proteins." Emili and his colleagues isolated 4 different cellular compartments—cytosol, plasma membrane, nucleus and mitochondria—via gradient separation, and then compared the proteomic differences between these organelles in different mouse tissues (Kislinger *et al.*, 2006). Their study yielded tissue- and organelle-specific data for nearly 5,000 different proteins, and their comparisons of proteomic profiles against high-quality microarray data revealed a surprisingly tight relationship between mRNA and protein expression levels.

Kathryn Lilley, of the University of Cambridge, encountered similar problems to Emili's in her initial studies of *Arabidopsis* sp. proteomics. "We were seeing the same proteins over and over again, because we were just sampling the abundant cytosolic proteins," she explains. She and her colleagues used localization of organelle proteins using isotope tagging, a technique called LOPIT, to perform an organelle enrichment study of their own, with a particular emphasis on the identification of membrane proteins (Dunkley *et al.*, 2006). They consistently detected approximately 700 proteins in multiple experiments, 60% of which were putative membrane proteins, and more than 75% of which could be confidently assigned to a particular organelle after careful computational analysis of the protein content in various cellular fractions.

Technical limitations have posed a serious obstacle to studies like these in the past, and Mann is quick to credit much of his data quality to the equipment at his disposal. "We used very new instrumentation," he says, "[and] so we were able to get much more accurate data." Emili agrees: "If I had my way, a mass spectrophotometer would be like a PCR machine, and every lab would have one. We were in a luxury position... being able to dedicate an instrument to this." All three researchers agree that this is a field rapidly coming of age. "I think it's an exciting time to be involved in organelle proteomics," says Lilley. "There are so many different biological questions that require a knowledge of where proteins are and where they traffic to upon given perturbations that have largely been ignored in the past because we haven't had the tools."

Authoritatively indexing the organelle proteome will require more effort, as well as powerful computational tools to maximize the value of the data. Mann sees this work as a starting point for answering far more interesting questions about cellular dynamics: "For example, if you have insulin signaling, how exactly does it signal into the mitochondria?" He concludes, "I think there will be more looking in these functional directions, and not just trying to build a catalog." Emili also sees this blossoming of 'reductionist' proteomics as an important step toward understanding fundamentals of global protein organization and behavior. "I think we're going to take it to the next level," he says. "My view of where the field is going is that in five years, we'll not only be measuring the levels of protein in various organs and cell types and tissues, but we'll [also] know who they're associated with and we'll have some holistic sense of the post-translational modifications."

Michael Eisenstein

RESEARCH PAPERS

Dunkley, T.P. *et al.* Mapping the *Arabidopsis* organelle proteome. *Proc. Natl. Acad. Sci. USA* **103**, 6518–6523 (2006).

Foster, L.J. *et al.* A mammalian organelle map by protein correlation profiling. *Cell* **125**, 187–199 (2006).

Kislinger, T. *et al.* Global survey of organ and organelle protein expression in mouse: combined proteomic and transcriptomic profiling. *Cell* **125**, 173–186 (2006).

© 2006 Nature Publishing Group http://www.nature.com/naturemethods

NEWS IN BRIEF

PROTEOMICS

Biochemical suppression of small-molecule inhibitors: a strategy to identify inhibitor targets and signaling pathway components

Peterson *et al.* describe a biochemical alternative to the widely used genetic suppressor screen. Cells treated with a small-molecule inhibitor are incubated with concentrated cytosolic fractions from untreated cells; closer analysis of the fractions that reverse the inhibitor phenotype can reveal drug targets—including multiprotein complexes—and proteins that act downstream of these targets.

Peterson, J.R. *et al.* *Chem. Biol.* **13**, 443–452 (2006).

GENE REGULATION

Preventing gene silencing with human replicators

Transcriptional silencing presents a serious obstacle to the efficacy and safety of insertion-based gene therapy. Previous research has shown that transcriptionally active chromosomal regions tend to undergo replication early in S phase, and Fu *et al.* demonstrate that the extent of silencing can be greatly reduced by the incorporation of active replicator sequences into transgenes.

Fu, H. *et al.* *Nat. Biotechnol.* **24**, 572–576 (2006).

IMAGING AND VISUALIZATION

Assembly of the brainstem cochlear nuclear complex is revealed by intersectional and subtractive genetic fate maps

Analyzing the development of complex tissues often requires the ability to distinguish between similar but distinct cell populations. As a tool for such mapping projects, Farago *et al.* have developed a indicator system that allows them to visually differentiate cells that simultaneously express two genes of interest from cells where only one of the two is being expressed.

Farago, A.F. *et al.* *Neuron* **50**, 205–218 (2006).

MICROFLUIDICS

Microfabricated bioprocessor for integrated nanoliter-scale Sanger DNA sequencing

Whereas some scientists foresee the impending demise of Sanger sequencing, Blazej *et al.* still see advantages in this venerable technique. They describe a microfabricated lab-on-a-chip system capable of accurate Sanger sequencing from one femtomole of template DNA and discuss the possibility of developing similar nanoscale bioprocessors for other genomic applications.

Blazej, R.G. *et al.* *Proc. Natl. Acad. Sci. USA* **103**, 7240–7245 (2006).

MICROSCOPY

STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis

Stimulated emission depletion (STED) considerably improves the resolution of fluorescence microscopy, allowing the visualization of objects tens of nanometers in diameter with a minimum of effort by the investigator. Willig *et al.* demonstrate the power of STED microscopy, imaging the clustering of synaptic vesicles at the presynaptic membranes of rat neurons.

Willig, K.I. *et al.* *Nature* **440**, 935–939 (2006).