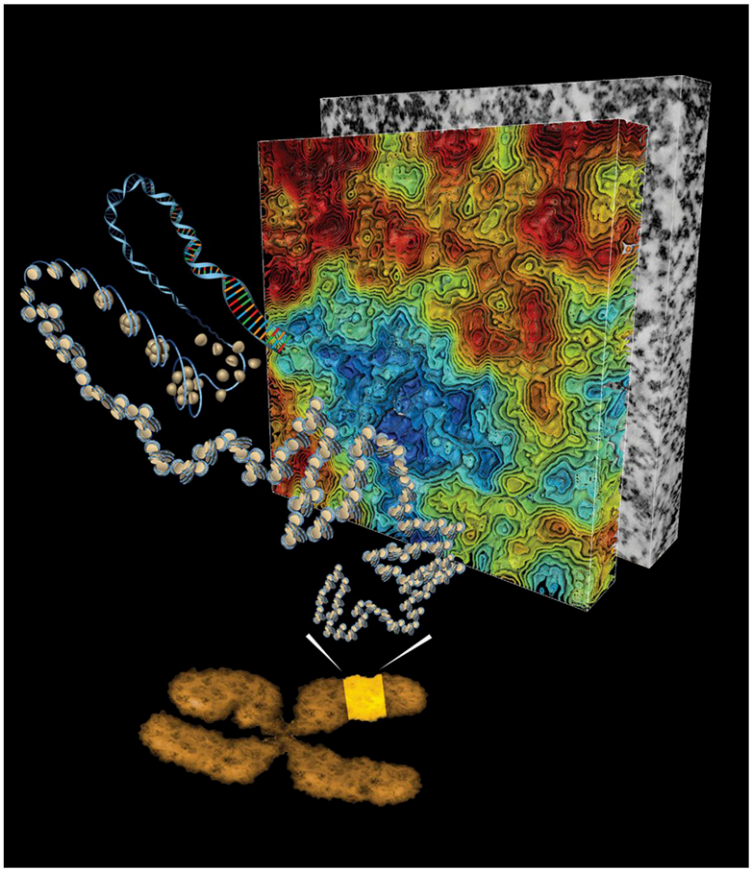
INNER WORKINGS

Researchers peek into chromosomes' 3D structure in unprecedented detail

Amber Dance, *Science Writer*

Clodagh O'Shea will never forget the moment in early 2015 when she peered at the cell's nucleus in a way no one had ever done before. Using a new technique to visualize three-dimensional (3D) chromosomes as they exist in the active, unadulterated nucleus, O'Shea was able to zoom in on individual nucleosomes where DNA wrapped around organizing proteins, then zoom out to see the entire scraggly mass. "It was just like seeing another planet, this whole new world," recalls O'Shea, a molecular biologist at the Salk Institute in La Jolla, CA. "You get lost in it."



A technique called ChromEMT reveals the details of chromatin ultrastructure, 3D packing, and organization of DNA. From ref. 1. Reprinted with permission from AAAS.

As her initial euphoria faded, O'Shea realized the shapes she saw were wholly unexpected. Based on widely accepted notions about how genetic material organizes in vitro, researchers had anticipated that the DNA strands would be neatly wound into regular fibers of 30, 120, and 300–700 nanometers in diameter. Instead, in the intact nucleus, chromosomes appeared as an amorphous tangle—at least at first glance. "Oh my god, how do we make sense of this?" she wondered. Further analysis revealed that chromatin formed chains of 5–24 nanometers across, winding throughout the nucleus; the chains packed tightly in some spots and more loosely in others (1). That doesn't mean the standard fibers don't exist, says O'Shea, but "there are probably more structural possibilities."

Since the 2001 publication of the human genome, researchers have navigated DNA mainly as digital strings of As, Ts, Gs, and Cs. Related research has led to intriguing advances, such as testing for genetic risk factors for disease and precision medicine. But the work hasn't met expectations when it comes to disease cures, notes Cliff Brangwynne, a chemical and biological engineer at Princeton University in Princeton, NJ. Brangwynne says that may be, in part, because researchers lack an understanding of how all those strings of nucleotides physically fit together in the microscopic bowl of spaghetti that is the nucleus. "That's been wholly underappreciated," he says. "There's a lot of excitement now in that space." Recent insights have started to piece together that puzzle.

Nucleosome Nuance

Brangwynne and O'Shea are part of a large effort to decipher the arrangements and interactions in that tangle of DNA strands—not just across space, but also over time—called the 4D Nucleome (2). Supported by the NIH's Common Fund for high-priority research, the program began in 2015 and was recently renewed for a second five-year stint. "It's ultimately about figuring out how the genome is organized and the logic of how that works," says Brangwynne. Nearly 30 teams spent the first five years building tools to determine how two meters of human DNA fold into in a nucleus about 5–10 microns across. Many teams are applying advanced microscopy to visualize the tangle directly.

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"It's the frontier of genomic science," says Bing Ren, a molecular biologist at the University of California, San Diego (UCSD), School of Medicine and another researcher on the project. "The imaging field is moving very, very fast."

Just as the double-helix structure of individual DNA strands offered clues about their manner of replication, nucleome researchers expect that studying the structure of the entire complement of chromosomes will offer hints about how the proximity of two or more genetic elements controls transcription, or how proteins might navigate the forest of chromatin to reach target genes. Ren predicts that over the next five years, 4D Nucleome grantees will begin to link those structures to human diseases that remain unsolved by sequence alone. For example, researchers have already shown that rearrangements of normal DNA architecture can disrupt gene expression and cause limb abnormalities such as extra fingers and toes (3). Other conditions, such as cancer, might also result from altered chromatin structure, Brangwynne speculates.

DNA Dust

O'Shea, originally a cancer biologist specializing in virus-delivered gene therapies, started pursuing nucleome studies when she became curious how those viruses found their nuclear targets. That led to a deceptively simple question: "What is the structure of DNA?"

Before her team could seek the answer, they'd have to find a way to make the invisible visible. Electron microscopy requires heavy metals to create contrast, and there was no such technique to specifically label natural DNA structures in intact cells. Conventional stains usually bind to lipids, proteins, or RNA. A stain composed of osmium ammine does bind DNA, but only after harsh acid treatment that would destroy the native DNA structure.

So, to create a label for DNA in an intact nucleus, staff researcher Horng Ou developed a method that relies on a series of steps: A red dye binds DNA indiscriminately. Activating the dye with red light causes it to produce reactive oxygen species that remain near the DNA molecule. These cause polymerization of another chemical component, DAB. Within a few minutes, the nucleus darkens as the DAB polymer coats the strands. DAB, unlike DNA, is amenable to staining with osmium. And that makes electron microscopy images possible.

"It's kind of dusting the DNA," says O'Shea, who likens the DAB application to a scene from *Indiana Jones and the Last Crusade*. En route to the Holy Grail, Indy discovers an invisible bridge. He tosses gravel over the path to make it obvious on the return journey.

In any one slice across the nucleus of a human airway cell, DAB-labeled chromatin looks like scattered sand, surrounded by the nucleus's membranes. To really understand and inspect the chromatin in fine detail, the researchers wanted to see it in three dimensions—even walk through it as if perusing a gallery of interwoven sculptures. The imaging capabilities of collaborator Mark Ellisman, director of the National Center for Microscopy and Imaging Research at UCSD, made that possible. Using electron microscopy tomography (EMT), Ellisman's team can build a 3D picture of a 70-nanometer-thick section. The researchers call their combined technique ChromEMT.

Donning a virtual reality headset, the researchers can wander through the chromatin, where those dots of sand converge into filaments and fibers. They look up or down to follow DNA's twists and turns, or cock their heads to peer out through a nuclear pore. Wearing the headset, says O'Shea, it's easy to pretend they're transcription factors seeking a path through empty space—pseudocolored in blue—while bumping into red and yellow bits of DNA.

Taking their technique for a test-drive, the team compared the structures of two major forms of DNA: chromosomes condensed for mitosis versus the sprawled-out chromatin of interphase. Previous studies had reconstituted structures in vitro and measured chromatin fibers via X-ray crystallography or electron microscopy; others used cryo-EM or other imaging techniques. But those approaches were limited because of the removal of other cellular components, the small volume visualized, poor contrast of the DNA, or inability to directly detect it.

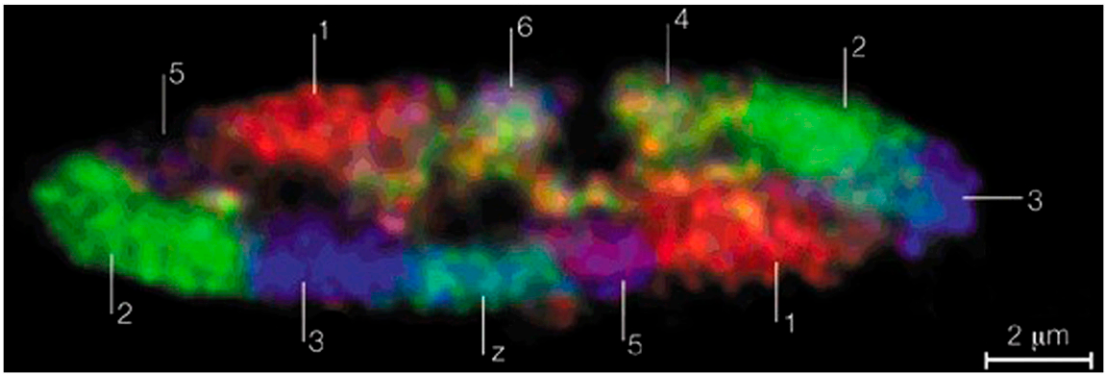
Using ChromEMT to reveal the native nucleome in exquisite detail, O'Shea's team found that the two chromosome arrangements were surprisingly similar in their disordered nature, lacking the expected categories of fibers. The mitotic DNA was simply more concentrated. That helps explain why chromatin can condense into mitotic chromosomes so quickly—taking a half-hour or so in cultured cells—says O'Shea; it simply scrunches more tightly, without a major restructuring. More tightly packed strands during interphase, she proposes, are less accessible for transcription (1).

Now, in as-yet-unpublished studies, the team has begun to label individual genes and RNAs with iron-containing particles. Inactive genes appear to cluster together in a sphere. When those genes are activated for transcription, the chromatin opens up, and globs of other dense material appear. The team suspects these globs are nuclear speckles: dynamic, irregularly shaped, protein-rich zones where enzymes process RNAs before export to the cytosol.

The researchers have even observed unexpected membrane bubbles floating amid the genetic strands. "What on Earth that is, and what it's doing, I don't know," says O'Shea. But they never would have found them without directly looking via ChromEMT.

Enhancing Chromatin

In a sparse, windowless basement lab at UCSD's new Center for Epigenomics, engineer Yuanyuan Han has custom built a microscope to precisely label specific parts of chromatin. A series of clear tubes holds probes that paint 5-kilobase segments of DNA, three at a time in red, green, and blue, so the team can precisely trace the curves and loops of 3,000 kilobases or more. With this trichromatic artistry, they hope to reveal how genetic elements called "enhancers" interact with far-off sequences to promote transcription.



This mid-plane light optical section through a chicken fibroblast nucleus shows chromosome territories, with homologous chromosomes seen in separate locations. Reprinted by permission from ref. 5: Springer Nature, *Chromosome Research*, copyright 2001.

The chromatin tracing technique was developed in the labs of biophysicist Xiaowei Zhuang at Harvard University in Cambridge, MA, and developmental biologist Alistair Boettiger at Stanford University in Palo Alto, CA. It's based on fluorescence in situ hybridization, or FISH, which researchers use to tag chromosomes from tip to tip with fluorescence labels.

Zhang and Boettiger used this technique, labeling much smaller segments of DNA, to reveal the chromatin architecture of so-called "topologically associating domains" (TADs), which could explain why some genes are co-expressed or co-silenced. DNA sequences in the same TAD interact with each other more than with sequences beyond the domain's boundaries, and share regulatory features, according to studies of entire cell populations. But the nature of TADs, at the single-cell level, was not understood.

The team not only observed TAD-like structures in individual cells but also saw that the precise arrangements varied from cell to cell. That may be a result of random variation or a case of nucleome rearrangement to suit a cell's particular needs, says Boettiger; the researchers are still trying to understand the cause. The researchers also corroborated the findings of cell population-level studies with respect to the edges of TADs: Their boundaries often correlate with binding sites for the transcriptional repressor CTCF and the protein complex cohesin, which also serves to hold the two pieces of X-shaped chromosomes together (3).

Because the nuclei are intact, with DNA arranged as it normally is, chromatin tracing should reveal where enhancers tend to hang out and which genes they might influence. The UCSD team, working with Zhuang's assistance, is still brainstorming other questions they can ask with the novel technology, says Adam Jussila, a bioinformatics graduate student. But already they're seeing hints as to how DNA structure controls transcription.

For example, graduate student Hui Huang has experimented with the gene *SOX2*—best known for its role in returning cells to an embryonic, pluripotent state—by inserting four "insulator" elements (4). They block enhancers and shut down transcription—but how? Based on Huang's images, Jussila speculates that insulators act like a rubber band tightened around the DNA, perhaps making the double helices less flexible so transcription enzymes can't bind. The researchers are now testing this hypothesis.

Dancing Along DNA

Based on these and other experiments, researchers have come to see chromatin as nothing like the static pictures in textbooks. "The chromosome is much less like a building and more like a dancer," says Boettiger, who has shown that disruptions in TAD boundaries can lead to developmental abnormalities in fruit flies. In a typical human nucleus, 46 chromosome "dancers" step according to a molecular chorography, but the individual movements have a lot to do with random chance, too.

By decoding that dance, Ren says, researchers will be able to add a new layer to the human genome. Currently, a gene can be annotated based on its location in the linear sequence and its function. Thanks to 3D imaging methods and other techniques from the 4D Nucleome Project, the next level of annotation might include an address in a particular TAD or a list of enhancers that are often found nearby. And therein, researchers might find clues to the origins of varied diseases or potential drug targets.

After five years developing the toolkit, the next five years of the project, Ren says, will be about "linking the study of human chromosome organization to human disease, translating this into clinically relevant discoveries." It's an ambitious goal, he acknowledges, but one with great potential payoff.

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