PROTEIN BIOCHEMISTRY

Test tubes go microscopic

Researchers in Japan have created arrays of microscopic 'test tubes' that permitted them to accurately quantify the efficiency of single enzyme molecules.

Scientists seem to be obsessed with performing experiments at increasingly smaller scale in an effort to probe a system's innermost workings or reduce reagent usage in high-throughput experiments. Many of us have experienced the agony of trying to pipet ever-shrinking sample volumes into arrays of test tubes. Now, in the ultimate pipetting nightmare, researchers at the University of Tokyo report in *Nature Biotechnology* the creation of arrays containing hundreds of microchambers, or test tubes, that are measured not in microliters or even nanoliters, but in femtoliters (Rondelez *et al.*, 2005a).

Because of the many favorable characteristics of poly-dimethylsiloxane (PDMS), Noji and colleagues chose this widely used material to create these femtoliter chambers. Using technology similar to that used to make microchips, the researchers prepared a silicon wafer mold with arrays of micron-sized cylindrical protrusions. They used this as a template to mold sheets of PDMS containing hundreds of micron-sized chambers that hold as little as 1.4 femtoliters of liquid when filled. Fortunately for researchers interested in using this technology, it's not necessary to pipet into these microscopic 'test tubes'. All you have to do to fill them is place a drop of your sample on a glass slide, lay the patterned PDMS sheet upside-down on the slide and press it down. They discovered that this alone is enough to effectively seal the liquid into the individual chambers and prevent cross-contamination.

These microchambers quickly became handy in Noji's lab, which is interested in studying the properties of single enzymes and how these properties influence function. So far, when examining single enzyme molecules, it had been impossible to study product accumulation because the product could not attain concentrations high enough to be measured. But Noji and

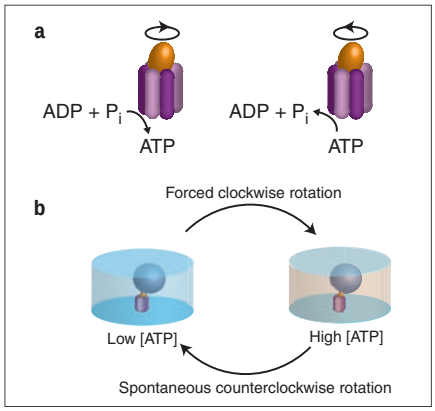

Figure 1 | Confined operation of F1-ATPase. (**a**) Diagram of F1-ATPase rotating clockwise, producing ATP, or counterclockwise, consuming it. (**b**) By attaching F1-ATPase linked to a magnetic bead to the glass bottom of a microchamber, magnetic tweezers could be used to force clockwise rotation and produce ATP.

RNA INTERFERENCE

THE MIGHTY MICRO

Analyzing the binding of microRNAs (miRNA) to their mRNA target sites reveals that many different factors determine what constitutes a good fit. A computational prediction method based on these factors shows that many *Drosophila melanogaster* genes are targets for miRNA regulation.

Its name suggests insignificance; one would think that in the age of super-sizing, something called microRNA, sporting a mere 22 nucleotides, would not attract a lot of attention. And yet, the more we learn about these miRNAs, the more impressive their role in genetic regulation becomes. Essential biological processes, such as embryonic development, cannot happen without miRNAs. The question of which genes are targeted by miRNAs has been of interest to many researchers, including the group of Stephen Cohen at the European Molecular Biology Laboratory.

For Cohen and his colleagues, the first brush with miRNA came when they found that a gene responsible for tissue growth in flies encodes an miRNA. Their next step was to find targets for this miRNA, but this proved to be harder than anticipated, because the miRNA, despite its short sequence, did not perfectly match any mRNA. After developing a rough target-prediction method, Cohen decided on a more systematic approach for the detailed analysis of criteria for binding between a target and an miRNA (Brennecke *et al.*, 2005). The Cohen team developed a screen that allowed them to determine how well a particular miRNA recognized its target; by introducing changes at certain positions in the miRNA, they could identify positions critical for target binding.

The profile of a functional miRNA-target interaction yielded a few surprises, as Cohen pointed out: "I think the most striking finding is that sites which consist of no more than eight base pairs can function." An eight-base-pair match at the 5′ end of the miRNA seems to be the best indicator for a good target site. But not all is lost for an miRNA with some mismatches in the first eight 5′ bases, as Brennecke *et al.* show that weak 5′ binding can be compensated for by strong binding at the 3′ end of the miRNA. These experiments underscore the complexity of miRNA target site prediction: out of the 22 possible, as few as 8 base pairs between miRNA and mRNA may be sufficient, and in certain cases paired bases at the 5′ and 3′ ends of the miRNA are needed. Moreover, bulges formed by mismatches are

colleagues reasoned that with femtoliter chambers this would be possible, and to test this, they added a low concentration of the enzyme β-galactosidase to the chambers along with a substrate that becomes fluorescent when cleaved by the enzyme. Within 60 seconds they could see a discrete level of fluorescence in each of the chambers. When they plotted the increase in fluorescence over time for each chamber, it became apparent that each one contained zero, one, two or three enzymes. It was clear that these chambers would be ideal for studying their enzyme of choice, F_1-ATPase.

F_1-ATPase is a tiny rotary enzyme, which produces ATP that serves as a major store of potential chemical energy in many biological reactions. But the efficiency with which this enzyme produces ATP from ADP has never been accurately measured. Noji and colleagues used the microchambers to confine single F_1-ATPase molecules and rotated the enzyme with magnetic tweezers to produce ATP (Rondelez *et al.*, 2005b). Because the ATP remained in the microchamber with the enzyme, once they stopped rotating the enzyme, it used the ATP to rotate in the opposite direction (**Fig. 1**). By comparing the number of rotations in each direction, they were able to accurately measure the efficiency of this enzyme for the very first time. This clever experiment demonstrates quite clearly the potential of these tiny 'test tubes' to examine the function of individual enzymes.

Daniel Evanko

RESEARCH PAPERS

Rondelez, Y. *et al*. Microfabricated arrays of femtoliter chambers allow single molecule enzymology. *Nat. Biotechnol.*, **23**, 361–365 (2005a).

Rondelez, Y. *et al*. Highly coupled ATP synthesis by F_1-ATPase single molecules. *Nature* **433**, 733–777 (2005b).

tolerated in certain positions but not in others. The Cohen team incorporated these findings into computational models for prediction of miRNA targets and concluded that a large number of genes in the fly are likely to be regulated by miRNAs.

In the end, any computationally predicted miRNA target has to be verified, and Cohen and coworkers are now testing some of their predicted targets experimentally. Their screen will tell them which of the predicted targets the miRNA actually binds to, but as Cohen points out, "It will not necessarily mean that a particular site is regulated in the natural circumstances in a cell." To prove that miRNA is working on its target *in vivo*, one will have to first mutate the endogenous miRNA and then demonstrate upregulation of the target gene.

Several groups are now working on such loss-of-function miRNA mutants to do proper genetic testing, whereas others are working to refine the computational prediction methods, and others still are focused on identifying more miRNA genes. It will take such multidisciplinary efforts to reveal just how influential the seemingly humble microRNA is.

Nicole Rusk

RESEARCH PAPERS

Brennecke, J. *et al*. Principles of microRNA-target recognition. *PLoS Biology* **3**, e85 (2005).

NEWS IN BRIEF

GENOMICS

Whole-genome patterns of common DNA variation in three human populations

In an effort to generate a more comprehensive and relevant collection of single-nucleotide polymorphism (SNP) information, Hinds *et al.* conducted a detailed analysis of three distinct populations of unrelated individuals of European, African and Asian ancestry. They present the findings from their initial analysis of the extent of variation for over 1.5 million SNPs, data which they have made publicly available online.

Hinds, D.A. *et al. Science* **307**, 1072–1079 (2005).

IMAGING AND VISUALIZATION

Near infrared–emissive polymersomes: self-assembled soft matter for *in vivo* optical imaging

The development of a versatile and stable reagent with near-infrared fluorescence would be a considerable boon for imaging studies that require deep penetration of living tissues. Ghoroghchian *et al.* describe the generation of self-assembling polymersomes that incorporate an oligo(porphyrin)-based near-infrared fluorophore and demonstrate the potential of these bodies for *in vivo* imaging applications.

Ghoroghchian, P.P. *et al. Proc. Natl. Acad. Sci. USA* **102**, 2922–2927 (2005).

PROTEOMICS

Interaction network containing conserved and essential protein complexes in *Escherichia coli*

By adapting the yeast-based tandem affinity purification (TAP) technique for use in *E. coli*, Butland *et al.* have assembled a detailed interaction network that describes the associations between nearly 650 different bacterial proteins, confirming several predicted associations and identifing many interaction 'hubs' that seem to be broadly conserved among prokaryotes.

Butland, G. *et al. Nature* **433**, 531–537 (2005).

RNA INTERFERENCE

A universal plasmid library encoding all permutations of small interfering RNA (siRNA)

Many current RNA interference (RNAi) studies begin with a target gene and attempt to identify a suitable siRNA. Chen *et al.* present a tool for researchers who want to work in the reverse direction—a library encoding nearly every possible 19-nucleotide siRNA sequence—and describe how such libraries could prove valuable for conducting phenotype-driven RNA interference studies.

Chen, M. *et al. Proc. Natl. Acad. Sci. USA* **102**, 2356–2361 (2005).

MICROBIOLOGY

Screening for quorum-sensing inhibitors (QSIs) by use of a new genetic system, the QSI selector

Genes involved in quorum sensing, the process by which many bacteria organize their pathological progression in response to population density, are now recognized as an important potential antibiotic target. Rasmussen *et al.* demonstrate several novel genetic screens for the identification of QSIs.

Rasmussen, T.B. *et al. J. Bacteriol.* **187**, 1799–1814 (2005).