GENOMICS

Finding function in the folds

Single-cell modeling of chromosomal organization could help scientists untangle how genomic organization informs function and vice versa.

Fluorescence *in situ* hybridization revolutionized the way scientists looked at the nucleus by allowing them to visually situate specific sequences or chromosomal structures within the milieu of the entire genome. More recently, methods such as Hi-C have added an extra dimension to chromosomal imaging: with high-throughput sequencing, one can analyze DNA sequences that have been chemically tethered together on the basis of their physical proximity within the nucleus. By piecing together which sites are adjacent to each other, one can then begin to assemble three-dimensional chromosomal maps.

Hi-C, however, can give only a composite picture based on data from large numbers of chromosomes, and Peter Fraser's team at the Babraham Institute realized that such information was of limited value for dissecting relationships between genomic organization and function. "We realized we were never going to get there, because every cell is different and you end up with this average structure that probably doesn't actually exist in any one cell," says Fraser. When the idea of doing single-cell Hi-C first came up a meeting with collaborator Ernest Laue of the University of Cambridge, it was essentially as a joke—teasing apart interaction data from two sets of nearly identical autosomes would make data analysis a potential nightmare. But when Fraser's team members realized that they could eliminate this problem by focusing on the lone X chromosome in male cells, they partnered with Laue and Amos Tanay at the Weizmann Institute to make single-cell Hi-C reality.

In-depth analysis of the nuclei from ten helper T cells revealed relatively high conservation in terms of local intrachromosomal interactions that give rise to discrete structural 'domains'. On the other hand, the researchers observed far more variability in terms of the interactions between those domains and between chromosomes, which in turn relates to

EPIGENETICS

MODIFYING CHROMATIN TO SHUT OFF ENHANCERS

Transcription activator–like effectors (TALEs) fused to enzymes that change chromatin signatures interrogate the role of enhancers in gene expression.

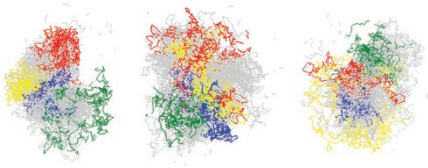
One way to determine whether something has value is to do without it and see what happens. The same principle holds in biology, but the 'doing without' part can be tricky to accomplish. Bradley Bernstein and his team at Harvard Medical School adapted genome-engineering tools to eliminate the function of enhancers.

Enhancers are regulatory DNA elements that bind transcription factors and other *trans*-acting proteins to augment gene expression. Unlike promoters—regulatory regions immediately upstream of the transcription start site of a gene—enhancers can be located distantly from the genes they regulate.

Recent work showed that enhancers are marked by characteristic chromatin signatures such as a mono- or dimethylated lysine 4 on histone H3 (H3K4me1 or H3K4me2, respectively) or an acetylated lysine 27 on H3 (H3K27ac). In the course of the Encyclopedia of DNA Elements (ENCODE) project and the Roadmap Epigenomics Project, researchers have identified close to 1 million putative enhancer elements. "But," notes Bernstein, "that does not tell you whether they are really functional; there is a lot of noncoding genome out there and a lot of potential elements to test."

Traditional reporter assays, in which an enhancer element is cloned in front of a reporter gene, can show which elements can be active, but they don't demonstrate which endogenous genes are being regulated.

Bernstein wanted to look at enhancers in their endogenous context. He reasoned that specific removal of the histone modifications that mark enhancers would lead to an enhancer's inactivation. He and Eric Mendenhall, now an Assistant Professor at the University of Alabama, collaborated with Keith Joung, also from Harvard Medical School, to design TALEs fused to a lysine-specific demethylase that target candidate enhancers in a leukemia cell line. The demethylase also recruited deacetylases, and both histone marks were removed.



Models of X chromosome structures from three different cells, generated using single-cell Hi-C data. Image courtesy of P. Fraser.

their positioning within the nucleus. Fraser and colleagues used these data to model the three-dimensional organization of the X chromosome and found that they could accurately distinguish regions situated near the 'surface' of the chromosomal tangle from those buried in the 'core'.

Domains near the surface were more likely to be in contact with other chromosomes, and after correlating their structural data with histone methylation signatures, the researchers learned that regions of active gene transcription were often situated at such interchromosomal interfaces. These active regions tended to interact with other active regions, and the relatively few inactive genes near the surface also tended to be in contact with each other. More generally, the specific X chromosomal domains situated at these interfaces varied considerably between cells. "It could be that we're looking at a dynamic structure that's constantly having different domains bubble up to the surface and be transcribed," says Fraser.

However, he notes that these are preliminary results that will require careful verification via higher-resolution analysis of larger numbers of cells. In parallel, the team is embarking on Hi-C analyses of experimentally generated haploid embryonic stem cells, which should yield far more sophisticated models. "All of these interchromosomal contacts will help us to freeze the individual chromosomal models into a single conformation," says Fraser. "I think it's going to be very exciting once we can get several or even hundreds of whole nuclei."

Michael Eisenstein

RESEARCH PAPERS

Nagano, T. *et al.* Single-cell Hi-C reveals cell-to-cell variability in chromosome structure. *Nature* **502**, 59–64 (2013).

The team members targeted 40 enhancers in a leukemia cell line then sequenced the transcriptome to identify genes whose expression changed in response to the inactivation of nine different enhancers. In four of the cases, they detected a clear downregulation of genes in proximity to the enhancer, but the other five did not show a detectable effect.

Although this shows that the principle of inactivating an enhancer via its chromatin signature works, the rules underlying the approach are not yet fully understood, and not all of the engineered reagents were effective.

Interestingly, the researchers found effects only in genes close to the enhancer. Bernstein speculates that to capture enhancers working over longer distances, as some developmentally important enhancers are known to do, they would have to use a different cell model.

Another exciting possibility for Bernstein is to combine this approach with chromosome confirmation capture methods and quantitative trait loci mapping. This would allow further validation and could improve methods for predicting enhancer targets.

The applications go beyond inactivation. By recruiting methyltransferases, enhancers can also conceivably be activated. "This might be a way to achieve specificity in regulation," says Bernstein, "to engineer highly specific regulators that only act on a given gene in a very specific context."

For Bernstein and his colleagues to reach the ultimate goal that prompted them to start this work—the detailed characterization of thousands of enhancers—the method will have to be scaled up. The newest genome engineering tool, CRISPR (clustered, regularly interspaced, short palindromic repeats) systems, might; but more work may be needed to improve the efficiency with which these can change the epigenome.

The hope is that before long these tools will allow systematic probing and modulation of the flood of *cis*-regulatory elements emerging from genome-wide mapping studies.

Nicole Rusk

RESEARCH PAPERS

Mendenhall, E.M. *et al.* Locus-specific editing of histone modifications at endogenous enhancers. *Nat. Biotechnol.* doi:10.1038/nbt.2701 (8 September 2013).