Subcellular maps

Methods to systematically map the distribution of proteins in cells are evolving.

Human beings have been mapping the earth, the oceans and the heavens for centuries, but it is only in recent decades that we have extended our explorations to the cell, at least in any molecular detail.

Not merely bags of cytoplasm, cells are highly organized. They are compartmentalized into organelles and domains with particular molecular compositions and physical properties, and they are also dynamic, with constant molecular flux into and out of such organizational units. There is little doubt that cellular processes depend on organization: cellular secretion takes place via a series of membrane-bounded compartments, and many signaling processes are organized via molecular scaffolds. Even gene expression is intricately linked with the spatial organization of the genome. Although cellular organization and protein localization have been appreciated since the dawn of cell biology, the ability to make systematic molecular maps of protein distribution across a cell is still evolving.

Early work using cell fractionation followed by biochemical analysis of protein locations has been rejuvenated by the use of mass spectrometry for a more comprehensive picture of protein distribution across the cell (*Cell* **125**, 187–199, 2006). Workhorse methods such as antibody-based immunocytochemistry are being deployed in systematic efforts to generate subcellular maps. And genetically encoded tools such as ascorbate peroxidases and biotin ligases have been harnessed for proximity-based biotinylation, followed by capture and mass spectrometry, to determine what proteins are present at probed locations of a cell (*Science* **339**, 1328–1331, 2013; *J. Cell Biol.* **196**, 801–810, 2012). These tools are being constantly improved (*Nat. Methods* **12**, 51–54, 2015) and are beginning to be applied in subcellular mapping experiments (*Proc. Natl. Acad. Sci. USA* **112**, 12093–12098, 2015). It will be interesting to watch how the problem of specificity in proximity-based methods is contended with and to study the maps that emerge from all these efforts.

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Maps of the cell will help us navigate biological processes.

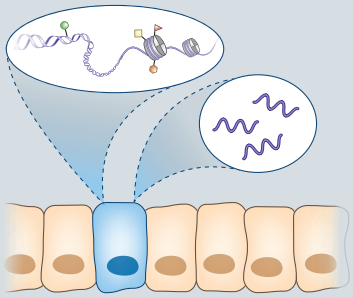
Although subcellular maps of protein locations will give us an unprecedented picture of the territory, proteins are only one of several types of molecule that make up the cellular landscape. Even with such maps, lipids and sugars will remain a relative *terra incognita*. But in explorations of the cell, protein maps are certainly the place to start. **Natalie de Souza**

Integrated single-cell profiles

Integrated molecular profiles of single cells will provide mechanistic insights into gene regulation and heterogeneity.

Like a new pair of glasses, single-cell sequencing is being used by more and more scientists to take a fresh look at their research. Examining tissues at cellular resolution can give insight into heterogeneity and allow researchers to directly define cell identity, including novel cell types, by comparing molecular states. Methods for single-cell DNA and RNA sequencing are maturing, and a rash of epigenetic methods have recently reached the single-cell milestone. We look forward to further improvements in epigenetic profiling as well as new approaches for extracting multiple profiles from the same cell.

The sequencing of RNA from individual cells is already robust, routine and possible at large scale; it can be used as a phenotypic readout to infer cellular functions and identity. Methods for examining gene regulation in single cells now include the study of DNA methylation, chromatin accessibility, histone modifications and chromosome structure. Although the achievements are extraordinary, most of these approaches would benefit from better genomic coverage and cleaner signal. Analytic issues also need to be solved; high technical noise, data sparsity due to undersampling, and biological variation (for example, from cell cycle differences, batch effects and biochemical stochasticity) are just some of the challenges.



Marina Corral Spence/Nature Publishing Group

Single cells will soon be interrogated for a number of molecular states.

Gleaning mechanistic or causal insights from bulk cell epigenetic data has proven difficult. Many data types are correlated and have complex relationships with gene expression in cell populations. The ability to profile epigenetic features and RNA in the same cell could provide more direct, mechanistic interpretations of how epigenetic states affect gene expression, or how RNA feeds back on epigenetic changes. In addition, gene expression could in principle be used to assign cells to subpopulations in a sample, and pairing single-cell RNA analysis with additional assays probing epigenetic features—DNA methylation in one experiment and DNA accessibility in another, for example—could identify relationships between the epigenetic features in each subpopulation, as well as their potential effect on gene expression.

Combined single-cell approaches will be important for the study of stem cell biology, development and tissue heterogeneity. With enough sampling, it should be possible to resolve how epigenetic changes affect gene expression at individual loci. **Tal Nawy**