully at what these methods are telling us."



A simulation (*top*) shows how a single DNA loop (*above*) twists and folds.

Aiden says his lab's new Hi-C results agree with the microscopy findings. Nonetheless, "we shouldn't fool ourselves into thinking that the Hi-C data is the be all and end all," he points out. "You want to have multiple lines of experimentation that can confirm one another or contradict one another."

NIH's 4D Nucleome program aims to provide that. This 5-year, $24-million-a-year effort, announced in July 2014, will improve the existing techniques and, possibly, come up with new ones. It's called 4D because the nucleome structure changes as cells age, differentiate, and divide, and researchers want to understand how and why. "The object is to make these techniques widely available," says Rafael Casellas, a molecular biologist at the National Institute of Arthritis and Musculoskeletal and Skin Diseases in Bethesda, Maryland. They are sorely needed, Dekker says. Just as origami paper comes to life only when folded, he says, "nothing in our genome makes sense except in 3D." ■

ILLUSTRATIONS: (TOP TO BOTTOM) ADRIAN SANBORN, MIRIAM HUNTLEY, AND EREZ LIEBERMAN AIDEN; M. ATAROD