Cell

One (Cell) in a Million

Imagine that you have a bag of candy. Every once in a while, you grab a few pieces and eat them without thinking too much about it. They taste good, maybe vaguely like strawberries. You think the candy might be a pale pink. Now, you grab another handful. This time, you take a closer look and are surprised to find out that most of them are white and sweet, though unflavored—and all of the strawberry taste comes from a few rare bright pink candies.

Averaging across populations can give a great idea of that population's central tendencies, though it can mask important differences among the individuals within that population, and it tells you nothing about the organization or behavior within any particular individual. It's a pretty simple concept when applied to a dozen pieces of candy, but it's much more complex when thousands or even millions of cells are involved. Yet the capacity to interrogate biological phenomena on a single-cell level is increasingly feasible and has already changed our understanding of cellular function and differentiation—not to mention challenging the prevailing notions of several established cell types.

Image from iStock/ZHARATE.

The most popular way to examine single cells has so far been to quantify RNA in real time as cells transcribe it. RNA sequencing (RNA-seq) has been the closest thing to an industry standard in this still-emerging field (Tang et al., 2010), but its drawbacks include low throughput and massive, high-dimensional datasets that can be hard to interpret. Some recently described microfluidic devices have helped to overcome the throughput difficulty. For example, the groups of J. Christopher Love and Alex Shalek developed the Seq-Well platform, comprised of wells with sub-nanoliter volumes and semipermeable membranes, to better capture and analyze hundreds of cells at the same time with single-cell resolution (Gierahn et al., 2017). Another approach to increase throughput, CROP-seq, uses pooled CRISPR-based screens to insert unique tags into each cell's genome followed by RNA-seq to track how those edited genes were transcribed (Datlinger et al., 2017). This strategy effectively combines the old bulk method of investigating entire pools of cells at the same time with the new paradigm of understanding what each of those cells is doing.

Alternatively, instead of improving RNA sequencing, some researchers have sought to use visual methods to image and quantify RNA in situ. These image-based approaches, mostly based on the well-known fluorescence in situ hybridization (FISH) technique, have the additional advantage of being able to track where in the cell the transcription is happening. While the information generated by these techniques can be easier to understand and offer unique spatial insight, they share some difficulties with methods based on RNA-seq, especially low throughput. One reported extension of FISH, termed MERFISH, alters the underlying chemistry of the FISH technique and expands the number of relevant fluorescent wavelengths. This technique can profile more than 100,000 human single cells in less than a day, a 100-fold increase in throughput over previously described methods (Moffitt et al., 2016). A unique challenge of optical systems compared with sequencing-based systems is the inability to study opaque or physically inaccessible cells. However, recent work from Long Cai's group described a system called MEMOIR, where these obscured cells record lineage information with molecular "scratchpads," which can later be read and interpreted with FISH (Frieda et al., 2017). MEMOIR was demonstrated to reconstruct the lineage of different cells that proliferated from the same pool of mouse embryonic stem cells.

While the field is still a long way from applying personalized single-cell omics to precision medicine, it has already produced some new fundamental insights into how cells behave. A recent study from Sarah Teichmann's group (Lönnberg et al., 2017) explored the bifurcation between commitments to different T cell lineages during infection with *Plasmodium* (the causative agent of malaria) by sequencing RNA transcribed in single naive T cells. One critical finding is that T cell lineages are not mutually exclusive within a given T cell population; instead, multiple T cell subtypes can exist in the same population if single naive cells are exposed to different differentiation factors. And an

ambitious project from the Science for Life laboratory in Sweden combined RNA-seq with immunofluorescence to update the Human Protein Atlas with a 3D spatial distribution of where proteins localize in human cells (Thul et al., 2017).

These methods and technologies may help us understand some of the most intractable problems in biology: Why do some cells mutate to become cancerous?; What explains the (seemingly stochastic) rise of bacterial ''persister'' cells in response to antibiotic treatment?; And are there any undiscovered cell types lurking within us? Each starts with a single cell.

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