CELL BIOLOGY

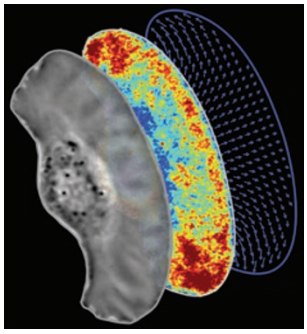
A fluid situation

By monitoring the size-dependence of particle distribution in the lamellipodium, fluid flow in moving cells can be measured.

Cell migration is a critical part of several biological processes. In recent years, studies of motile cells have focused largely on the role of the cytoskeleton in this process. Cells also contain fluid, however, which can respond to hydrodynamic and osmotic pressure in the moving cell. "But this intracellular fluid," says Kinneret Keren at the Technion Institute in Israel, "has been largely invisible."

Keren and her colleagues set out to study fluid flow in the lamellipodia of moving cells, with the idea that such a measurement may help illuminate the role of fluid flux in actin-based cell motility. They found, however, that this was no easy task.

Fluid movement in cells and embryos is typically studied by tracking the movement of single particles. But because of the dense actin meshwork in the lamellipodium, only very small particles (30 nanometers in diameter) could be used in this case. In contrast to the larger particles that are more typical in such studies (100 nanometer–or even micrometer-sized), the small particles diffuse so rapidly that tracking them for the purpose of detecting biased movement is very difficult in practice.



The intracellular fluid flow in the lamellipodium of rapidly moving fish keratocytes was measured by quantifying the steady-state size-dependent distribution of inert probes. Shown are a phase-contrast image, a ratio image (large probe, 655 QDs; small probe, AlexaFluor 488 dye) showing enhancement of large probes toward the leading edge and sides of the cell, and the fluid flow field. Image courtesy of K. Keren.

So the researchers decided to make diffusion work for rather than against them. They reasoned that a net fluid flow in the lamellipodium would have different effects on particles of different sizes. Larger particles with slower diffusion (but still of a size that could enter the lamellipodium) would be affected by flow, whereas smaller, rapidly diffusing particles would have a distribution that is less sensitive to flow. By measuring the ratio between different-sized fluorescent probes introduced into the lamellipodium, Keren and colleagues could determine whether there was indeed such a differential effect. In fish keratocytes, a cell type with rapid, consistent motion, they saw an enhanced localization of larger particles toward the leading edge. From this observed distribution, they inferred a forward-directed fluid flow in the lamellipodium.

GENOMICS

THE TRUE RNA-SEQ

With a modified polymerase and optimized oligonucleotide chemistry, Helicos' single-molecule sequencer takes on RNA.

RNA sequencing (RNA-seq) is actually a misnomer for the increasingly popular technique to determine the sequence of transcripts. It is not the RNA that is being sequenced but its reverse-transcribed cDNA derivative.

Presently available second-generation sequencing platforms all require many copies of the molecule that is to be sequenced and thus include an amplification step in their protocols. As RNA cannot be amplified, the detour via cDNA is necessary, and though the protocols for cDNA generation are well worked out, they are not immune to errors and bias, which can make data interpretation difficult.

Helicos BioSciences has recently introduced a third-generation, single-molecule DNA sequencer, the HeliScope, and now a team led by Fatih Ozsolak and Patrice Milos at the company have adapted the protocol to allow direct RNA sequencing, thus avoiding the cDNA detour and allowing a straight look at the transcriptome.

It is not a given that what works with robust DNA will also work with more fickle RNA. Whereas the scientists did not have to change the principle of Helicos' sequencing by synthesis, Milos says that the main challenge was in modifying all the components of the system—buffer, polymerase and nucleotide chemistry—so that they would work in the context of RNA; the exact nature of these modifications is not being disclosed.

The team started with synthetic 40-mer RNA oligoribonucleotides that they poly(A)-tagged to capture them on the poly(T) surface of the sequencer's flowcell. Their prototype flowcell was small, allowing only thousands of reads, as opposed to the 600–800 million reads in the HeliScope, but the average read number per area on the flowcell was very similar, indicating that the prototype can be scaled up. The average read length was around 20 nucleotides, with an error rate of approximately 4%.

Moving to a biological sample the researchers then sequenced poly(A)-containing RNA from yeast starting with 2 nanograms of material, about 100-fold less than other next-generation sequencing platforms require for RNA-seq. A three-day run yielded just over 41,000 reads of which 48% aligned to the yeast genome. Milos says that the team is now working on scaling the prototype methods up for the HeliScope.

Higher sequencing depth will be beneficial for error correction and quantitative transcript analysis, but another challenge, especially for the discovery of new transcripts and isoforms,

Based on a biophysical model built to describe the flows, the researchers postulated that myosin II might generate the pressure at the rear of the lamellipodium that drives forward flow. Indeed, treatment with blebbistatin, an inhibitor of myosin II activity, resulted in a depletion of large particles at the leading edge of the cell. "The fact that the particle distribution was reversed in blebbistatin, which is what we expected, gave us a lot of confidence that we are actually measuring fluid flow with this approach," says Keren. Notably, although blebbistatin treatment slows down the movement of fish keratocytes, it does not halt them completely. At least in the *in vitro* context, therefore, and at least for this cell type, fluid flow is not essential for movement.

Fish keratocytes are among the fastest moving cells. They are involved in wound healing and migrate in a sheet at the surface of the animal. It is possible, as Keren speculates, that the fluid flow seen *in vitro* may also exist at the leading edge of the sheet *in vivo*. It will be of interest to extend this approach to other motile cell types, but this is likely not to be trivial. The measurement requires approximately one minute for particle equilibration within the lamellipodium, so it requires steady movement of the cells over this time frame.

"We study a very simple and probably idealized *in vitro* system for cell movement," says Keren, "but it allows us to show that you can measure and model fluid flow in the moving cell. So for the first time, you can see what the fluid is doing."

Natalie de Souza

RESEARCH PAPERS
Keren, K. *et al.* Intracellular fluid flow in rapidly moving cells. *Nat. Cell Biol.* **11**, 1219–1224 (2009).

is the short read length. Other next-generation sequencing platforms have used paired-end reads, short reads from either end of a longer molecule, to improve isoform discovery. Scientists at Helicos are currently developing a similar but distinct approach for the single-molecule sequencer.

Their strategy involves the capture of long molecules in the flowcell followed by sequencing of the initial 30 nucleotides. Then they turn off the laser, which captures the signal of the incorporated fluorophore-labeled nucleotides, and add unlabeled nucleotides to extend the strand for a defined length, after which they turn the laser back on and sequence the next 30 bases. The end result is intermittent sequence information on a long molecule that will make its characterization much easier than if it has to be assembled from short reads. Milos predicts that this strategy will, for example, be invaluable for finding long intergenic noncoding RNAs, transcripts that span the interval between exonic regions, and she adds: "I think people are very interested to learn if these are indeed true cellular RNAs."

This is just one application for single RNA molecule sequencing; it is likely that in 2010, when Helicos will make this technology available to customers, many more will become apparent.

Nicole Rusk

RESEARCH PAPERS
Ozsolak, F. *et al.* Direct RNA sequencing. *Nature* **461**, 814–818 (2009).

NEWS IN BRIEF

SYSTEMS BIOLOGY

Metabolic network in 3D

Zhang *et al.* used structural genomics to solve three-dimensional structures of the 478 proteins (120 by experiment and 358 by modeling) involved in the central metabolic network of the bacterium *Thermotoga maritima*. This comprehensive structural analysis allowed them to assign metabolic functions to the proteins and identify essential genes as well as investigate questions about the mechanism of metabolic network expansion and the evolution of protein folds.

Zhang, Y. *et al. Science* **325**, 1544–1549 (2009).

STEM CELLS

Reprogramming with OCT4

Four transcription factors, OCT4, SOX2, c-Myc and KLF4, are necessary to reprogram somatic cells into induced pluripotent stem (iPS) cells. But for clinical applications involving iPS cells, it is desirable to avoid overexpressing the oncogenes encoding c-Myc and KLF4. Now Kim *et al.* report that *OCT4* (*POU5F1*) alone is all you need to reprogram human neural stem cells into neural iPS cells that look like and behave like human embryonic stem cells.

Kim, J.B. *et al. Nature* **461**, 649–653 (2009).

CHEMICAL BIOLOGY

Inducing cell signaling

Hashiro *et al.* describe an alternative concept for inducing specific cell-signaling pathways, without requiring protein engineering: they take advantage of the fact that many proteins activate downstream signaling pathways upon localizing to the plasma membrane. By placing a synthetic ligand at the plasma membrane, they can induce translocation of the endogenous protein to the plasma membrane and subsequent activation of the downstream signaling cascade.

Hashiro, S. *et al. J. Am. Chem. Soc.* **131**, 13568–13569 (2009).

PROTEOMICS

Natural product discovery using proteomics

Nonribosomal peptide synthetases and polyketide synthetases are very large enzyme machines in microorganisms that synthesize interesting and potentially pharmacologically valuable metabolites. Bumpus *et al.* describe an assay to enrich for such high-molecular-weight proteins, identify them by mass spectrometry and use the sequence information to link gene expression to the metabolic product.

Bumpus, S.B. *et al. Nat. Biotechnol.* **27**, 951–956 (2009).

GENOMICS

Metagenomics of bug splatter

With a unique approach to sampling the diversity of species in a local environment, Kosakovsky Pond *et al.* collected biological matter (from insects, bacteria and other species) from the front bumper of a moving vehicle and subjected it to phylogenetic profiling. They built a complete pipeline for metagenomic analysis, which involved DNA sequencing of the bumper samples, quality control, sequence alignment via database matching and taxonomic assignment; all tools are available in the Galaxy platform.

Kosakovsky Pond, S. *et al. Genome Res.* advance online publication (9 October 2009).