duction is caused by a failure of KRPs to inhibit CDKA;1. As expected, plants defective in RBR1 also showed supernumerary female meiocytes, a phenotype that was not previously reported (*7*). A decrease of CDKA;1 activity in the *rbr1* mutant did not reduce the number of abnormal meiocytes, demonstrating that the defect does not depend on cell proliferation. Rather, the defect is caused by failure of a mechanism that restricts cell differentiation before meiosis. Strikingly, this mechanism is driven by the action of RBR1 as a direct repressor of the gene *WUSCHEL* (*WUS*). *WUS* encodes a homeobox transcription factor that is crucial for specifying stem cells in the shoot apical meristem (*8*). *WUS* plays a role in ovule development, but its role in promoting meiocyte differentiation has not been clear (*9, 10*). WUS protein localization is restricted to epidermal cells of the ovule by the action of RBR1 and CDK inhibitors, confirming that its regulation is also important for the specification of the female germ line.

RBR1 controls context-dependent signaling pathways by interacting with diverse binding partners, giving rise to a large collection of cell lineages and cell identities that are crucial for the plant life cycle. Does the RBR1-WUS circuit interact with RNA-dependent DNA methylation and chromatin to avoid gametogenesis in somatic cells (*11*, *12*)? Although RBR1 is required for regulating chromatin remodeling late during gametogenesis (*13*), it is not clear how plant Rb proteins could epigenetically connect environmental response, cell cycle progression, and internal differentiation signals.

The findings of Zhao *et al.* provide a previously unknown link between stem cell and germ cell differentiation through the extended aura that surrounds Rb and homeobox proteins. These proteins are indeed among the most versatile regulators for controlling animal and plant development. ■

REFERENCES

1. X. Zhao *et al.*, *Science* **356**, eaaf6532 (2017).
2. X. Zhao *et al.*, *PLOS Genet.* **8**, e1002847 (2012).
3. J. Sage, *Genes Dev.* **26**, 1409 (2012).
4. D. Z. Zhao *et al.*, *Genes Dev.* **16**, 2021 (2002).
5. C. J. Wang *et al.*, *Development* **139**, 2594 (2012).
6. E. Demesa-Arevalo, J.-P. Vielle-Calzada, *Plant Cell* **25**, 1274 (2013).
7. C. Ebel, L. Mariconti, W. Gruissem, *Nature* **429**, 776 (2004).
8. K. F. Mayer *et al.*, *Cell* **95**, 805 (1998).
9. R. Groβ-Hardt, M. Lenhard, T. Laux, *Genes Dev.* **16**, 1129 (2002).
10. D. Lieber *et al.*, *Curr. Biol.* **21**, 1009 (2011).
11. V. Olmedo-Monfil *et al.*, *Nature* **464**, 628 (2010).
12. A. Schmidt *et al.*, *PLOS Biol.* **9**, e1001155 (2011).
13. A. J. Johnston *et al.*, *Curr. Biol.* **18**, 1680 (2008).

10.1126/science.aan2734

NANOTECHNOLOGY

Extracting the contents of living cells

Alumina "nanostraws" enable longitudinal intracellular monitoring of live cells

By **Stuart G. Higgins**[1,2] *and* **Molly M. Stevens**[1,2,3]

Being able to monitor cells at different times is key to tracking fundamental cellular processes such as differentiation and cellular senescence, as well as disease progression and the effectiveness of drugs. However, most approaches are destructive and involve lysing the cells. Different time points can be studied by using parallel cell cultures, but the inferred changes could also be the result of cell heterogeneity (*1*, *2*). Techniques for extracting small quantities of the cytosol for long-term tracking of a single cell's response must manipulate picoliter-scale volumes, maintain high cell viability, and give an accurate reflection of the cell's multiple biological components, as well as avoid influencing the ongoing development of the cell (see the figure) (*1*, *3*). Cao *et al.* approached this problem by culturing cells on top of a random arrangement of hollow cylinders, which they call nanostraws (*2*). These 150-nm-diameter alumina tubes can sample 5 to 10% of proteins, messenger RNA (mRNA), and small molecules from the cells but only reduce cell viability by ~5%. Their approach allows intracellular sampling and characterization at multiple time points from the same cells to track changes.

In Cao *et al.*'s technique, the cells are electroporated as they sit on top of the nanostraws. This process locally opens the cell membrane so that the intercellular contents can diffuse passively through the nanostraws into an adjacent buffer. Modeling a cell volume on the order of ~1 pl, the authors estimate that 7% of analytes diffuse into the buffer. The buffer can be analyzed by using either fluorescence (when studying cells modified to express green fluorescent protein), enzymatic assays, or polymerase chain reaction (PCR). Their study culminates by comparing the mRNA expression in human-induced pluripotent stem cell–derived cardiomyocytes from their nanostraw extraction method to an analysis of lysed cells. Of the 44 mRNA sequences identified, only seven were underdetected as compared with the lysed-cell control. These mRNAs were relatively larger molecules, which suggests that slower diffusion rates could be responsible for their absence. Subcellular localization of mRNA within the cell may also play a role in their detection efficiency.

[1]*Department of Materials, Imperial College London, London SW7 2AZ, UK.* [2]*Department of Bioengineering, Imperial College London, London SW7 2AZ, UK.* [3]*Institute of Biomedical Engineering, Imperial College London, London SW7 2AZ, UK. Email: m.stevens@imperial.ac.uk*

"Nanostraw extraction arguably has the advantage of offering much greater throughput..."

Despite this discrepancy, Cao *et al.* demonstrate a valid approach to live intracellular sampling, for which only a handful of techniques currently exist. Those alternatives, in which a portion of the cell contents is collected, as opposed to the introduction of a target-specific marker, include the use of glass nanopipettes (*4*), fluid force microscopy (*1*), and the use of carbon nanotube probes (*5*).

Glass micropipettes are commonly used to manipulate larger cells, but their relatively large dimensions (0.5 to 5 μm) can result in cellular damage (*3*). An alternative is the nanopipette, which uses a quartz capillary with a 100-nm-diameter opening to extract components (*4*, *6*, *7*). Actis *et al.* used nanopipettes in combination with a scanning ion conductance microscope setup to extract femtoliter quantities of intracellular material. In their approach, a nanopipette filled with an organic solvent forms a phase-interface with the aqueous cell contents. By applying a bias across the tip and sample, the position of this interface can be shifted, resulting in extraction. A key advantage of this approach is the precision with which they could target organelles within the cell, enabling them to assess heterogeneous variants in RNA and mitochondrial DNA expression in cultured HeLa cells (*4*).

Fluid force microscopy uses a hollow atomic force microscopy tip to controllably penetrate the cell membrane and selectively withdraw the intracellular contents by applying a negative pressure to the tip (*1*, *8*). Guillaume-Gentil *et al.* (*1*) reported successfully withdrawing up to 90% of the cytoplasmic content of HeLa cells without adversely affecting cell viability or behavior over a 5-day period in comparison with a control, although this experiment was an extreme case. They could also extract structures from both the cytoplasm and nucleus and image them with transmission electron microscopy and used enzyme assays and PCR to determine enzyme activity and mRNA presence, respectively, in their samples.

Carbon nanotubes have similarly been used for minimally invasive cell monitoring. Singhal *et al.* developed a "carbon nanotube endoscope" by attaching a ~50-μm-long multiwalled carbon nanotube to the ends of glass pipettes (*5*). They could extract fluorescently labeled Ca^{2+} from the cytosol and image this process in situ, although the reported attoliter volumes limited further extraction and analysis.

Nanopipettes, fluid force microscopy, and carbon nanotube endoscopes all offer high levels of control over cell selection and intracellular extraction volume but come at the expense of throughput. Nanostraw extraction arguably has the advantage of offering much greater throughput, at the expense of not being able to directly choose which cells are addressed (many cells are attached to the membrane). However, this limitation could be overcome with greater control over the spatial location of the nanostraws, combined with a microfluidic approach for systematically processing the extracted buffer. This capability is particularly important for cell screening applications in which high numbers of cells per plate are required.

Many other approaches can assess the intracellular environment, including fluorescent markers, quantum dots, nanoparticles, fluorescent Förster-resonance energy transfer (FRET) pairs, and thermally sensitive fluorescent markers conjugated to specific antibodies or RNA strands (*3*). These approaches offer high specificity, contrast, and resolution, but any given experiment is limited to identify a small number of prespecified targets, and care must be taken to avoid introducing materials that adversely affect the cell environment.

Although intracellular extraction methods provide clear advantages, there is still a need for further investigations into the impact of introducing high-aspect-ratio structures into cells. Whether the membrane wraps around the protruding structure, or is penetrated by it, is still a topic of much discussion (*9*). For nanostraws, membrane penetration is rare and depends on the cell adhesion behavior (*10*). In the case of this nanostraw extraction technique, electroporation was necessary to facilitate any extraction, which suggests that membrane penetration was unlikely. The lack of penetration is not inherently a limitation but does suggest that if this approach is to be applied to different cell lines, care must be taken to ensure that the convoluted plasma membrane surface, as well as electroporation, do not adversely influence the cell phenotype. ■

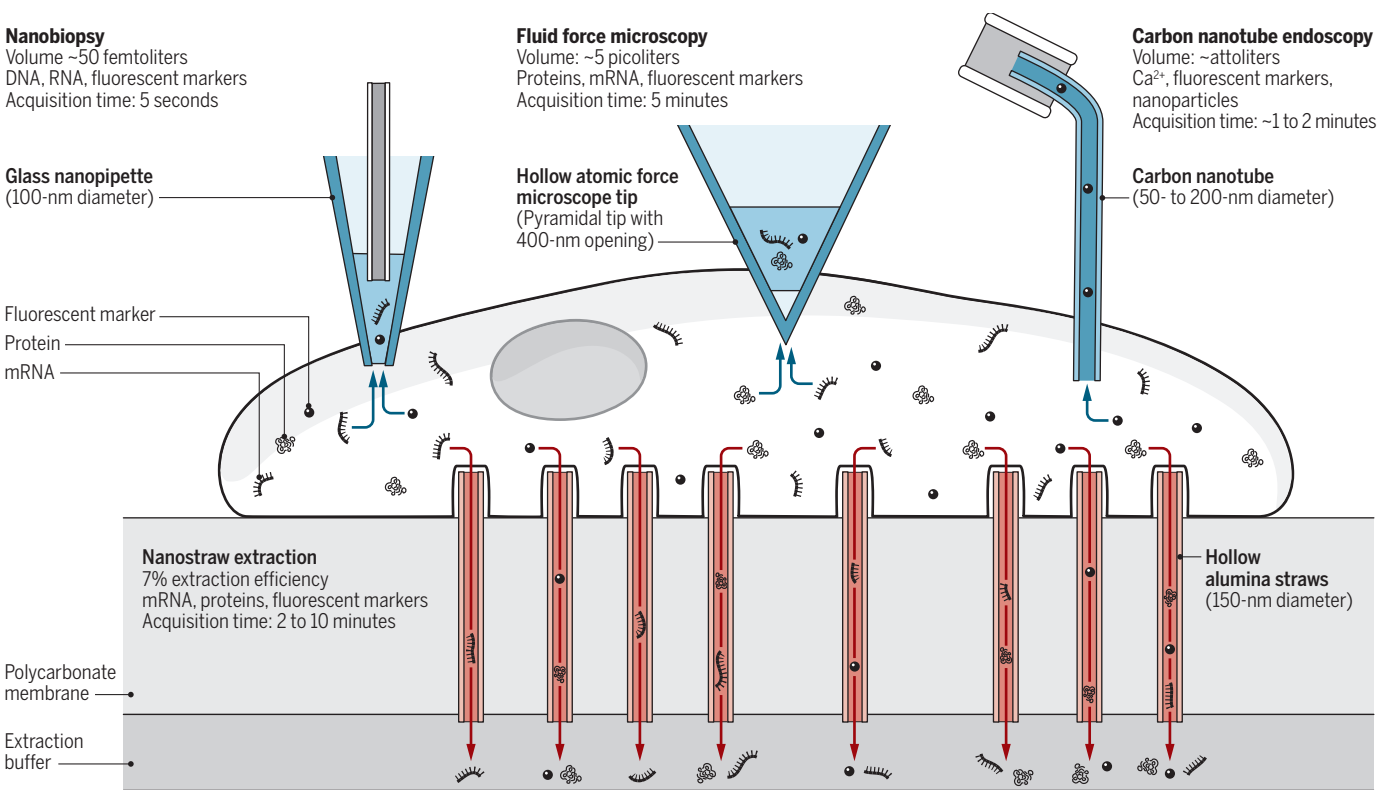
REFERENCES

1. O. Guillaume-Gentil *et al.*, *Cell* **166**, 506 (2016).
2. Y. Cao *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **114**, E1866 (2017).
3. J. Liu, J. Wen, Z. Zhang, H. Liu, Y. Sun, *Microsys. Nanoeng.* **1**, 15020 (2015).
4. P. Actis *et al.*, *ACS Nano* **8**, 546 (2014).
5. R. Singhal *et al.*, *Nat. Nanotechnol.* **6**, 57 (2011).
6. W. Kang, R. L. McNaughton, H. D. Espinosa, *Trends Biotechnol.* **34**, 665 (2016).
7. Y. Nashimoto *et al.*, *ACS Nano* **10**, 6915 (2016).
8. A. Meister *et al.*, *Nano Lett.* **9**, 2501 (2009).
9. C. Chiappini *et al.*, *Nat. Mater.* **14**, 532 (2015).
10. A. M. Xu *et al.*, *Nat. Commun.* **5**, 8 (2014).

10.1126/science.aan0228

Four ways for removing cell contents

Cao *et al.* used 150-nm-diameter alumina nanostraws combined with electroporation to extract cellular contents for analysis. This method complements nanobiopsy, fluid force microscopy, and carbon nanotube endoscopy.



GRAPHIC: A. KITTERMAN/*SCIENCE*

Extracting the contents of living cells
Stuart G. Higgins and Molly M. Stevens (April 27, 2017)
Science **356** (6336), 379-380. [doi: 10.1126/science.aan0228]

Editor's Summary

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.