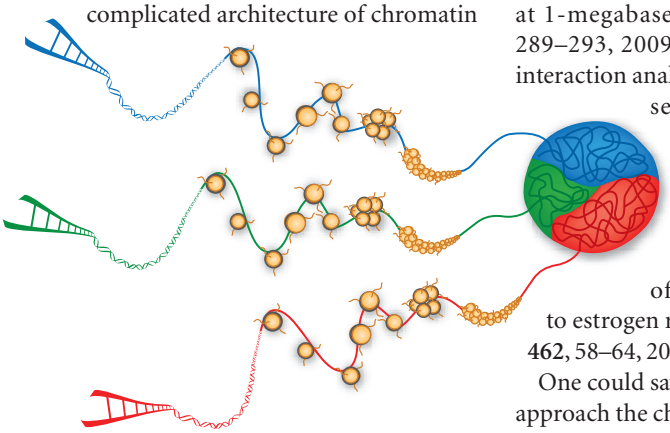
Mapping genomes in 3D

Refinements in methods to uncover the higher-order structure of the genome will allow functional insight into genomic architecture at high resolution.

The importance of the chromatin interactome for genome function has long been recognized. Techniques to unravel the complicated architecture of chromatin (genomic DNA looped around proteins), such as chromatin conformation capture (3C) and its higher-throughput derivatives 4C and 5C, provide long-range chromatin interactions but are not scalable to the entire chromatin interactome.



Hi-C and ChIA-PET will unravel the complex structure of genomes.

Late in 2009 two methods burst on the scene that promise such genome-wide interaction maps. Hi-C, a 3C-based method that captures global interactions, shows the higher-order folding principles of chromatin, independent of any particular protein, at 1-megabase resolution (*Science* **326**, 289–293, 2009). ChIA-PET (chromatin interaction analysis using paired-end ditag sequencing) resolves protein-mediated functional interactions at base-pair resolution. Its developers have so far applied it to draw the organization of the genome in response to estrogen receptor activation (*Nature* **462**, 58–64, 2009).

One could say that Hi-C and ChIA-PET approach the chromatin interactome from two opposite directions—one providing the bird's-eye view of how chromatin is folded in the nucleus, the other looking at the effect of a particular protein on genomic architecture. To increase their impact and provide a detailed, functional map of the chromatin interactome, both methods will have to move toward the center. Higher sequencing power, on the order of hundreds of millions of reads, and new analysis methods will increase the resolution of Hi-C, possibly to the 1-kilobase resolution ultimately desired by its creators. The application of ChiA-PET to proteins with more generic function, such as polymerases, will help to identify all chromatin interactions involved in processes such as transcription. Merging the maps will provide one with the best of both worlds—all possible genome-wide interactions independent of proteins paired with the functional maps induced by the activation of certain proteins.

These maps will provide the basis for understanding the structure of each chromosome during biological processes as well as in diseases such as cancer in which chromosomes often become scrambled and rearranged. Seeing how this rearrangement affects chromatin architecture, and what the functional implications are, may yield unique insights into what drives disease progression. **Nicole Rusk**

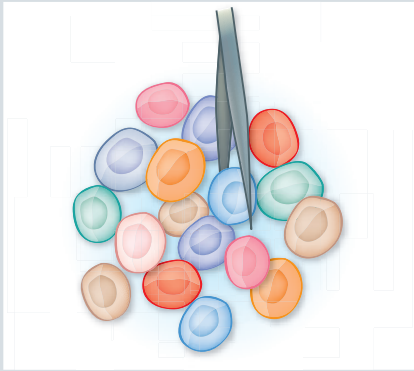
Single-cell methods

The ability to study single cells will permit a better understanding of cellular heterogeneity.

Cells, even genetically identical cells in an *in vitro* population, are not homogenous entities. There are by now several instances in which studies of enzyme activity, gene expression or response to signaling, among other readouts, indicate that there is substantial variability from cell to cell. This may be particularly true for cell types such as stem or progenitor cells, for which changes in state are an inherent part of their biology, but it is also true for other mammalian primary cell types, for cell lines in culture and for prokaryotic cells and yeast as well.

In order to more finely dissect cellular biology, therefore, methods that can be used on single cells are needed. Just about any 'omic' technique, from genomic approaches such as RNA-Seq to proteomic or metabolomic profiling, is likely to be usefully applied to single cells, as it will give a large-scale picture of how cells differ from each other and how this may contribute to cellular function. But humbler approaches, too—RT-PCR, for instance, or amplification-based methods for protein detection—can give insight into cellular variation if applied at the single-cell level. Some of these technologies, such as single-cell RNA-Seq, have been shown to be possible, whereas others, such as single-cell proteomics, are still over a distant horizon. As these methods develop, an added perk is that they will be generally useful for analyses where the amounts of starting material are very limited.

It may be argued that imaging is the ultimate single-cell method, and indeed, in this case the challenge is principally in the development of image acquisition and analysis methods that allow the resulting high-content data to be collected at sufficiently large scale and to be properly interpreted so that meaningful conclusions can be drawn. As many existing methods converge onto the single cell both *in vitro* and *in vivo*, and as bioinformatic and modeling approaches are applied to understand how the measured heterogeneity contributes to cellular function, a more dynamic and nuanced picture of biology is likely to emerge. **Natalie de Souza**



Making measurements in single cells.

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