NEUROSCIENCE

Neurotechnology to address big questions

Profiling of single neurons in tissue allows structure and function linkage in brain circuits

By **Thomas Knöpfel**

Emotions, cognition, and consciousness emerge with the processing of neuronal information provided by memory readouts and senses. How this actually works seemed for a long time to be a question too big to ask. Advances in neuroscience have always been driven by methodological inventions, and current efforts to develop neurotechnologies are motivated by experimental strategies, including analytical dissection (that is, inverse engineering), large-scale interrogation, and synthetic reconstruction of the mammalian cerebral cortex (which is important for higher cognitive functions) and connected brain structures (*1*, *2*). As part of this neurotechnological endeavor, mapping the transcriptome of neurons with single-cell resolution and with known three-dimensional tissue localization has been a long-sought enabling technology (*3*). On page 380 of this issue, Wang *et al.* (*4*) present the technology and workflow to access transcriptional states of more than 100 genes from up to 30,000 cells in a cubic millimeter of cortical tissue. This advanced methodology will facilitate studies that improve our understanding of the neuronal hardware and, when combined with other emerging neurotechnologies, will enable big questions in neuroscience to be addressed.

Wang *et al.* integrated knowledge of mouse cortex-specific gene expression, targeted signal amplification, in situ transcriptomics, and hydrogel-tissue chemistry (*5*) into a workflow for single-cell resolution spatial transcriptomics, called STARmap (spatially resolved transcript amplicon readout mapping). Targeted signal amplification was achieved by generating circular complementary DNAs (cDNAs) after recognition of a

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specific transcript [messenger RNA (mRNA)] by a probe from a library of gene-specific primers. These cDNAs were then rolling-circle-amplified so as to generate a DNA nanoball (amplicon) that contains multiple copies of the cDNA templates (*6*). Importantly, this process takes place in structurally conserved brain tissue. Formation of the circular cDNAs included a barcode as a distinct identifier, which was decoded with a newly designed error-reduced in situ sequencing technology to identify specific mRNAs (*5*).

Since the development of techniques that involve mRNA harvesting from single cells for sequencing—single-cell reverse transcription–polymerase chain reaction (scRT-PCR)—

transcriptomics has been instrumental to classify the many functionally distinct cell types found in the cerebral cortex (*7*). scRT-PCR in combination with electrophysiological recordings has the advantage to directly link specific gene expression patterns and cellular functional characteristics (including information on how specific neurons integrate synaptic information and on their synaptic connectivity) but is not practical for more than 100 cells (*7*). Scaling this approach is required for a systematic analysis of cellu-

lar diversity and brain state–dependent transcription. STARmap can provide information about the transcriptome (up to 10,000 genes) of up to 30,000 cells in a cortical volume prepared from an individual mouse brain.

Hydrogel-tissue chemistry (*5*) was needed to fix the amplicons in the cubic millimeter tissue samples. Classification of cortical cell type based on anatomical location, gene expression pattern, and functional properties has been performed in the past by accumulating data across many experiments and many specimens (*7*). This knowledge served the authors to identify a set of 160 target genes, including 112 putative cell-type marker genes and 48 genes that are expressed in response to neuronal activity patterns. It is not known which of the selected marker genes are most indicative for specific cell types and which of the activity-regulated genes are recruited in which cell types and under specific patterns of neuronal activity. Therefore, Wang *et al.* used this set of target genes both to benchmark their methodology and workflow but also to contribute to efforts aimed at detecting and classifying cell types and corresponding tissue-organization principles in the neocortex of adult mouse brain (see the figure).
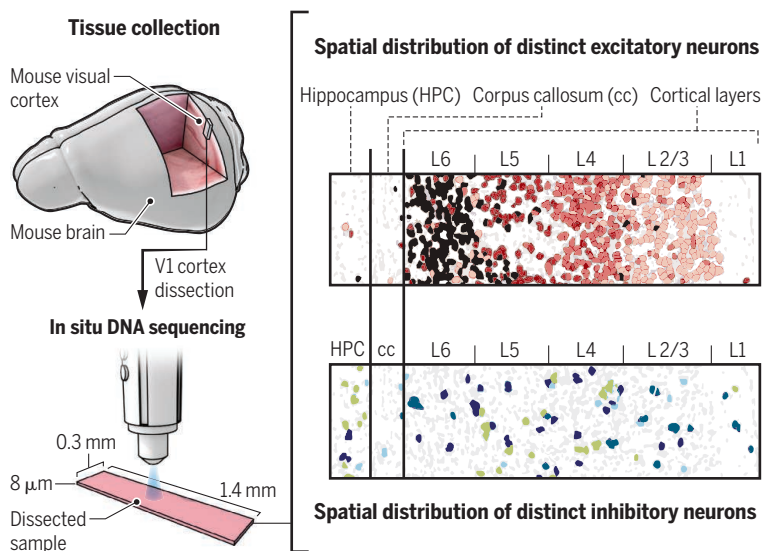
A weakness of STARmap is the lack of direct linking of transcriptomes with cellular protein expression and function. However, because several of the chosen marker genes encode proteins—including ion channels, receptors, and neurotransmitter-related enzymes that indicate functional properties—an indirect link between transcriptomics and function is already provided. In future implementations, a larger number of genes indicating functional properties may replace marker genes with low cell type–specificity to increase the possibilities for functional inference.

The high data content from a single tissue specimen provided by STARmap allows for correlation of gene activation patterns within an individual's specific brain state at the time

Profiling brain tissue

Wang *et al.* provide a method to determine the activity of marker genes within a sample of brain tissue. This allows identification and mapping, for example, of subtypes of excitatory or inhibitory neurons in the cortical layers, corpus callosum, and hippocampus.

Tissue collection

Mouse visual cortex

Mouse brain

V1 cortex dissection

In situ DNA sequencing

0.3 mm
8 μm
1.4 mm
Dissected sample

Spatial distribution of distinct excitatory neurons

Hippocampus (HPC) Corpus callosum (cc) Cortical layers

L6 | L5 | L4 | L 2/3 | L1

HPC | cc | L6 | L5 | L4 | L 2/3 | L1

Spatial distribution of distinct inhibitory neurons

of tissue preparation. As a proof of principle, the authors compared the transcriptome from two cohorts of mice: The first was exposed for 1 hour to light after 4 days of housing in darkness, whereas the second cohort was housed continuously in darkness before tissue collection. STARmap analysis confirmed and refined our knowledge about the recruitment of activity-dependent genes and their enhancer RNAs (which control transcription of their target genes). In the future, a larger range of physiological brain states as well as rodent models of brain diseases, and even human tissue, may be analyzed this way.

The STARmap data are a valuable source for data mining to identify patterns and higher-level structural and functional principles, many of which are likely still hidden behind the complex nature of cortical circuits and their function. The first applications of STARmap already revealed an intriguing finding: a short-range self-clustering organization of inhibitory neuron subtypes (3). These clusters may just be relicts of cell differentiation and cortical morphogenesis with little functional consequences, but more exciting are the possible functional implications. Among these is the increased opportunity of these inhibitory neuron subtypes to form direct electrical connections with cell bodies and the proximal processes of neighboring cells. These gap junctions are involved in the generation of high-frequency rhythmic circuit activities (8).

STARmap is well suited for further interfacing with other recent technologies that have been developed to address the big questions on brain functions. The technology closest in reach for combination with STARmap is functional in vivo optical imaging with activity indicators (9). This combination could be tremendously helpful for directly linking gene expression patterns with cell activity patterns and brain circuit functions. Another exciting possible application of STARmap could be the mapping of trans-synaptically distributed (10) and activity-dependent expressed barcoded optical activity reporters. This combination of cutting-edge technologies would allow efficient linking of cellular activity with connectivity, cell-type classification, and proteomic state. ∎

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GENE EXPRESSION

Dynamic condensates activate transcription

Transcriptional components exhibit transient phase separation to drive gene activation
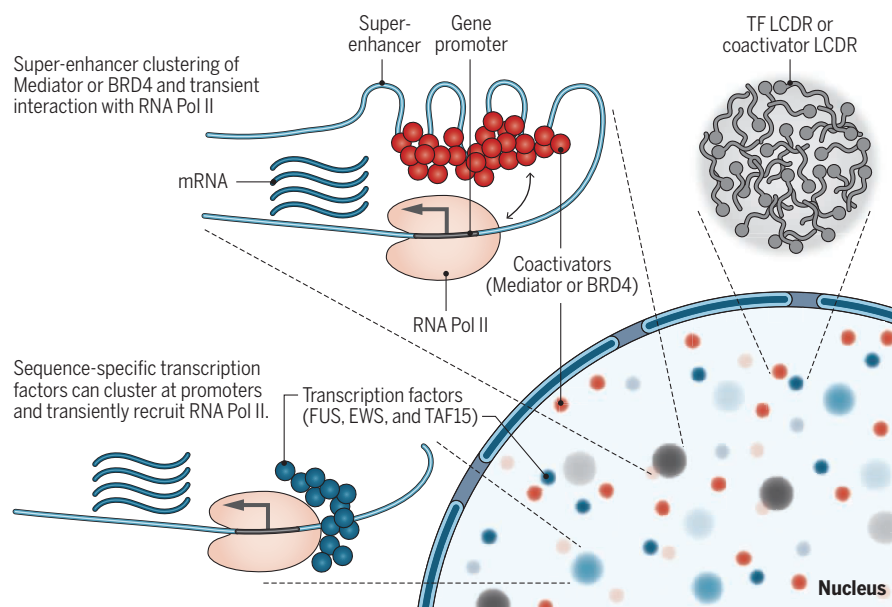
By **Aaron J. Plys**[1,2] *and* **Robert E. Kingston**[1,2]

Every aspect of human function, from proper cell differentiation and development to normal cellular maintenance, requires properly timed activation of the necessary genes. This requires transcription of genomic DNA into messenger RNA (mRNA), accomplished by RNA polymerase II (RNA Pol II), which initiates transcription at gene promoters. This highly regulated process requires hundreds of proteins that must go to the promoter in a coordinated manner. Although many of these proteins are already organized into large and stable protein complexes, and so travel as a group, the process still requires coordination of many individual proteins and preformed complexes so that they are all in the same place on genomic DNA at the same time. This problem has been appreciated for years and has led to models such as "transcription factories," where components are organized and ready to act on a gene that goes to the cellular location of the factory (1). On pages 378, 379, and 412 of this issue, Chong *et al.* (2), Sabari *et al.* (3), and Cho *et al.* (4), respectively, argue that special protein domains, which interact with each other to form fleeting or more persistent interactions, form biomolecular condensates that concentrate the transcription machinery. Some of these condensates might even form droplets, generating a liquid phase separated from the rest of the nucleus. Phase separation is a phenomenon familiar to anyone who has made a salad dressing: The oil and vinegar exist as two separated liquids. Phase separation in cells creates membrane-less organelles that, in this case, provide the organization necessary for productive transcription (5).

Several distinct types of proteins are needed for transcriptional activation. Gene-specific transcription factors (TFs) bind to

Dynamic transcription machinery clustering during gene activation

Transcription factors (TFs) and coactivators condense into high-concentration clusters in the nucleus. Condensation is mediated by low-complexity disordered regions (LCDRs) in these proteins. These clusters can incorporate RNA Pol II through transient interactions to efficiently activate gene transcription.

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