

gite. Since 1993, he's had a harder time of it. "We have flat-out failed at finding fullerenes" in anything since, he says. "We've given up. The only one that's been strikingly successful is the Becker group."

Becker and Poreda have indeed reported considerable success. They have published detections of fullerenes in three different organic-rich meteorites and in debris from three large impacts—the putative P-T impact (at Meishan, Graphite Peak, and Sasayama in Japan), the Cretaceous-Tertiary (K-T) impact at the end of the dinosaur age (five locations), and in the 250-kilometer Sudbury impact crater of Canada. Some analyses have supported the existence of natural fullerenes. Geochemist David Mossman of Mount Allison University in Sackville, Canada, and his colleagues reported in the March issue of *Geology* that they found fullerenes in a Sudbury sample, although they found none in a shungite that they also examined. And Dieter Heymann of Rice University in Houston, Texas, and his colleagues reported fullerenes at the K-T boundary in a 1994 *Science* paper (29 July 1994, p. 645).

But the number of failures no doubt outnumbered the reported successes at finding fullerenes. No one but Becker and Poreda has identified fullerenes in meteorites, despite considerable effort, most of it unpublished. Roger Taylor, one of the group at the University of Sussex, U.K., that first made fullerenes in the lab, reported in 2000 that a K-T sample lacked fullerenes at the part-per-trillion level. And geochemist Kenneth Farley of the California Institute of Technology in Pasadena has come up empty after looking for the helium-3 (but not fullerenes) supposedly trapped in fullerenes at the P-T boundary at Meishan and another location in China. "Fullerenes in the geologic record are still awaiting confirmation," says organic geochemist Iain Gilmour of the Open University in Milton Keynes, U.K., who has had his share of failures in meteorites and at the P-T. "There's not a great incentive for people to chase things and not find them," he says.

Why all the conflicting results? Becker points the finger at her critics. "We're not talking about the same samples or doing the same experiment," she says. "There's no attempt to replicate my results. You have to do the organic chemistry" the way she does in order to produce a clean extract containing the fullerenes.

Many researchers see a larger problem in the samples being analyzed. No two analysts work on exactly the same boundary sample, despite sometimes receiving parts of a field sample from the same source. But Becker says they will be distributing Graphite Peak P-T samples to a number of different groups. There is also talk, but nothing more, of some-

one providing samples for a blind test, as happened in the course of the K-T debate.

#### Just one impact extinction?

Acceptance of the K-T impact has led to impacts being proposed for most other major extinctions. Just last June, a group of scientist proposed that a comet triggered the Paleocene-Eocene extinction of 55 million years ago, and another presented evidence for the latest of several impacts in the mid-Devonian 380 million years ago. But all depend on iridium anomalies that are too small, unconvincing shocked minerals, or impact markers as yet not generally accepted.

Twenty years after the K-T impact gained convincing support, some impact geologists are getting discouraged by their failure to find a second example. "I've tried for 10 years to look for impact layers," says Schmitz. "I almost ruined my career. I have lots and lots of negative data in my drawers. This is evidence for the uniqueness of the K-T boundary."

But Schmitz is not giving up. Basu and colleagues "say the haystack is a needlestack," he says. "Who knows, maybe they are right," and bits of killer meteorite will soon be turning up everywhere.

—RICHARD A. KERR

## Proteomics

# Public Projects Gear Up to Chart the Protein Landscape

Researchers in industry as well as those in the public sector seek the protein equivalent of the human genome sequence

**MONTREAL, CANADA**—With the human gene sequence now in hand, researchers have moved on to a new goal: identifying all the body's proteins. The task is massive. Not only does each of the body's 252 cell types harbor its own complement of proteins,

will identify proteins that can serve as both markers for disease and targets for new drug therapies, the pharmaceutical industry jumped into the research a few years ago, investing hundreds of millions of dollars in high-speed protein-tracking technology (*Science*, 7 December 2001, p. 2074).

More recently, the public funding agencies have gotten into the act, launching their own large-scale proteomics projects. They say they had little choice, as the corporate push has left university researchers out in the cold. "Companies don't open their resources for academics," says Marius Ueffing, a proteomics researcher at the German Society for Proteome Research and the Institute of Human Genetics in Munich.

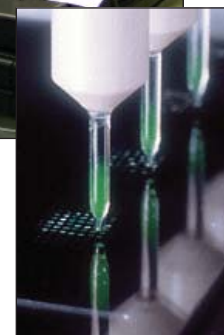
To fill that gap, four separate public international proteomics initiatives have been launched over the past year and a half. Three of them, spearheaded by researchers in the United States, Germany, and China, are



**Speed boost.** An international effort to churn out 10,000 antibodies to human proteins could enable the development of protein-tracking technology, such as the antibody microarrays produced by this machine.

but their expression patterns also vary with age, nutrition, health, and disease.

These difficulties haven't deterred researchers who want to determine the body's complete set of proteins—the proteome, as it's called. Buoyed by hopes that their efforts



will identify proteins that can serve as both markers for disease and targets for new drug therapies, the pharmaceutical industry jumped into the research a few years ago, investing hundreds of millions of dollars in high-speed protein-tracking technology

aimed at tracking down all the proteins in human blood plasma, the brain, and liver. A fourth effort seeks to create antibodies against thousands of human proteins, a resource that should help researchers devise other protein-tracking tools.

Additional proteome projects are looming, with kidney, muscle, heart, and saliva among the possible targets. "We are being bombarded by groups that want to have this or that initiative," says Samir Hanash, president of the Human Proteome Organization (HUPO), an international coordinating group. Once researchers have collected snapshots of the ever-changing proteomes of different tissues and cells, they hope to assemble them into a kind of full-length movie showing the ebb and flow of proteins in the body.

In addition to these major efforts, research groups are also pursuing numerous smaller efforts, such as tracking down all the proteins in subcellular structures—including the mitochondria and Golgi—as well as in various microbes (see sidebar). "It's a very, very hot field," says Fuchu He, director of China's Beijing Institute of Radiation Medicine and head of HUPO's liver proteome project.

Initial plans and results from many of these projects were on display at HUPO's 2nd Annual World Congress held here 8 to 11 October. Just how they will unfold is uncertain. "We're still early on and testing the waters," Hanash says.

Indeed, large-scale programs are likely to be far more difficult to pull off with proteins than with genes. The chemistry is more variable, for one. Some proteins reside in watery environments such as blood, for example, whereas others hide out in the fatty membranes surrounding cells. Proteins range considerably in size, from 5000 daltons to 1 million or more. They differ in their electrical charges. And most challenging of all, most proteins exist only in vanishingly small quantities. "This is a nightmare analytically," says Thomas Conrads, a biochemist at the National Cancer Institute at Frederick (NCI-Frederick) in Maryland.

The equipment is more demanding, too. Whereas genome researchers could concentrate their resources on a single technology, sequencing machines, that's not possible in proteomics. "No method right now is able to analyze a complete single proteome," says Thierry Rabilloud, a proteomics expert at the French Atomic Energy Commission in Grenoble. As a result, proteomics researchers must make hard choices about what to go after. "You have to reduce the proteome to manageable units and define achievable goals," Hanash says.

That's where HUPO has stepped in. Launched in 2001, this loosely knit feder-

ation of proteome researchers doesn't dole out any research funds itself. That money comes from traditional biomedical funding agencies in participating countries. But HUPO helps set priorities, coordinate research, set standards for handling and processing samples, and arrange for the use of common bioinformatics tools to ensure that researchers can directly compare their results.

HUPO didn't waste any time picking favorites. The organization quickly targeted blood plasma as its first priority, aiming to discover blood-borne proteins indicative of disease. The \$1 million pilot project was launched in April 2002 and currently consists of researchers from 47 labs around the

electrospray mass spectrometer. That laborious effort ensures that researchers don't see only common proteins such as albumin, which constitutes up to 50% of the total protein in plasma.

But discarding the high-abundance proteins comes at a cost. When the NCI-Frederick team took a closer look at such proteins, they found that they readily bind a wide variety of low-abundance proteins, acting like molecular sponges. The researchers identified 341 different proteins and peptides bound to albumin alone. Other sets of proteins bind to other highly abundant proteins such as antibodies. "This was rather stunning to us," Conrads says. "Each one of these proteins is binding different peptides.

So there does seem to be some precise interaction." Although it's still too early to be certain, Conrads says it might be possible to use common proteins to track down the low-abundance proteins and peptides that most groups are interested in as potential diagnostic markers.

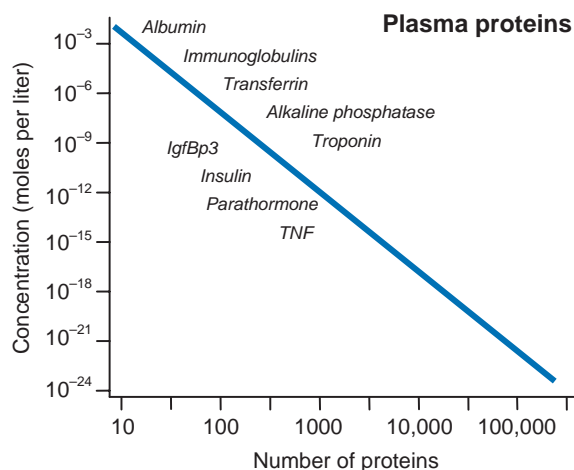
PPP director Gilbert Omenn of the University of Michigan Health System in Ann Arbor says that other techniques will have their own strengths and weaknesses. It's still too early to make meaningful comparisons, he adds. He expects that the final results of the

plasma analyses should be in by the end of the year.

PPP aims to move on to a full-scale plasma project, attempting to correlate changes in the abundance of select proteins with various diseases. There could be a snag, though. The project could cost about \$50 million, Omenn says, and he hasn't yet identified a funding source. But he is confident that "there will be a major follow-up."

HUPO's Human Brain Proteome Project (HBPP) is also up and running. Begun in April as a pilot project, HBPP builds on a brain proteome project begun in 2000 and backed by \$17 million from the German ministry of research. The collaboration aims to sort out technology and standards issues, focusing at first on tracking down the proteins in the substantia nigra and hippocampus, the brain regions that degenerate in Parkinson's and Alzheimer's diseases.

Project co-leader Helmut Meyer, a protein chemist at the University of Bochum in Germany, says that HBPP researchers hope to find proteins that mark the early disease stages, because most damage to brain cells occurs before the first symptoms show up. If



**Where's Waldo?** Most plasma proteins, including potential diagnostic markers and drug targets, are present in only tiny quantities.

globe, including 28 in the United States.

For now, HUPO's Plasma Proteome Project (PPP) is focused on comparing the strengths and weaknesses of different protein-hunting technologies, such as two-dimensional gel electrophoresis and liquid chromatography for separating proteins, and various versions of mass spectrometry for identifying them.

In July, PPP's leaders mailed out standardized plasma samples to participating labs and asked each to use its technique of choice to separate and identify as many proteins as possible. HUPO leaders plan to set recommendations about which technologies are most appropriate for tracking down different subsets of plasma proteins. Initial results, which started coming in last month, suggest that the task won't yield simple answers, however.

Conrads, for example, described how the NCI-Frederick team had managed to sift out some 1444 plasma proteins using a standard technique—first separating out the high-abundance proteins, dividing the rest of the sample into numerous fractions, and then scanning each one using an

## A Sharper Focus

The large-scale proteomic surveys coordinated by the Human Proteome Organization (HUPO) have a long way to go to produce their promised medical benefits (see main text). But more-focused proteomic projects may have a more immediate impact. Researchers around the globe are hard at work using proteomic technologies to identify the proteins that make up the mitochondria, the cell's energy powerhouse, and other subcellular structures. A talk given last month at HUPO's annual meeting by University of Montreal cell biologist Michel Desjardins provides an example of how proteomic tools can lead to crucial new insights into cell biology as well as potential medical benefits.

Over the last few years, Desjardins and colleagues used a standard combination of techniques known as two-dimensional gel electrophoresis and mass spectrometry to separate and identify proteins from phagosomes, transitory sacklike structures involved in engulfing and eliminating foreign invaders ranging from dust particles to pathogenic bacteria. A 2001 paper by the scientists in the *Journal of Cell Biology* revealed that they had identified some 150 proteins.

When the researchers scanned the literature to find out the functions of these proteins, one mystery stood out. A protein called flotillin was known to be present on the outer cell membrane,

where it apparently helps other membrane-bound proteins assemble into so-called lipid rafts. Rafts, which may help coordinate cell-signaling pathways by organizing the membrane proteins that transport substances such as hormones and growth factors into cells, had not previously been spied on the surface of phagosomes. But, Desjardins says, flotillin's presence there suggested that phagosomes might also contain them. A series of traditional cell biology tests confirmed the hypothesis. The work shows that proteomics has "enormous potential" for leading biologists to new molecular insights, says cell biologist Kathryn Howell of the University of Colorado Health Sciences Center in Denver.

But Desjardins's team went one step further. The researchers knew that certain pathogens, such as the bacterium that causes leishmaniasis, are able to survive in phagosomes. So they decided to test whether they do so by disrupting raft formation. And here too they received a pleasant surprise. A series of cell biology studies revealed that a molecule called lipophosphoglycan (LPG) that is abundant on the surface of *Leishmania* bacteria inhibits the formation of lipid rafts. In a way not yet understood, this prevents the organism from being killed in the phagosome.

For drugmakers interested in fighting *Leishmania*, which is thought to infect some 2 million people around the world, LPG "presents an ideal target," Desjardins says. For example, it may be possible to design a small drug molecule that blocks its effects on rafts. **—R.F.S.**

the researchers find these markers they can ask, "Are they already visible when people are 30 or 40 years old?" Meyer says. The HBPP team will also scan cerebral spinal fluid and blood plasma for proteins linked to brain diseases.

The early results reveal some tantalizing hints of what's to come. At the meeting, HBPP co-director Joachim Klose, a protein scientist at Humboldt University in Berlin, described a series of experiments with mice. Klose and colleagues tracked changes in the abundance of 250 brain proteins as the mice grew from embryos to aging adults. As expected, the researchers found that the overall amount of proteins remained essentially constant from a few days after birth until the animals died.

Even so, the abundance of a large percentage of different proteins changed considerably during the animals' early growth. But the researchers were somewhat surprised to find that nearly 20% of brain proteins continued to change their abundance levels when the animals were in the final stages of life. The results, Klose says, suggest that changes in members of this protein subset could be linked to disease.

The Human Liver Proteome Project (HLPP), meanwhile, is backed by an initial round of \$25 million in funding from the Chinese government for a 3-year pilot study to be completed in 2005. The effort was launched in May and is aimed at setting up the collaborations,

standards, and procedures for tallying the thousands of proteins expressed in human liver cells. So far, 79 labs, 37 of them in China, have signed on to the liver proteome effort. The project's leader, Fuchu He, says he expects a full-scale production phase to follow from 2006 to 2010.

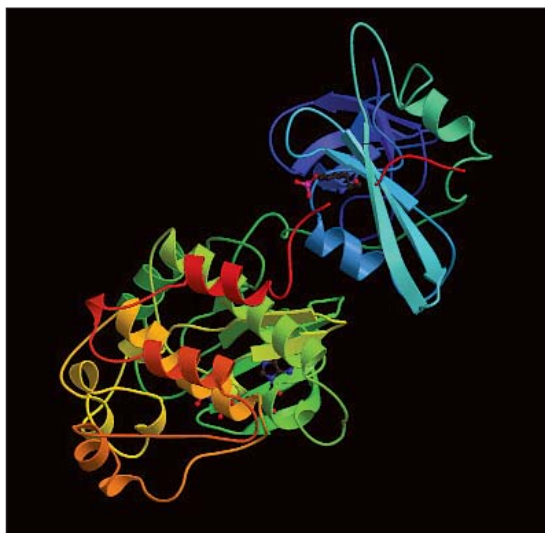
As with the other proteome efforts, the idea is to ultimately link liver-specific pro-

duction phase. This commitment, He says, stems from the fact that liver diseases kill hundreds of thousands of people in China each year.

Proteomics experts caution, however, that they can't link a change in protein expression to disease from just one or two samples. "If you want sound results, you will have to repeat it five or 10 times," Klose says. That's not likely to be accomplished with the large proteome projects.

But confirming a protein linkage to disease should become much easier if HUPO's fourth initiative—to make a vast library of antibodies against human proteins—succeeds. Such antibodies could be used to track a particular protein in many people as a way of confirming its involvement in a disease. In addition, Omenn says, this project will help researchers in each of the other initiatives create protein microarrays and other tests for tracking the ebb and flow of thousands of proteins simultaneously in different tissues.

Both HBPP and HLPP have dedicated a significant portion of their early funds to antibody production. And a group led by Ueffing is applying to companies and the European Union for an initial round of \$12.5 million in research funding on what it hopes will eventually become a \$60 million initiative to raise antibodies against 10,000 human proteins. If researchers line up the money, the antibodies produced could make life easier for proteomics researchers. **—ROBERT F. SERVICE**



**On the hunt.** Proteomics researchers hope that their quest to find thousands of novel proteins will turn up good candidates for new therapeutic drugs such as the so-called Src kinase, which has been implicated in some cancers.

teins to diseases such as hepatitis and liver cancer. According to He, the Chinese government has promised to kick in another \$250 million if HLPP makes it into the pro-