'Omics' of the mitochondrion

Two complementary proteomics approaches promise to move us closer to definition of the complete complement of proteins that make up a mitochondrion.

Benedikt Westermann and Walter Neupert

What is the set of proteins that constitute an organelle in its entirety? Having the answer to this question is the dream of many cell biologists. The advent of the 'omics' era has changed the dream to an experimentally tractable problem. Several systematic approaches are being pursued to define the complements of proteins associated with particular intracellular structures or functions. Two studies published in this issue[1,2] hold the promise that the combination of biochemical and genetic approaches will be extremely powerful to obtain a complete picture of the mitochondrial proteome in the near future.

The discovery of mitochondria as ubiquitous and defined entities of nucleated cells dates back to the end of the 19th century. Some 50 years ago, mitochondria were recognized as the power plants of the cell. In the late sixties, mitochondrial DNA was discovered, and in the eighties the human mitochondrial genome was sequenced. It became clear that this genome encodes only a handful of mitochondrial proteins (i.e., 8 in yeast and 13 in human), whereas the vast majority of proteins are encoded by the nuclear DNA, synthesized in the cytosol and imported into the organelle. At the same time, mitochondrial diseases were found to be due to mutations both in the mitochondrial genome and, more recently, in the nuclear genome. In recent years, research on the roles of mitochondria in apoptosis, aging, and the pathogenesis of several diseases (including Parkinson's, Alzheimer's and cancer) has gained much interest. One can foresee that elucidation of the mitochondrial proteome will be the next big step toward an in-depth understanding of this complex organelle because it will be the basis for the molecular dissection of known and novel mitochondrial functions in the coming decades.

How many different proteins make up a mitochondrion? The most comprehensive study performed so far was aimed at localizing the proteome in yeast cells. In a large-scale study, 2744 epitope-tagged yeast proteins covering roughly 45% of the proteome were immunolocalized. A mitochondrial location was found for 332 proteins, representing 13% of the set analyzed. From this analysis, it can be estimated that yeast mitochondria contain about 800 distinct proteins[3]. Given the complexity of differentiated mammalian cells, the number of distinct proteins in human mitochondria is likely to be considerably higher; perhaps between 1000 and 2000, or even higher.

The present paper by Taylor *et al.* [1] pursues a biochemical approach to obtain the most complete catalog of mitochondrial proteins reported so far. Highly purified mitochondria isolated from human heart were solubilized with a mild detergent, protein complexes were partially separated by sucrose gradient centrifugation, and proteins were resolved by one-dimensional gel electrophoresis. Mitochondrial proteins were identified by mass spectrometry combined with rigorous bioinformatic analysis. Thus, a total of 615 mitochondrial or mitochondria-associated proteins were identified. These include a significant number of potentially new mitochondrial proteins, the biochemical functions of which remain to be defined.

Several similar studies aimed at a systematic identification of mitochondrial proteins by proteomic approaches have been reported. The most comprehensive study reported so far was on rat liver mitochondria and yielded a list with 192 proteins[4]. Remarkably, Taylor *et al.* were able to triple the size of that list. There is no doubt that this is a major achievement. However, at least half of the mitochondrial proteome remains unknown. The elucidation of the complete set of mitochondrial proteins will certainly be sped up by the combination of several approaches coming from different disciplines. Thus, the accompanying paper by Umezawa and colleagues[2] is very timely. These latter researchers developed an elegant genetic screening method that holds the potential to identify novel mitochondrial proteins on a large scale from cDNA libraries.

Their approach is based on reconstitution of a split green fluorescent protein (GFP) reporter in mitochondria. Sequences from cDNA libraries are randomly fused to the amino-terminal half of GFP. If the expressed fusion protein contains targeting information for the mitochondrial matrix, the GFP moiety will meet its carboxy-terminal counterpart there. Full-length GFP is reconstituted in the matrix by a protein splicing element present in both reporter constructs. This methodology has the advantage that only mitochondria-positive clones yield a fluorescence signal. These clones can be isolated by automated cell sorting, a technique that allows a wide net to be cast. Relevant genes are subsequently identified by molecular genetic techniques. Although the system has not yet been applied in a comprehensive manner, the authors demonstrate its suitability for rapid identification of novel proteins containing mitochondrial targeting information.

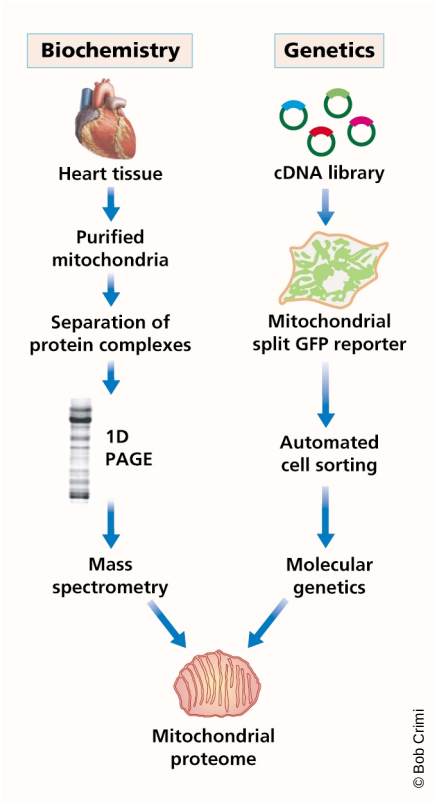

Figure 1. Two roads towards an elucidation of the mitochondrial proteome. A biochemical[1] and a genetic[2] approach promise to define the complement of proteins that make up a mitochondrion.

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Both biochemistry and genetics have proven to be instrumental in identifying mitochondrial components. However, some limitations are associated with the strategies discussed above. Proteins of low abundance might be missed because they are present in amounts too low for identification by gel electrophoresis and mass spectrometry, and their genes might be underrepresented in cDNA libraries. Furthermore, both methods might yield a significant number of false-positive candidates; mitochondrial preparations may be contaminated by other organelles, or some sequences taken out of their normal context may artificially target a passenger protein to the mitochondrion. The genetic system, at least in its present form, requires that the GFP fusion protein is exposed to the matrix and thus fails to identify outer membrane and intermembrane space proteins as well as certain inner membrane proteins.

Other recent efforts to achieve a genome-wide identification of mitochondrial components have relied on the analysis of yeast mutant collections defective in mitochondrial functions[5,6] or on the changes of the transcription profiles dependent on respiratory activity[7,8]. These studies, however, are restricted to proteins involved in oxidative phosphorylation and cannot discriminate between mitochondrial proteins and factors that are indirectly involved, (e.g., nuclear transcription factors).

Although comprehensive biochemical and genetic approaches combined with computational predictions are providing a more complete picture of the mitochondrial proteome, important challenges remain. First, more information must come from comparative proteomics: analysis of the proteomes of different organs will be important in correlating different structures with different sets of proteins. And second, mitochondrial proteomes need to be studied under the various physiological or pathological conditions to teach us more about the roles of mitochondria in health and disease. At the same time, it will be important to assign biochemical functions to the many newly identified proteins. Clearly, knowing the complete set of proteins that constitute a mitochondrion will open a new era of mitochondrial biology.

1. Taylor, S.W. *et al. Nat. Biotechnol.* **21**, 281–286 (2003).
2. Ozawa, T., Sako, Y., Sato, M., Kitamura, T., & Umezawa, Y. *Nat. Biotechnol.* **21**, 287–293 (2003).
3. Kumar, A. *et al. Genes Dev.* **16**, 707–719 (2002).
4. Fountoulakis, M., Berndt, P., Langen, H., & Suter, L. *Electrophoresis* **23**, 311–328 (2002).
5. Steinmetz, L.M. *et al. Nat. Genet.* **31**, 400–404 (2002).
6. Dimmer, K.S. *et al. Mol. Biol. Cell* **13**, 847–853 (2002).
7. Hughes, T.R. *et al. Cell* **102**, 109–126 (2000).
8. Epstein, C.B. *et al. Mol. Biol. Cell* **12**, 297–308 (2001).

Bringing diabetes therapeutics to the big screen

Large-scale genomic screening of secreted proteins for therapeutic potential in diabetes reveals a surprise—bone morphogenetic protein-9.

Leif Groop

Type 2 diabetes is one of the world's most common diseases, affecting more than 150 million people across the globe, and this number is predicted to double within the next 15 years. No cure is available for type 2 diabetes, and management of the disease typically involves diet control, exercise, home blood glucose testing, and in some cases, medication with recombinant insulin or oral pharmaceuticals. The application of genomics-based target discovery to type 2 diabetes has long promised to provide new targets for medical intervention. In this issue, Chen *et al.*[1] apply such an approach to functional screening for secreted proteins with therapeutic potential in type 2 diabetes. Their screens reveal bone morphogenetic protein-9 (BMP-9) as a promising target for further investigation in diabetes drug discovery.

The epidemic increase in the prevalence of type 2 diabetes is attributed to a synergism between genetic predisposition and obesity common in affluent western society[2]. However, the root cause of the genetic predisposition has thus far eluded most scientific enquiry. In type 2 diabetes, elevated glucose concentration (chronic hyperglycemia) results from impaired secretion of insulin and insulin resistance in target tissues like muscle and liver. As early as the mid-19th century, Claude Bernard suggested that the liver plays a central role in this scenario[3]. A putative hormone, hepatic insulin-sensitizing substance (HISS), which is released from the liver and enhances glucose uptake in peripheral tissues, has been discussed and debated for half a decade[4]. The nature of this "hormone" has, however, remained obscure. The BMP-9 molecule identified by Chen *et al.* in this issue represents a putative HISS.

To identify factors that could influence key steps involved in diabetic pathogenesis, Chen *et al.* first searched for secreted proteins among more than 3 million expressed-sequence tags (ESTs) from 1,000 different cDNA libraries in the Human Genome Sciences (Rockville, MD) database. They scanned all the resulting open reading frames (ORFs) starting with an ATG for the presence of an N-terminal secretory signal peptide using two different bioinformatic algorithms: a hidden Markov model and the SignalP classifier program. Complementary DNAs of the ESTs that scored positive by both methods were sequenced to confirm that they were full-length. Approximately 8,000 sequences containing complete coding regions, starting with a putative signal peptide, were then assessed in a functional screening program (Fig. 1).

In the next step, the 8,000 secreted proteins were transiently transfected into human embryonic kidney (HEK) 293 cells and the supernatants tested in high-throughput cell-based assays for their ability to modulate glucose metabolism. The researchers assessed the proteins for a role in suppression of hepatic glucose production by screening for their ability to inhibit the expression of a key rate-limiting enzyme in gluconeogenesis, phosphoenolpyruvate carboxykinase (PEPCK) in liver rat hepatoma H4IIe cells. They also tested their capacity to activate the serine/threonine kinase Akt (which stimulates glycogen synthesis in muscle) and to stimulate the transcription of two proteins essential for fat synthesis, malic enzyme (ME) and fatty acid synthase (FAS).

BMP-9 scored as a hit in the assays, with an observed effect that was comparable to that seen with insulin. Moreover, purified recombinant BMP-9 was shown to cause a sustained lowering of plasma glucose concentrations in normal (C57BL/6) and diabetic (db/db) mice. In normal mice, there was a delayed dose-dependent response, with a hypoglycemic effect 24 hours after treatment, and in the diabetic mice glucose concentrations declined within the first 30 hours after treatment. In addition to mimicking the action of insulin, BMP-9 also stimulated insulin release in Wistar and Zucker diabetic rats.

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