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GE PRIZE ESSAY

Zoom!

Exploring how the genome folds by using three-dimensional genome sequencing.

Erez Lieberman Aiden

GE & Science Prize for Young Life Scientists

GE Healthcare and *Science* are pleased to present the prize-winning essay by Erez Lieberman Aiden, a regional winner from North America, who is the Grand Prize winner of the GE & *Science* prize for Young Life Scientists.

Growing up, I watched the 1968 film *Powers of Ten*. The camera begins with a couple on a picnic and then zooms out: to the picnic ground (10^1 meters), Chicago (10^5 m), Earth (10^7 m), and, eventually, the universe (10^{26} m), only to zoom back in until it reaches the interior of a proton (10 to 16 m). Breathtaking structures emerge at each scale. I realized that if, one day, I could hold that magical camera—examining a phenomenon at a new scale, however briefly—I would see things that had not been seen before.

Where to look? The film provides a clue: at 10^{-6} m, we enter the cell nucleus, but see little before reaching the double helix (10^{-8} m) (*1*, *2*). Something is missing. DNA must fold further; if it did not, human genomes would be 2 m long and would not fit in the cell, much less the nucleus.

Since 1968, we have learned that DNA wraps around histones, packing ~10^2 base pairs into the 10^{-8}-m nucleosome (*3*–*6*). We also know that individual chromosomes occupy distinct subnuclear volumes called chromosome territories which pack ~10^8 base pairs into 10^{-6} m (*7*, *8*). In between? We lack suitable experiments: x-ray crystallography and nuclear magnetic resonance work best in vitro with shorter polymers, and contemporary microscopists can only track a few genomic loci at a time (*9*, *10*).

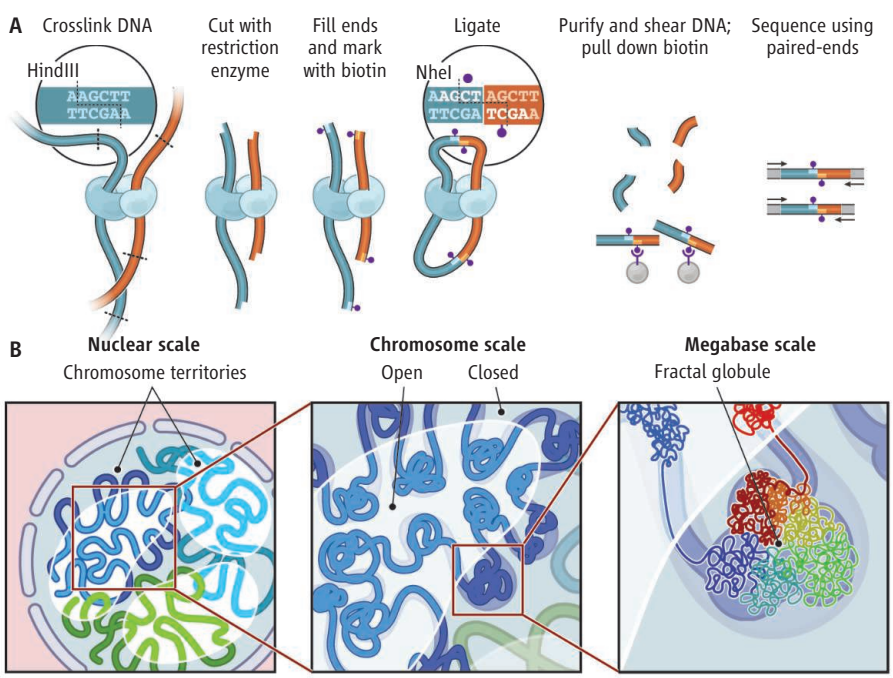
Fortunately, decades of work are enabling new approaches. In 1966, Wang and Davidson measured "cyclization"—how fast an oligonucleotide's sticky ends form loops—to argue that naked DNA folds into shapes resembling a random walk (*11*). Promoter-enhancer interactions between DNA ends catalyze cyclization in vitro (*12*). In 1993, Cullen *et al.*'s nuclear ligation assay (NLA) brought cyclization into the cell by transforming in vivo proximity relations between genomic loci into chimeric oligonucleotide sequences. NLA entails stabilizing nuclear structure using spermine and spermidine, followed by restriction, proximity ligation, and the use of universal polymerase chain reaction (PCR) primers to amplify junctions containing a target fragment (*13*). Chromosome conformation capture (3C) replaced spermine and spermidine with formaldehyde, enabling dilution prior to ligation and markedly improving signal (*14*). Coupling microarray and sequencing technology to Cullen *et al.*'s universal PCR approach (4C) or to multiplexed ligation mediated amplification (5C) increased throughput (*15*, *16*). But these methods still require target loci.

As a student in Eric Lander's lab, I wondered whether this approach could be modified to create a genome-wide proximity map that would enable zooming in on scales between chromosome territories and nucleosomes. Targeted primers would not work; instead, junctions would be biochemically marked. With collaborators at the Broad Institute, I outlined such a method: "Hi-C."

Next, I collaborated with Nynke van Berkum, a postdoc in Job Dekker's lab, to refine this strategy and bring it to life (*17*). In Hi-C, cells are cross-linked; DNA is restricted, leaving overhangs; overhangs are filled in, incorporating a biotinylated nucleotide; and the blunt ends are ligated in dilute conditions (Fig A). Because the site of ligation is marked with biotin, ligation junctions—evidence of physical contact between two loci—can be purified and sequenced. The resulting maps reveal features at three scales: the nucleus, individual chromosomes, and single megabases (Fig B).

At the nuclear scale, we found that loci on the same chromosome—even opposite ends—interact more than loci on different chromosomes. This is the proximity-pattern fingerprint of chromosome territories, confirming that Hi-C's picture resembles the microscope's. But we can zoom further.

Examining individual chromosomes, we found that open chromatin interacts more with open chromatin, and closed with closed. This suggests that open and closed chromatin are segregated during interphase. Unlike



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Zooming in on the human genome. (**A**) The Hi-C protocol. (**B**) The architecture of the genome is represented at three scales. Chromosomes (dark blue, light blue, green) occupy distinct territories (Left), and weave back and forth between the open and closed compartments (Middle). At the megabase scale, chromatin tends to fold into fractal globules (Right). Panels adapted from (*17*).

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most epigenetic marks, in which local biochemical changes activate or repress a region (*18*), this compartmentalization is a spatial mark: epigenetics through origami.

Last, we zoomed in on a megabase. Surprisingly, our data lacked the hallmarks of a classic condensed polymer: It resembled neither a random walk, nor its condensed-polymer cousin, the highly knotted equilibrium globule. What could we be looking at?

Eventually, I found a paper theorizing that extremely long polymers initially condense into a configuration known as the fractal globule, (*19*). This state has remarkable properties: dense, but completely unknotted, one can unfold and refold sections without disturbing the rest (and, at 10^{-7} m, it fits snugly in the *Powers of Ten* gap between 10^{-6} and 10^{-8} m). A back-of-the-envelope calculation (subsequently confirmed by simulations) suggested that our data was consistent with a fractal globule.

Though it may sound abstract, the fractal globule is easy to explain to graduate students because it closely resembles the only food we can afford: ramen. Ramen is densely packed: A 4–by–4-inch package contains ~100 feet of noodles (*20*). Also, as any grad student can tell you, undercooked ramen noodles are unknotted—you can grab a forkful of noodles, check if it is cooked, and replace it without disturbing the rest. But after cooking, they become highly knotted, clumping together on your fork. Thus, ramen noodles are unstable: Given enough thermal energy (i.e., cooking) they reach a knotted equilibrium. But, like the genome, when the conditions are right, the unknotted state can persist for a very long time: Ramen has an admirably long shelf life.

Sequencing genomes in three dimensions is becoming increasingly common. Several methods are emerging (*21*). For instance, chromatin interaction analysis by paired end-tag sequencing (*22*) uses antibodies to target contacts mediated by a particular protein. Spatial maps will help us unravel fundamental mysteries in biology, such as how the cells in our bodies—genetically identical—perform different functions; they will also help us understand cancer, where this differentiation has gone awry. At finer scales, we will be able to comprehensively map long-range gene regulatory elements buried in so-called "junk" DNA.

More than just a cinematic device, the camera in *Powers of Ten* is an apt metaphor for science itself, which progresses by probing the world at one scale after another. Humans are naturally found at 10^{0} m and, after thousands of years, have managed to explore ~25 orders of magnitude in either direction: from the 1-MeV neutrino (10^{-24} m), to the observable universe (10^{26} m). We suspect that this is all there is: that in this universe of scales, humankind lies at the center. Perhaps we are right. But as our forebears—who marked the edges of their maps with phrases like "hic sunt dracones" ("here be dragons")—learned time and time again, at the fringes of our maps the world is full of surprises. So on we must go, building better cameras. Zoom!

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2011 Grand Prize Winner

Erez Lieberman Aiden, the author of the prize-winning essay, grew up in New York City and studied mathematics, physics, and philosophy at Princeton University. He received a Ph.D. from Harvard and the Massachusetts Institute of Technology, where he was advised by Eric Lander and Martin Nowak. He is currently a Fellow at the Harvard Society of Fellows and visiting faculty at Google.

Dr. Tsukahara was born in Japan and graduated from the University of Tokyo in 2005. He conducted his Ph.D. work in the laboratory of Yoshinori Watanabe at the Institute of Molecular and Cellular Biosciences, University of Tokyo, where he studied the molecular mechanism of chromosome segregation. Dr. Tsukahara is currently an assistant professor in the laboratory of Hiroyuki Takeda at the Graduate School of Science, University of Tokyo and is studying the epigenetic regulation of vertebrate development and differentiation.

Regional Winners

Europe: **Felipe Teixeira** for his essay "Mechanisms of Transgenerational DNA Methylation Inheritance." Dr. Teixeira was born in Rio de Janeiro, Brazil. He received a B.Sc. in biological sciences and a M.Sc. in genetics from the Federal University of Rio de Janeiro. Dr. Teixeira completed his Ph.D. (University of Paris XI) under the supervision of Vincent Colot, first at the Unité de Recherche en Génomique Végétale (Evry, France) and then at the Ecole Normale Supérieure (Paris, France). Dr. Teixeira is now a postdoctoral fellow in Ruth Lehmann's lab at the New York University Medical Center/Skirball Institute, where he is studying the mechanisms involved in the regulation of transposable elements during *Drosophila* germline development.

Japan: **Tatsuya Tsukahara** for his essay "CDK Directs the Chromosome Passenger Complex to Centromeres for Chromosome Bi-Orientation."

All Other Countries: **Eran Eden** for his essay "Proteome Dynamics and the Fate of Individual Cancer Cells in Response to a Drug." Dr. Eden was born in Haifa, Israel, and received a B.Sc. in computer science, a B.A. in biology, and an M.Sc. in computer science from the Technion, Israel, Institute of Technology. His Ph.D. research, conducted under the guidance of Uri Alon at the Weizmann institute of Science in Rehovot, Israel, focused on studying the proteome dynamics and half-lives of human cancer cells. Since completing his Ph.D., Dr. Eden cofounded MeMed Dx, a startup company in the field of personalized diagnostics of infectious disease.

For the full text of essays by the regional winners and for information about applying for next year's awards, see *Science* Online at www.sciencemag.org/feature/data/prizes/ge/index.dtl.

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ERRATUM

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GE Prize Essay: "Zoom" by E. L. Aiden (2 December 2011, p. 1222). An editorial error was introduced during production. On p. 1222, the zoom for the interior of a proton was given as "10 to 16 m." The correct number is 10^{-16} m. The number has been corrected in the HTML version online.