TECHNOLOGY FEATURE

CHROMOSOMAL DNA COMES INTO FOCUS

Imaging techniques to probe the shape of chromatin are revealing the dynamism of the DNA–protein complex.

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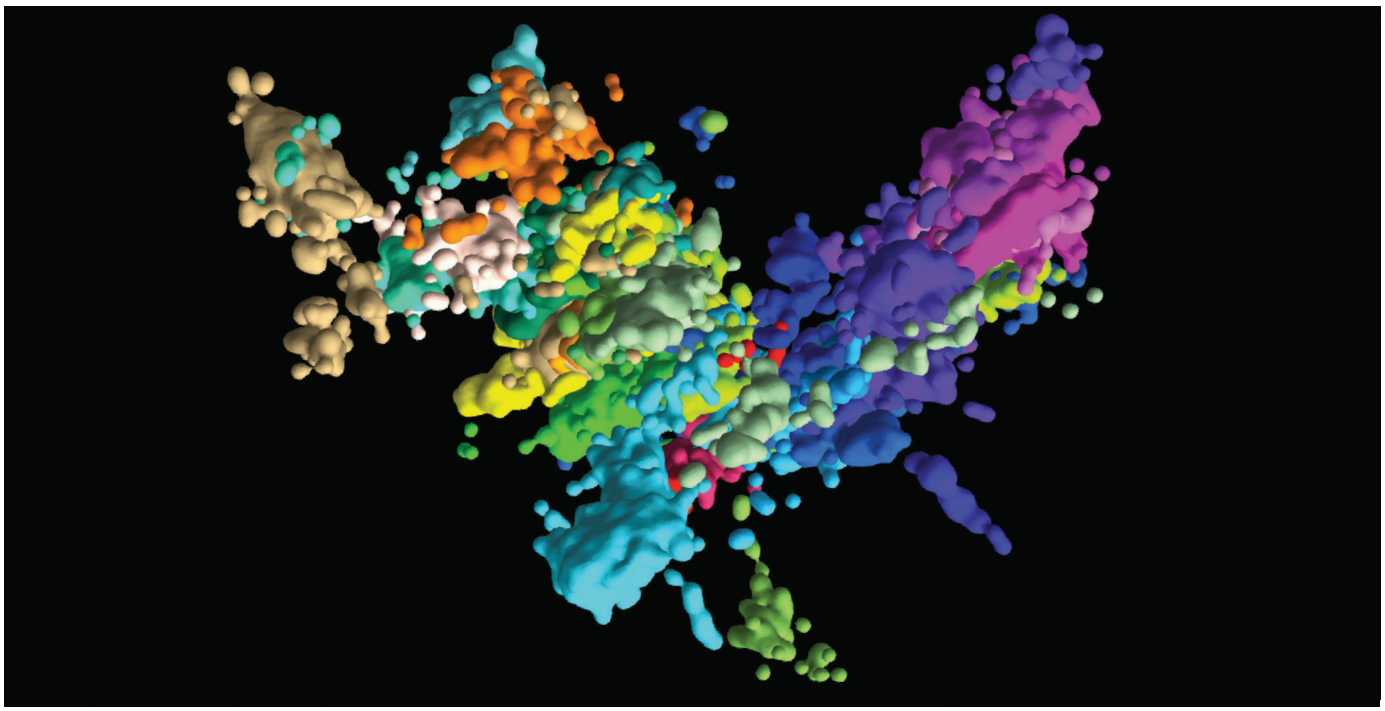


This multicoloured image of chromatin was created using multiplexed fluorescence *in situ* hybridization and super-resolution microscopy.

BY JEFFREY M. PERKEL

Molecular models suggest that chromosomes assemble in an ordered, hierarchical way: DNA wraps around proteins called histones to form nucleosomes, which fold into 30-nanometre fibres, then 120-nanometre 'chromonema', and further into larger chromatin structures until they reach their most tightly coiled form — the characteristic X-shaped bodies.

Under the high-resolution microscopes of biophysicist Xiaowei Zhuang, these chromosomes resemble something from the mind of surrealist painter Salvador Dalí. Zhuang, who is at Harvard University in Cambridge, Massachusetts, is one of a growing number of researchers charting the topology of the genome to decode the relationship between chromatin structure and function. Using a highly multiplexed form of fluorescence *in situ* hybridization (FISH) in combination with super-resolution microscopy, Zhuang's team mapped several million bases of human chromosome 21 at 30 kilobase resolution, tracing their shape like a dot-to-dot puzzle[1]. The resulting multicoloured image resembles one of the melting clocks in Dalí's 1931 *The Persistence of Memory*.

But that was in just one cell. In each cell that Zhuang's team looked at, the chromosome assumed a different shape — each one a different solution to some ineffable cellular calculation. "There is very strong cell-to-cell heterogeneity," Zhuang says.

Ting Wu, a geneticist at Harvard Medical School in Boston, Massachusetts, who combined a similar super-resolution FISH approach with sequencing analysis to map a chunk of human chromosome 19 to 10 kilobase resolution in late 2018, observed similar heterogeneity[2]. The chromosomes in that study look more like space-filling protein models, and when the team overlaid markers of inactive and active chromatin, they observed distinct patterns. "We have never seen a structure of that 8.6-megabase region twice," says Wu. "The variability, which people had thought was there, and there are hints of, is truly astounding." Brian Beliveau, a genomic scientist at the University of Washington, Seattle, and a co-author of the paper, says bluntly: "Chromosomes are almost certainly like snowflakes."

A DEEPER LOOK

In biology, function derives from form. It is shape, as a result of amino-acid sequence, that determines whether a given protein acts as a structural scaffold, signalling molecule or enzyme. The same is probably true of the genome. But until recently, there was no easy way for researchers to determine that structure.

Using a sequencing-based method called Hi-C, which calculates the frequencies at which different chromosomal segments ▶

interact in space, researchers discovered that chromatin organizes into relatively stable structures called topologically associating domains (TADs), and larger domains called compartments. But Hi-C works by averaging chromosome conformation across millions of cells. To see chromatin forms as they exist in cells, researchers must study them individually.

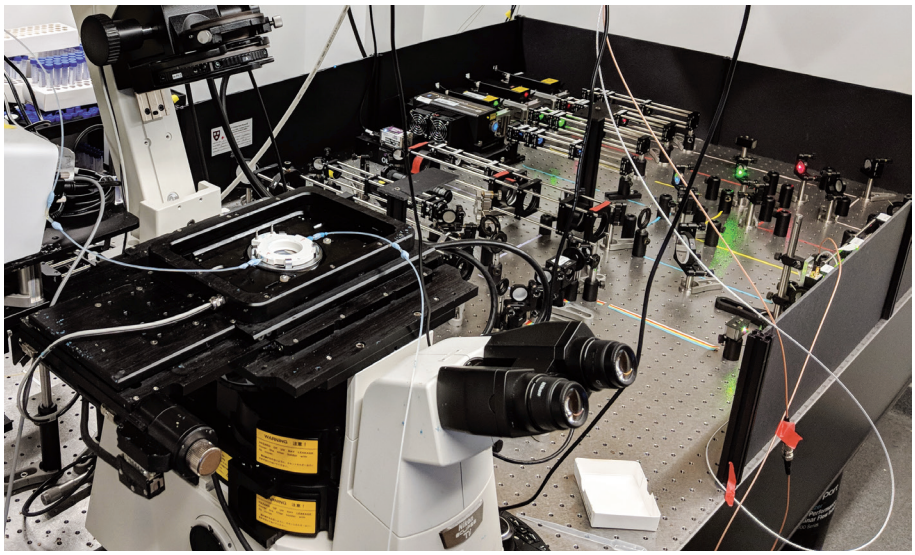
Wu and Zhuang focused on relatively small regions of the genome — a few million base pairs out of three billion. They had previously used a similar approach to map whole chromosomes at lower resolution[3]. Biophysical chemist Sunney Xie at Peking University in Beijing takes an even bigger-picture view.

Xie and his colleagues have used Dip-C — a sequencing method akin to single-cell Hi-C — to computationally map chromosomes at 20 kilobase resolution in individual cells with two sets of chromosomes[4]. The method's sensitivity is such that it picks up both intra- and interchromosomal contacts — about one million per cell — from which the team can infer the organization of the entire nucleus. It looks, in computer-generated renderings, like a multicoloured skein of yarn. "We know how that 6×10^9 bases are located in the nucleus," Xie says.

And in olfactory neurons, they found[5] that structure reflects cellular biology. Whereas most cells pack their thousand-odd olfactory-receptor genes at the periphery of the nucleus, olfactory neurons mostly pack them near the nuclear centre, where they are silenced — except, presumably, one that remains free to produce the neuron's olfactory receptor. "Chromatin structure determines cell function," Xie says.

But unlike in proteins, that structure is highly variable. In one 2019 study[6], Elizabeth Finn, a postdoctoral fellow in the laboratory of Tom Misteli, director of the Center for Cancer Research at the National Cancer Institute in Bethesda, Maryland, and her colleagues selected 125 pairs of contacts from Hi-C maps and used a high-throughput FISH platform to map the physical locations of contacts in human cells. In general, they found, regions that were strongly associated in Hi-C tended to be close together in space, whereas more weakly associated regions tended to co-localize less frequently.

"So it correlates," Misteli says — but not completely. In many cells, no interaction was observed. That's not surprising, says Job Dekker, a chromatin biologist at the University of Massachusetts Medical School in Worcester, and a co-author of the study. Even a strong Hi-C signal might reflect just a handful of cells. And the genome is almost certainly dynamic, Dekker adds. A configuration that exists at one moment might disappear minutes later, as the cell samples the genomic landscape. "What we do think is that most of these structures can happen in all cells, but occur transiently."



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A super-resolution microscopy system for imaging chromatin.

Other mechanisms are probably also at play, says Misteli. Some loci, for instance, are characteristically spaced so far apart it would be difficult for them to interact through chromosome diffusion alone.

What researchers are looking for, then, are the overarching patterns. At the Salk Institute for Biological Studies in La Jolla, California, molecular biologist Clodagh O'Shea has developed a method for mapping chromatin structure in single cells with nanometre precision. The method involves coating cellular chromatin in a thin metal shell — as she puts it, like Han Solo encased in carbonite in the 1980 film *The Empire Strikes Back*. Working with Mark Ellisman at the University of California, San Diego, her team then uses 3D electron microscopy to make a CT scan of that metallic cast, and computer algorithms to track its tortuous path through the nucleus.

The resulting structure can reveal neither which chromosomes are which, nor where any particular piece of DNA is located. But by studying its shape across size scales from nucleosomes to nuclei, the team discerned that cellular chromatin is much more chaotic than conventional wisdom would suggest[7]. In the paper, the researchers describe "a disordered granular chain with varying diameters between 5 and 24 nm and many different nucleosome particle arrangements, unknown densities, and structural conformations".

Still, by comparing chromatin's properties across different stages of the cell cycle, the team found that chromosomal structure seems to vary strongly with local DNA concentration. Small changes in concentration could push the DNA into a more or less fluid state — a finding that provides a potentially simple explanation for the speed and regulation of chromatin dynamics.

Now researchers are working out ways to peer even deeper into the genome. O'Shea, for instance, has developed a genetically encoded fluorescent metal nanoparticle called FireNano, which will allow live-cell tracking of specific genetic loci followed by higher-resolution electron-microscopy studies. And Alistair Boettiger, a developmental biologist at Stanford University in California who was a postdoc with Zhuang, has developed a method to boost the resolution of multiplexed FISH to 2 kilobases while also capturing gene-expression data to dissect the role of structure in transcription regulation[8].

Under the aegis of the 4D Nucleome programme, funded by the US National Institutes of Health, Dekker says, researchers are throwing everything they can think of at genome organization. "People have started to realize, you can't answer this question by just doing an imaging experiment, or just doing a Hi-C experiment, or just doing a biochemical experiment or coming up with a clever molecular or biophysical model that you simulate in the computer. We will have to work together and do all these things in some kind of a coherent manner, because it's probably going to involve all these disciplines."

With such a broad and growing toolbox, says Wu, chromatin biology is having "a watershed moment". "This is such an exciting time in the field," she says. "The pace and number of these views of the genome, of different parts of the genome, different chromosomes, in different cells and different developmental stages, has been wonderful." ■

Jeffrey M. Perkel *is technology editor at* Nature.

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