To understand biological heterogeneity, researchers are learning how to profile the molecular contents of individual cells.

James Eberwine, a neuroscientist with a penchant for invention, helped to pioneer a technique that is now routine. In the early 1990s, he sucked the contents out of a single cell with a pipette, and examined the expression of a handful of genes using molecular techniques that amplify RNA. His data verified a long-held assumption — that electrical activity in a neuron simultaneously changes the abundance of multiple RNAs inside it.

But other researchers were sceptical. At the time, just about the only way to detect RNA in a single cell was by labelling molecules using fluorescence in situ hybridization. “People were used to using microscopy to look at RNA; they wanted to see it,” says Eberwine, who works at the University of Pennsylvania in Philadelphia.

Things have changed since then. Gene-expression analyses leaped forward in the mid-1990s with the invention of the microarray. And the rise of high-throughput RNA sequencing, or RNA-seq, which spits out the sequences of thousands of cellular RNAs at once, has enabled researchers to reveal the collection of active genes in a cell in a single readout.

Studies indicate how strongly cells can show their individuality. Brain cells may express as few as 65% of the same genes as their neighbours, according to an unpublished analysis by Eberwine. In the immune system, cells placed in the same category on the basis of surface markers can express different sets of genes, and have different responses to vaccines. And as tumour cells evolve, their genomes quickly evolve.
become twisted in unusual ways.

Single-cell techniques let researchers track and catalogue this heterogeneity. They may be the only way to get at some fundamental questions, such as what makes individual cells different biochemically and functionally. How much is each cell influenced by its microenvironment, and what is the role of stochasticity — random ‘noise’ in the behaviour of cellular molecules?

These questions are getting more attention (see page 139). In 2009, Eberwine co-organized a meeting on single-cell analysis at Cold Spring Harbor Laboratory in New York, along with Sunney Xie, a single-cell biochemist at Harvard University in Cambridge, Massachusetts. The meeting drew 47 attendees. This July, 120 people went to the second such meeting. And the US National Institutes of Health (NIH) has launched an initiative to support single-cell techniques (see “The NIH gets singular”).

But single-cell analysis is still an emerging field. Many researchers say that protocols from academic labs are often superior to commercial kits. “With any nascent field, there are lots of different approaches,” says Eberwine. “People are trying lots of things to see if they can make the techniques more sensitive, more representative of the state of a cell, easier and cheaper.”

**PROFILING HETEROGENEITY**

The classic biochemical approach is limited, say single-cell researchers. Grinding up and analysing the contents of large pools of cells — a procedure undertaken in thousands of labs every day — averages out the results, says Timm Schroeder, director of the Institute of Stem Cell Research at the German Center for Environmental Health in Munich. But, says Schroeder, “it’s the individual cell that makes a decision” such as whether to fire an electrical impulse, migrate or differentiate into a new cell type.

Looking at single cells to uncover the impetus for such decisions means doing fussy experiments on a very small object: a cell might span about 10 micrometres and contain less than 1 picolitre (1 × 10⁻¹² litres) of cytoplasm. And some key regulatory molecules are scarce — just a few, hard-to-detect RNAs can exert a big effect on a cell.

Many established techniques are only now being applied to single cells. Fluorescent tagging and microscopy can be used to analyse molecules that have already been characterized. To profile previously unexamined molecules, there is transcriptome analysis — cataloguing the set of RNAs expressed in a cell — as well as high-throughput methods based on microfluidics or flow cytometry. But getting such techniques to work on single cells is not easy, says Schroeder, whose research involves long-term imaging of individual bone-marrow cells. Single-cell applications are, he says, “at least one level more demanding and complex than the conventional approaches”.

And the unexplored biological terrain is vast. “We don’t even know what we are getting into in terms of heterogeneity,” says Sherman Weissman, a geneticist at Yale University in New Haven, Connecticut.

Whatever the study, the first step is generally getting hold of the cells. When Eberwine first struggled with studying gene expression in a neuron 20 years ago, it was difficult even to get intact RNA out of a single cell. Eberwine solved the problem by capturing the material in the same pipette used to measure electrical activity. Now, researchers can use a variety of techniques to pick out single cells, from enzymatic digestion, which releases cells from tissues, to laser-capture microdissection. But it is still tricky, says Eberwine. “A major technical issue is how do you do that initial capture.”

Eberwine has used his pipette-capture system to study individual warm-sensitive neurons⁵, which regulate core body temperature and underlie fever. Together with Tamas Bartfai, a neuroscientist at the Scripps Research Institute in La Jolla, California, and his colleagues, Eberwine examined the cells’ transcriptomes. The researchers identified transcripts for G-protein-coupled receptors — potential drug targets — that went undetected in screens of pooled cells.

As techniques improve, they are letting researchers explore the heterogeneity within a cell. By cutting branches, or dendrites, off neurons, Eberwine and his colleagues have discovered that RNA in dendrites can retain nucleotide sequences that target the RNA to that location⁶. They could not have found such information by analysing the RNA of an entire neuron.

Developing technology will produce even more fresh data. Most biologists will need to work closely with computational biologists to evaluate the huge data sets that will result from cataloguing thousands of molecules in numerous single-cell experiments.

**EXTRA-LOUD AMPLIFICATION**

Perhaps the best-known single-cell profiling technique is transcriptomics. Azim Surani, a developmental biologist at the University of Cambridge, UK, uses this method to examine cells of the early embryo, which are hard to study in large batches because they are so rare. He is tracing how, such cells turn into pluripotent embryonic stem cells in culture.

Surani has adapted a single-cell protocol for the polymerase chain reaction (PCR) to work with RNA-seq. To do this, he has collaborated with technical experts such as Kaiqin Lao, a molecular cell biologist at Applied Biosystems in Foster City, California (a subsidiary of Life Technologies in Carlsbad). In the cells of the early mouse embryo, the team detected the expression of some 12,300 genes — 75% more than were detected by microarray techniques⁷. Lao says he can now get his PCR technique to work with 1 picogram of RNA — one-tenth the amount of RNA in a typical cell.

The published protocol amplifies molecules only if they are no more than 3 kilobases long, so it misses about 40% of transcripts, says Lao. He and his colleagues are using different...
enzymes to increase that; Lao can now amplify 10-kilobase transcripts, corresponding to about 99% of transcription, he says.

Another technique to amplify a cell’s RNA is antisense RNA (aRNA), an in vitro transcription technique from Eberwine and his colleagues, in which a cell’s RNA is copied into a stable DNA library, with each DNA molecule containing a short sequence recognized by an RNA polymerase. The polymerase uses the DNA library to make multiple copies of the RNA.

Each approach has its advantages, and its problems. Bias can be introduced to PCR when certain sequences dominate during amplification, so approaches based on this technique are less quantitative than aRNA. But aRNA is less efficient than PCR, and can take days, notes Weissman.

Commercially available aRNA kits include TargetAmp from Epicentre Biotechnologies of Madison, Wisconsin (owned by Illumina of San Diego, California), and MessageAmp from Ambion of Austin, Texas, which is owned by Life Technologies. Both can work for single cells, says Eberwine. Companies such as NuGEN in San Carlos, California, and Sigma-Aldrich in St Louis, Missouri (in partnership with Rubicon Genomics of Ann Arbor, Michigan), have products designed for small amounts of RNA, and some say that their systems can work for single cells. It is unclear when Life Technologies might release a product based on Lao’s method, but both Eberwine and Lao report that their single-cell techniques are being used successfully in other labs.

THE GENOME GAP

Many researchers want to analyze not just the transcriptome of a cell, but the underlying genome. This would be particularly relevant for cancer cells, with their warped DNA, and Life Technologies is offering US$1 million to the first researchers to sequence the entire genome and RNA content of a single cancer cell using the company’s technology.

Nicholas Navin, a geneticist at the MD Anderson Cancer Center in Houston, Texas, is one of only a handful of researchers who have sequenced the genomes of single cells from eukaryotic organisms. This year, in collaboration with Michael Wigler, a geneticist at Cold Spring Harbor, and his colleagues, Navin sequenced the DNA of 100 individual cells from different parts of each of two human breast tumours, tracing how the cancer evolves as it spreads. It took several years and cost about $2,000 per cell; the cost has since fallen to about $200 per cell, he says. In the end, Navin was able to reliably cover about 6% of the genome of a cell — enough to assess some larger copy-number aberrations, but not to look at the accumulation of point mutations during tumour evolution.

The limitation, say Navin and other researchers, is the technique used to amplify the DNA: whole-genome amplification, which...
relies on an enzyme that copies some genomic regions but skips others. By tweaking this step, Navin says, he is now exceeding 50% coverage of the genome of a human cell, although his work has not yet been published.

Navin is not the only one tackling this problem. At the Cold Spring Harbor Meeting this year, Xie said that he and his colleagues had been able to sequence 85% of the genome of a mammalian cell. The paper describing it has not yet been published — but researchers who have seen the data are impressed. “Sunny nailed it,” says Lao.

This is welcome news to researchers such as Fred Gage, a neuroscientist at the Salk Institute for Biological Sciences in San Diego, California, who wants to sequence individual neurons. He has found that long interspersed elements (LINEs) — DNA sequences that can move around in the genome — form new insertions when neurons are born from neuronal stem cells. Every neuron probably contains unique LINE insertions, with most cells having between 80 and 300. “Every neuron is probably different from every other neuron,” says Gage.

**ONE DEVICE, LOTS OF INFORMATION**

Questions on single cells often lead researchers into difficult experimental terrain. For help in navigating such tricky territory, Gage recommends collaborating with the best technical experts; he is working with Roger Lasken, a leader in sequencing unculturable microbes at the J. Craig Venter Institute in San Diego.

But many researchers venturing into single-cell analysis will be on their own, so techniques will have to become more automated, integrated and kit-like, says Jonathan Sweedler, a chemist at the University of Illinois at Urbana–Champaign. “Researchers will be able to buy a device that has 48 steps incorporated into one platform,” he says. Widespread uptake of single-cell analysis will also require high-throughput analyses of dozens to thousands of cells.

“Working with small volumes gives you some real technical advantages,” says Quake, whose lab is harnessing microfluidics to develop a technique for single-cell transcriptomics, and has created a device to isolate and sequence single chromosomes.

**PUTTING IT ALL TOGETHER**

But high-throughput techniques will be limited if what they measure is too simple. To grasp how a cell works, “you need to understand not just chemistry, but spatial and temporal information,” says Daniel Chiu, a chemist at the University of Washington in Seattle. To integrate these analyses, his lab combines microfluidics, nanomaterials and optics. Chiu’s team has developed a technique for single-cell nanosurgery using a ‘vortex trap’, an optical method that can manipulate organelles or liquid droplets. The group has isolated single mitochondria from cells and prepared them for analysis on a ‘droplet nanolab’, which deploys the vortex trap to fuse droplets and change the concentration of reagents.

Chiu’s lab has also developed microfluidic devices for quantifying fluorescently tagged molecules, and for detecting and analysing cells that are rare in a population, such as tumour cells circulating in the blood (see ‘Beyond amplification’).

Ultimately, a combination of techniques will be necessary for researchers to attain their goal of measuring multiple parameters in a single, living cell. “The more parameters you can define — the transcriptome, the peptide-ome, how a cell looks, how it responds to drugs — the more information you are going to get out,” says Eberwine.

Eberwine is confident that these methods will emerge, even if it takes years. “I think we will be successful,” he says, “and if we are not, somebody else will be.”

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