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Research Focus

Mitochondrial building blocks

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Despite many genomic and proteomic attempts, approximately half of all mitochondrial proteins remain unidentified. Moreover, the composition of mitochondria varies in different mammalian cell types and the details of this tissue specificity are unclear. Two recent reports provide a major advance in our understanding of mitochondrial function. Sickmann *et al.* used an exhaustive proteomic approach and came very close to identifying the complete set of yeast mitochondrial proteins. Mootha *et al.* examined mitochondria from mouse brain, heart, kidney and liver cells, finding that a surprising fraction of the proteins are expressed in only a subset of tissues.

Mitochondria play pivotal roles in both the life and death of eukaryotic cells. During cell growth, they are best known for their predominant role in ATP production but they also contribute to a wide variety of other cell processes, such as ion homeostasis and the metabolism of amino acids, heme, lipids and urea [1]. Mitochondria are also central players in senescence, as well as in programmed cell death [2]. Not surprisingly, defects in human mitochondrial function lead to a multitude of problems, including cancer, cardiomyopathies, diabetes, obesity, and many types of neurodegenerative disease [3]. Recent genomic and proteomic studies suggest that mammalian mitochondria are composed of at least a thousand different proteins, representing ~5–15% of the eukaryotic proteome. Because mitochondrial DNA encodes only a handful of proteins (13 in humans and 28 in yeast), the genes for the vast majority of these proteins are located in the nucleus. Although many mitochondrial proteins are already known, particularly those involved in oxidative phosphorylation and the Krebs cycle, nearly half of all mitochondrial proteins remain unidentified. These missing proteins preclude a comprehensive understanding of mitochondria both in sickness and in health.

Over the past few years, a variety of approaches have been used in attempts to identify the full set of mitochondrial proteins, a few of which are highlighted in Table 1. One commonly used method is a proteomic survey of purified mitochondria, such as the recent analysis of human heart mitochondria [4]. Solubilized mitochondrial proteins are separated using procedures such as 1D or 2D polyacrylamide gel electrophoresis (PAGE) or liquid chromatography. Proteins extracted from gels or those located in different column fractions are digested by protease, and the peptides are then identified by mass spectrometry. In addition to biochemical techniques, several genetic and genomic approaches have been used to expand the catalog of mitochondrial proteins (see Box 1 for a brief description of a few of these methods). Computer analyses, such as the search for open reading frames containing mitochondrial targeting signals [5], have also uncovered new proteins. Specialized databases, including MITOP [6] and MitoProteome [7], have been developed to house all the known and predicted mitochondrial proteins.

As mentioned, despite all the different approaches that have been used, it is estimated that nearly half of all mitochondrial proteins still remain unidentified. Below we summarize two recent papers that go a long way towards

Box 1. Some genomic approaches for a mitochondrial inventory

Genomic GFP tagging and fluorescence microscopy
Using oligonucleotide-directed homologous recombination, each of the known 6234 open reading frames (ORFs) in yeast were tagged at the 3′ end with green fluorescent protein (GFP). The subcellular location of the fusion protein was determined in the yeast strains expressing detectable GFP (70% of the strains) by fluorescence microscopy. The distribution of many of the fusion proteins was confirmed using reference proteins fused to red fluorescent protein or organelle-specific dyes.

Protein splicing of split GFP in the mitochondrial matrix
cDNAs were randomly fused to the N-terminal half of GFP and were introduced into cells expressing the C-terminal half of GFP in the mitochondrial matrix. If the cDNA encoded a protein that was imported into mitochondria, full-length GFP was reconstituted in the matrix by protein splicing elements carried in both halves of GFP. Positive GFP signals were detected by automatic cell sorting.

Epitope tagging and immunofluorescence microscopy
By cloning polymerase-chain-reaction-amplified genes and by transpositional mutagenesis, ~60% of all yeast ORFs were epitope-tagged. The cellular location of each fusion protein was then determined by immunofluorescence microscopy, using antibodies to the epitope.

mRNAs that cofractionate with mitochondria
Polyribosomes bound to the surface of mitochondria are enriched with mRNAs encoding imported mitochondrial proteins. Using a genome-wide approach, new mitochondrial proteins were identified by the analysis of nuclear-encoded mRNAs that cofractionate with mitochondria.

Screening yeast mutants for mitochondrial function and morphology
A collection of deletion mutants, representing all of the nonessential yeast genes, was screened for its ability to grow on a nonfermentable medium, which is an indicator of mitochondrial function. In addition, mutants were stained with a mitochondria-specific dye, and those defective in the normal shape and distribution of mitochondria were examined by fluorescence microscopy.

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Table 1. Some previous attempts at a mitochondrial inventory

Approach	Number of mitochondrial proteins	Organism	Refs
Proteomics	615	Human	[4]
Genomic GFP[a] tagging and fluorescence microscopy	526	Yeast	[10]
Protein splicing of split GFP in the mitochondrial matrix	70	Mouse	[15]
Epitope tagging and immunofluorescence microscopy	332	Yeast	[9]
mRNAs that cofractionate with mitochondria	~200	Yeast	[16]
Screening yeast mutants for mitochondrial function and morphology	>300	Yeast	[17]

[a]Abbreviation: GFP, green fluorescent protein.

solving this problem, and significantly add to our understanding of mitochondrial complexity and function.

The yeast mitochondrial proteome

A major step towards a complete inventory of mitochondrial proteins has recently been accomplished using a proteomic approach with highly purified yeast mitochondria [8]. In this report by Sickmann *et al.* [8], 750 proteins were identified, of which 436 had been previously shown to be mitochondrial proteins. Based on the observation that all of the known subunits of the pyruvate dehydrogenase complex, the Krebs cycle enzymes and the inner membrane complexes of oxidative phosphorylation were detected, and that 65 of the 73 known mitochondrial ribosomal proteins were found, it is estimated that at least 90% of the total yeast mitochondrial proteins were identified in their study. Before this study, the largest proteomic survey identified 615 proteins using human heart mitochondria, representing only ~45% of the predicted total [4]. In this and other early attempts, it is likely that many mitochondrial proteins were not identified due to their low abundance or poor solubility. Therefore, to maximize the chance of identifying polypeptides with greatly different properties, Sickmann *et al.* used several methods to liberate mitochondrial proteins, including 1D-PAGE, 2D-PAGE, liquid chromatography and multidimensional chromatography, before their analysis by matrix-assisted time-of-flight or tandem mass spectroscopy. Two mitochondria-associated fractions (proteins removed from mitochondria by salt or protease treatment) were also analyzed.

Among the 750 proteins identified by Sickmann *et al.* there are several surprises. For example, more than 100 of the proteins had previously been reported to reside in a cellular compartment other than mitochondria. Although a few of these proteins are clearly plasma membrane, vacuolar or other cellular contaminants, most are likely to be *bone fide* mitochondrial proteins. Sickmann *et al.* chose several proteins that had been localized to another organelle and showed that each was imported into isolated mitochondria in an energy-dependent manner. Although a dual localization in mitochondria and other compartments can explain the discrepancy with some of these proteins, it is likely that many will turn out to be exclusively mitochondrial. For example, about half of the contentious proteins had been localized using only a tagging approach [9,10]. Because Sickmann *et al.* noted that many known mitochondrial proteins are found in the cytosol, endoplasmic reticulum, vacuole or nucleus when they are tagged, it should not be a surprise that other fusion proteins are mislocalized to the mitochondria – a clear warning to those of us who rely solely on tags to determine where our favorite protein resides.

Another surprise from the current work is that three proteins thought to reside fully inside the mitochondria (lactate dehydrogenase, cytochrome b_2 and the matrix-localized mtHsp70 protein) were detected in a surface-associated fraction with their N-terminal targeting signals still intact. Because the precursor form is not normally seen in cells, it was taken for granted that mitochondrial proteins are very rapidly imported and completely processed to their mature form. Clearly, this might not always be the case and provides a mechanism for some proteins to act at more than one mitochondrial location.

A survey of mitochondrial proteins from brain, heart, kidney, and liver

Although yeast mitochondria are poised to deliver the complete catalog of proteins, determining how mitochondria are customized for the metabolic demands of diverse cell types requires another line of attack. It is known that the activity of many biosynthetic and catabolic pathways, as well as the size, shape and number of the mitochondria, exhibit remarkable variation among different mammalian tissues. To pinpoint the molecular basis for this diversity, Mootha *et al.* [11] carried out a proteomic survey of mitochondria isolated from mouse brain, heart, kidney, and liver cells. A total of 399 proteins were identified and, when combined with the mitochondrial proteins already known, as well as those from the recent human heart proteome [4], the array of mammalian mitochondrial proteins now numbers more than 700. For many of these mitochondrial genes, the RNA expression profiles from a wide range of tissues are known. Mootha *et al.* identified additional candidates for mitochondrial proteins among subnetworks of genes with correlated expression patterns. For example, the group of genes known to be involved in oxidative phosphorylation, β-oxidation and the tricarboxylic acid cycle also contains 11 genes of unknown function. Although some of the 11 are likely to encode mitochondrial proteins, others might encode non-mitochondrial proteins, such as transcription factors required for the coordinated expression of the subnetwork. In either case, the RNA analysis is yielding exciting new information that complements the proteomic data.

Probably the most striking result from the proteomic comparison of mitochondria from brain, heart, kidney, and liver is the large number proteins that are missing from one or more tissues. When this information is combined with the RNA expression data, it appears that only half of all mitochondrial proteins are found in every cell type. Some of the similarities and differences were anticipated.

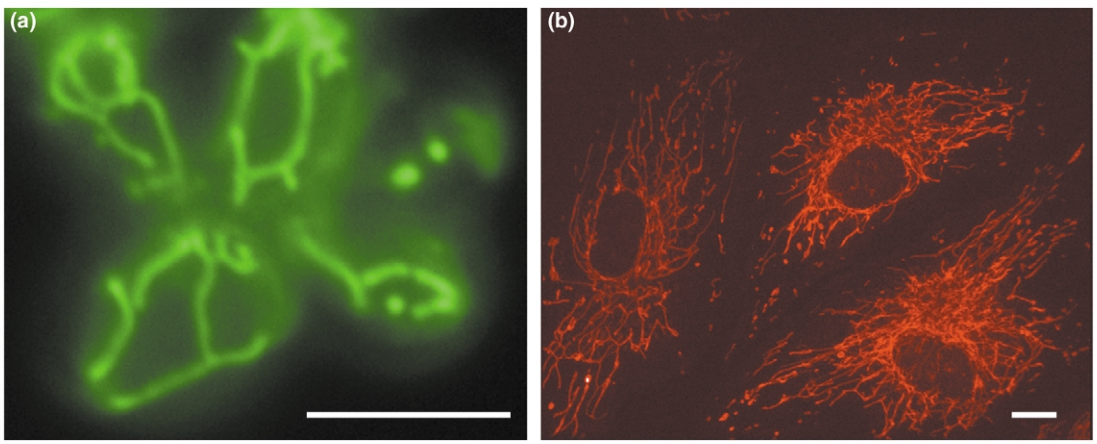

Figure 1. Fluorescent images of mitochondria. Images shown are of mitochondria in **(a)** *Saccharomyces cerevisiae* cells expressing a matrix-targeted green fluorescent protein and in **(b)** bovine pulmonary-artery endothelial cells stained with the mitochondria-specific dye MitoTracker™. Although most yeast cells contain ~5–10 tubule-shaped mitochondria, mammalian cells show much more variety in the shape of their mitochondria, ranging from the tubules seen in the endothelial cells to small spheres in liver cells and large, round organelles in corneal cells [13,14]. The number of mitochondria in mammalian cells can also vary greatly. Scale bars correspond to 10 μm.

For instance, among the ubiquitous proteins are those involved in bioenergetics, and examples of tissue-specific expression (e.g. the proteins required for steroidogenesis in adrenal cells or heme biosynthesis in bone marrow) are well known. However, the possibility that hundreds of mitochondrial proteins vary in their amounts from one cell type to the next was not expected. Just how unique mitochondria are in different organs awaits the quantitative proteomic and RNA analysis from many more tissues.

Concluding remarks

The survey of yeast and mammalian mitochondria has yielded more than 200 new proteins. A few of these have already been shown to be components of known pathways, such as a new subunit of complex I of the electron transport chain [12]. Others are likely to play roles in poorly understood processes, such as the control of organelle shape, number, and inheritance (Figure 1). One of the remaining challenges will be to determine the functions of all these new proteins. Because they number more than 200, novel methods could be needed to expedite these analyses. For example, it might be helpful to group genes with similar mRNA expression patterns, or proteins according to their location within mitochondria (i.e. outer membrane, inner membrane, intermembrane space or matrix). Systematic analysis of mitochondrial protein complexes might also provide important information. Ultimately, elucidating the function of many of the new mitochondrial proteins might require the slow grind of individual investigators chasing down the role of individual proteins.

Another remaining challenge is to complete the mitochondrial proteome. The mammalian inventory is the furthest from being done, with hundreds of proteins missing. More exhaustive proteomic analyses, similar to those used by Sickmann *et al.*, are clearly needed. However, even though almost all of the yeast mitochondrial proteins have now been identified, it might be very difficult to finish the job. Some of the missing proteins could be of such low abundance or solubility that new biochemical methods will be needed for their detection. Other mitochondrial proteins might require yeast cells to be grown under special conditions or on different media for their expression. Because mitochondria from different mammalian cell types show a wide range of tissue-specific diversity, completing the human mitochondrial proteome will be even more difficult. Many of the remaining mammalian and yeast proteins could, therefore, await identification by novel genetic or genomic methods. Nonetheless, the work by both Sickmann *et al.* and Mootha *et al.* provides a crucial framework for ultimately understanding the complexity of mitochondrial function and the contribution of this organelle to disease.

References

1 Schatz, G. (1995) Mitochondria: beyond oxidative phosphorylation. *Biochim. Biophys. Acta* 1271, 123–126
2 Newmeyer, D.D. and Ferguson-Miller, S. (2003) Mitochondria: releasing power for life and unleashing the machineries of death. *Cell* 112, 481–490
3 Wallace, D.C. (1999) Mitochondrial diseases in man and mouse. *Science* 283, 1482–1488
4 Taylor, S.W. *et al.* (2003) Characterization of the human heart mitochondrial proteome. *Nat. Biotechnol.* 21, 281–286
5 Claros, M.G. and Vincens, P. (1996) Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur. J. Biochem.* 241, 779–786
6 Scharfe, C. *et al.* (2000) MITOP, the mitochondrial proteome database: 2000 update. *Nucleic Acids Res.* 28, 155–158
7 Cotter, D. *et al.* (2004) MitoProteome: mitochondrial protein sequence database and annotation system. *Nucleic Acids Res.* 32, D463–D467
8 Sickmann, A. *et al.* (2003) The proteome of *Saccharomyces cerevisiae* mitochondria. *Proc. Natl. Acad. Sci. U. S. A.* 100, 13207–13212
9 Kumar, A. *et al.* (2002) Subcellular localization of the yeast proteome. *Genes Dev.* 16, 707–719
10 Huh, W.K. *et al.* (2003) Global analysis of protein localization in budding yeast. *Nature* 425, 686–691
11 Mootha, V.K. *et al.* (2003) Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell* 115, 629–640
12 Fearnley, I.M. *et al.* (2001) GRIM-19, a cell death regulatory gene product, is a subunit of bovine mitochondrial NADH:ubiquinone oxidoreductase (complex I). *J. Biol. Chem.* 276, 38345–38348
13 Bereiter-Hahn, J. and Voth, M. (1994) Dynamics of mitochondria in

living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microsc. Res. Tech.* 27, 198–219
14 Tandler, B. and Hoppel, C.L. (1972) *Mitochondria*, Academic Press
15 Ozawa, T. *et al.* (2003) A genetic approach to identifying mitochondrial proteins. *Nat. Biotechnol.* 21, 287–293
16 Marc, P. *et al.* (2002) Genome-wide analysis of mRNAs targeted to yeast mitochondria. *EMBO Rep.* 3, 159–164
17 Dimmer, K.S. *et al.* (2002) Genetic basis of mitochondrial function and morphology in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 13, 847–853

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O-GlcNAc modification: a nutritional sensor that modulates proteasome function

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The addition of *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) to serine and threonine residues is a post-translational modification of nucleocytoplasmic proteins that is thought to act in a manner analogous to protein phosphorylation. Recent work shows that many proteins of the metazoan proteasome are modified by *O*-GlcNAc and that the level of glycosylation is responsive to the nutritional state of the cell. Moreover, increased glycosylation of the 19S (or PA700) regulatory subcomplex has been correlated with decreased proteasomal activity, suggesting a new model of proteasomal regulation.

The addition of *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) to serine and threonine residues of nuclear and cytoplasmic proteins (Table 1) is an essential post-translational modification of metazoans [1]. *O*-GlcNAc levels change rapidly and dynamically in response to cellular stress [2], extracellular stimuli, morphogens, the cell cycle and development, which alludes to key roles for *O*-GlcNAc in regulating cellular function [1]. On key nuclear and cytoplasmic proteins, such as RNA polymerase II [3], endothelial nitric oxide synthase [4,5], SV-40 large T antigen [6], estrogen receptor-β (ER-β) [7] and the c-Myc proto-oncogene [8], glycosylation and phosphorylation have been mapped to the same amino acid, suggesting a complex interplay exists between these two post-translational modifications (Figure 1). Recent work from two groups shows that the metazoan proteasome is modified extensively by *O*-GlcNAc [9] and that increased glycosylation ablates the ATPase activity of the 19S (or PA700) subcomplex towards some substrates [10]. *O*-GlcNAc modification of proteasomal function appears to be dependent on the nutritional status of cells, providing further evidence for the roles of *O*-GlcNAc in nutritional sensing and subsequent cellular regulation.

Between 2 and 5% of the glucose transported into cells is converted to UDP-GlcNAc (the donor sugar for the biosynthesis of *O*-GlcNAc) through the hexosamine biosynthetic pathway [11]. Elevated extracellular glucose or glucosamine concentrations lead to increased modification of intracellular proteins with *O*-GlcNAc [12]. Moreover, the substrate specificity of *O*-GlcNAc transferase (OGT), the enzyme that catalyzes the addition of *O*-GlcNAc, changes at different UDP-GlcNAc concentrations [13]. Recent *in vitro* and *in vivo* data support a model in which increased UDP-GlcNAc levels, due to hyperglycemia, result in increased *O*-GlcNAc levels, leading to insulin resistance – a hallmark of type II diabetes [14–17]. These data and others have led researchers to propose that

Table 1. Key cellular proteins are modified by *O*-GlcNAc[a,b]

Functional protein group	Examples
Chaperones	HSP27; HSP70; HSP90; HSC70; HSP90
Transcription factors and polymerises	RNA polymerase II large subunit; Sp1; c-Fos; c-Jun; c-Myc; p53; β-catenin; NF-κB; YY1; RB; CREB
Nuclear pore proteins and chromatin-associated proteins	p62; Nup153; Nup180; Nup214; Nup358
RNA-binding proteins	hnRNP G; eIF4α; ribosomal proteins
Kinases and adaptor proteins	CKII; IRS1; IRS2; GSK3β; PtdIns 3-kinase
Cytoskeletal proteins	Keratins 8, 13, 18; neurofilaments H, M, L; band 4.1; talin; vinculin; ankyrin G; synapsin 1; myosin; E-cadherin; tubulin; tau; AP-3; AP-188; β-APP; β-synuclein
Others	eNOS; enolase; glycogen synthase; glut-1; OGT

[a]Abbreviations: AP, clathrin assembly protein; β-APP, β-amyloid precursor protein; CKII, casein kinase II; CREB, cAMP-response-element-binding protein; eIF4α, eukaryotic initiation factor 4α; eNOS, endothelial nitric oxide synthase; glut-1, glucose transporter-1; GSK3β, glycogen synthase kinase3β; hnRNP G, heterogeneous nuclear ribonucleoprotein G; HSP, heat shock protein; IRS, insulin-receptor substrate; NF-κB, nuclear factor-κB; Nup, nucleoporin; *O*-GlcNAc, *O*-linked β-*N*-acetylglucosamine; OGT, *O*-GlcNAc transferase; p62, nuclear pore protein 62; PtdIns 3-kinase, phosphatidylinositol 3-kinase; RB, retinoblastoma protein; YY1, yin yang-1.
[b]More than 100 proteins that are modified by *O*-GlcNAc have been identified. For comprehensive details, see Ref. [26].

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