The proteome of *Saccharomyces cerevisiae* mitochondria

Albert Sickmann*, Jörg Reinders*, Yvonne Wagner*, Cornelia Joppich†, René Zahedi*, Helmut E. Meyer†, Birgit Schönfisch‡, Inge Perschil‡, Agnieszka Chacinska‡, Bernard Guiard§, Peter Rehling‡, Nikolaus Pfanner‡¶, and Chris Meisinger‡

*Rudolf-Virchow-Center for Experimental Biomedicine, Universität Würzburg, Versbacher Strasse 9, D-97078 Würzburg, Germany; †Medizinisches Proteom-Center, Gebäude ZKF E/143, Ruhr-Universität Bochum, Universitätsstrasse 150, D-44780 Bochum, Germany; ‡Institut für Biochemie und Molekularbiologie, Universität Freiburg, Hermann-Herder-Straβe 7, D-79104 Freiburg, Germany; and §Centre de Génétique Moléculaire, Laboratoire Propre du Centre National de la Recherche Scientifique Associeté à l'Université Pierre et Marie Curie, 91190 Gif-sur-Yvette, France

We performed a comprehensive approach to determine the proteome of *Saccharomyces cerevisiae* mitochondria. The proteins of highly pure yeast mitochondria were separated by several independent methods and analyzed by tandem MS. From >20 million MS spectra, 750 different proteins were identified, indicating an involvement of mitochondria in numerous cellular processes. All known components of the oxidative phosphorylation machinery, the tricarboxylic acid cycle, and the stable mitochondria-encoded proteins were found. Based on the mitochondrial proteins described in the literature so far, we calculate that the identified proteins represent ≈90% of all mitochondrial proteins. The function of a quarter of the identified proteins is unknown. The mitochondrial proteome will provide an important database for the analysis of new mitochondrial and mitochondria-associated functions and the characterization of mitochondrial diseases.

Mitochondria play a central role in many cellular functions, including bioenergetics, apoptosis, and the metabolism of amino acids, lipids, and iron (1–4). Many diseases have been attributed to mitochondrial defects (5–8). According to the currently available information, however, only ≈50–60% of all presumed mitochondrial proteins have been identified so far (8–20). Thus, our knowledge about the physiological functions of mitochondria is limited, and many mitochondrial diseases cannot be analyzed on a molecular level.

The *Saccharomyces cerevisiae* genome was the first fully sequenced eukaryotic genome, and comprehensive approaches on the deletion and expression of nearly all ORFs have been performed (21–24). Many human genes involved in diseases possess functional homologues in yeast. Because of the excellent accessibility of yeast to biochemical and genetic analysis, it represents a major eukaryotic model organism for the identification and characterization of protein functions and cellular pathways.

So far, the largest proteomic study of a purified cell organelle was performed by Taylor *et al.* (13), leading to the identification of 615 mitochondrial and mitochondria-associated proteins with a coverage of up to 45% of the predicted human mitochondrial proteins (14, 16). Here we report the identification of the mitochondrial proteome of *S. cerevisiae* with a coverage of ≈90% (750 proteins in total).

Materials and Methods

Isolation of Highly Pure Yeast Mitochondria and Cellular Fractions. *S. cerevisiae* cells were grown in YPG medium (1% yeast extract/2% bactopeptone/3% glycerol, pH 5.0) to an OD of 1.5–2.0. For most experiments the strain YPH499 was used (25). Where indicated, a translocase of outer mitochondrial membrane (Tom)22$_{His-10}$ strain (MR103) was used (26). A crude mitochondrial fraction was obtained by differential centrifugation (12,000 × *g* pellet, P12) (27) and adjusted to a protein concentration of 5 mg/ml in 250 mM sucrose/1 mM EDTA/10 mM Mops, pH 7.2. After treatment with 10 strokes in a glass-Teflon potter, mitochondria were loaded on top of a three-step sucrose gradient [1.5 ml 60%, 4 ml 32%, 1.5 ml 23%, 1.5 ml 15% sucrose in EM buffer (10 mM Mops, pH 7.2/1 mM EDTA)] and centrifuged for 1 h at 134,000 × *g* (27). This step was performed twice. A total of 10 mg of highly pure mitochondria (protein amount) was used as material for this study.

For generation of the mitochondria-associated fraction, mitochondria (1 mg of protein per ml) were treated with either 20 μg/ml trypsin or 1 M NaCl in 250 mM sucrose/1 mM EDTA/10 mM Mops, pH 7.2 for 15 min on ice. After centrifugation for 15 min at 14,000 × *g*, the supernatant was used for further analysis. The isolation of yeast microsomal fractions (100,000 × *g* pellet, P100) and Western blot analysis were performed as described (26, 27).

MS Analysis and Data Processing. 2D PAGE followed by matrix-assisted laser desorption/ionization–time of flight MS, multidimensional peptide separation, tandem MS (MS/MS), the coupling of 1D PAGE with MS, and data interpretation were performed as described (28–31). For protease treatment, 50 μg of trypsin, chymotrypsin, Glu-C, or subtilisin per mg of protein was used at 37°C overnight or, in case of subtilisin, for 4 h. Monoisotopic peptide mass fingerprints obtained from in-gel digested proteins were used for searching the National Center for Biotechnology Information nonredundant protein database with PROFOUND (http://prowl.rockefeller.edu). The automatic database search of fragment-ion spectra was performed with the SEQUEST algorithm (version 27) on the complete yeast database (32, 33).

A total of 320 proteins (43% of 750) possessed a Mitoprot score between 0.7 and 1.0, suggesting the presence of a classical mitochondrial presequence (www.mips.biochem.mpg.de/cgi-bin/proj/medgen/mitofilter). A total of 255 proteins (34%) contained at least one predicted α-helical transmembrane segment according to YPD (www.incyte.com) and TMHMM prediction service (www.cbs.dtu.dk/services/TMHMM).

Import of Proteins into Isolated Mitochondria. ^{35}S-labeled preproteins were generated by *in vitro* transcription/translation from PCR products containing the SP6 promoter region (34) and incubated with mitochondria in import buffer (3% BSA/80 mM KCl/5 mM MgCl$_2$/10 mM Mops-KOH, pH 7.2/2 mM NADH/2 mM ATP). Subsequently, the samples were treated with 50

BIOCHEMISTRY
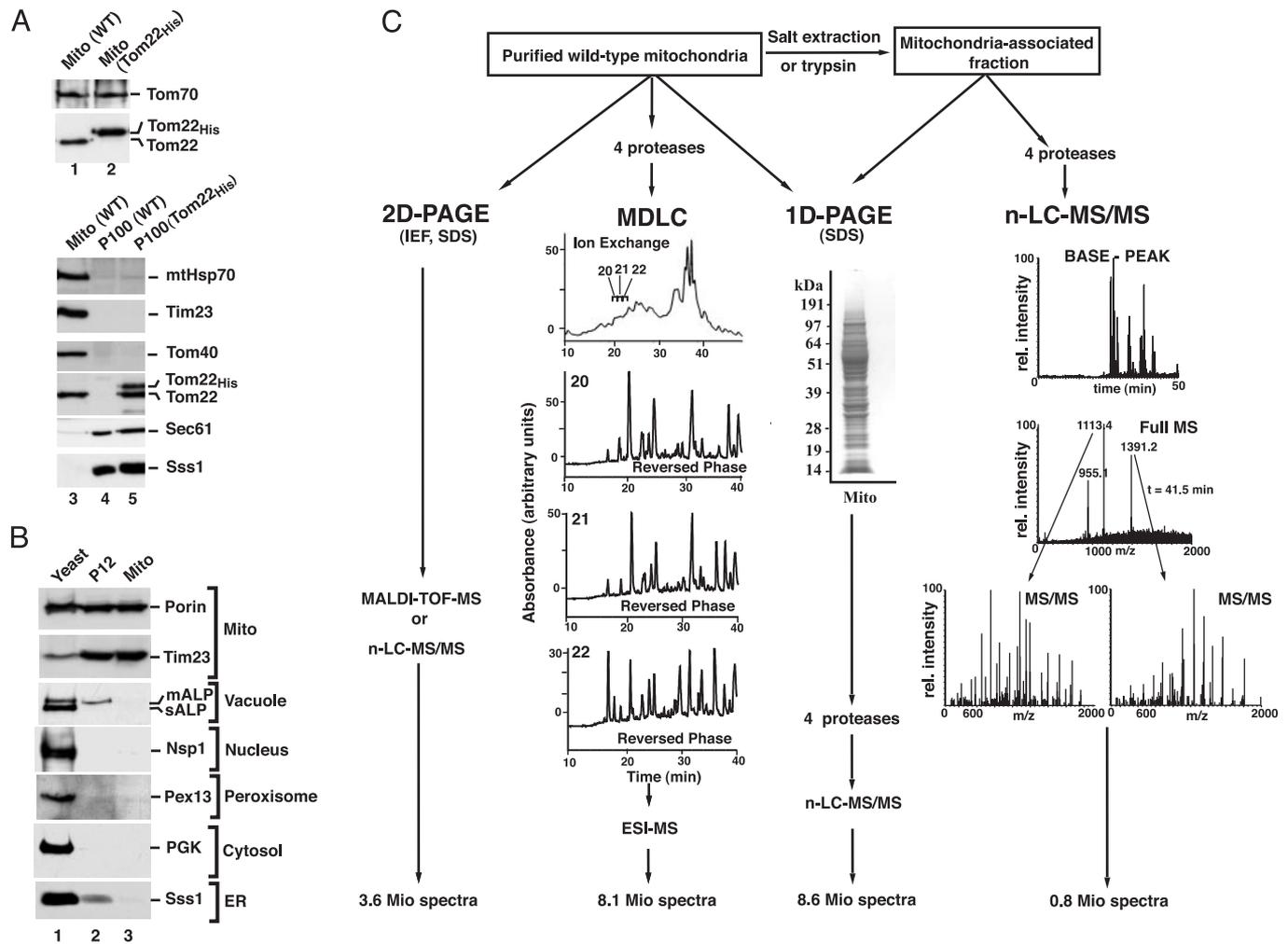
Fig. 1. Purification of yeast mitochondria, separation of proteins, and analysis by MS. (*A*) Subcellular localization of authentic and tagged Tom22. Mitochondria (Mito; 25 μg of protein) and enriched microsomal fractions (25 μg of protein; P100) from WT and Tom22$_{His-10}$ *S. cerevisiae* strains were separated by SDS/PAGE and transferred onto poly(vinylidene difluoride) membranes. Immunodecoration was performed with antisera against the indicated proteins and the ECL detection system (Amersham Pharmacia Biotech). Sec61 and Sss1 are subunits of the ER translocon. (*B*) Highly purified yeast mitochondria. Equal-volume aliquots from total yeast, P12, and highly purified mitochondria were separated by SDS/PAGE and blotted onto poly(vinylidene difluoride) membranes. mALP, mature vacuolar alkaline phosphatase; sALP, soluble vacuolar alkaline phosphatase; Nsp1, subunit of nuclear pore complex; Pex13, peroxisomal biogenesis protein (peroxin) 13; PGK, phosphoglycerate kinase 1. (*C*) Strategies for protein separation and MS analysis. Four separation methods were used to cover the mitochondrial proteins: isoelectric focusing (IEF) followed by SDS/PAGE (2D PAGE); digestion with four different proteases, followed by multidimensional liquid chromatography (MDLC) and electrospray ionization-MS (ESI-MS); 1D SDS/PAGE, followed by n-LC-MS/MS; generation of a mitochondria-associated fraction by treatment of mitochondria with trypsin or salt, followed by SDS/PAGE and n-LC-MS/MS. Typical examples of separation and spectra are shown.

μg/ml proteinase K for 15 min on ice. For dissipation of the membrane potential, the mitochondria were incubated with 1 μM valinomycin before the import reaction (34). The proteins were separated by SDS/PAGE and detected by digital autoradiography (PhosphorImaging). Blue native electrophoresis was performed as described (26).

Results and Discussion

Experimental Strategy. Kumar *et al.* (35) reported a large-scale localization of ≈45% of yeast proteins by tagging and immunolocalization; many of the proteins were overexpressed. They localized 332 proteins to mitochondria. We analyzed their database for known mitochondrial proteins and noticed that, upon tagging and (mild) overexpression, many mitochondrial proteins failed to be imported into the organelle and instead were localized to other cellular compartments, in particular the cytosol, nucleus, and endoplasmic reticulum (ER), e.g., the mitochondrial intermembrane space protein cytochrome b_2, the inner membrane proteins Tim50 (subunit of the presequence

translocase of inner membrane, Tim), Coq4 (coenzymeQ biosynthesis), Qcr6 (subunit of the ubiquinol cytochrome *c* reductase), and the ribosomal proteins MRPS28, MRPS8, and MRPL13 of the mitochondrial matrix. The conclusion that a subcellular localization of tagged proteins is less suitable at least for mitochondrial proteins was corroborated by the analysis of a yeast strain expressing the mitochondrial outer membrane protein Tom22 with a short tag at the C terminus (10-histidine tag; integrated into the chromosome). Although Tom22 was expressed from its own, fully intact promoter and the resulting strain grew like WT yeast (26), the protein was overexpressed and localized to two cellular compartments. Tagged Tom22 was present in mitochondria (Fig. 1*A*, lane 2), but also in the microsomal (ER) fraction where the tag was partially removed (Fig. 1*A*, lane 5), whereas the untagged Tom22 was selectively found in the mitochondrial fraction (Fig. 1*A*, lane 3 vs. lane 4).

We therefore concluded that for a reliable localization of mitochondrial proteins, we had to use the authentic, nontagged, and nonoverexpressed proteins. We optimized a procedure for
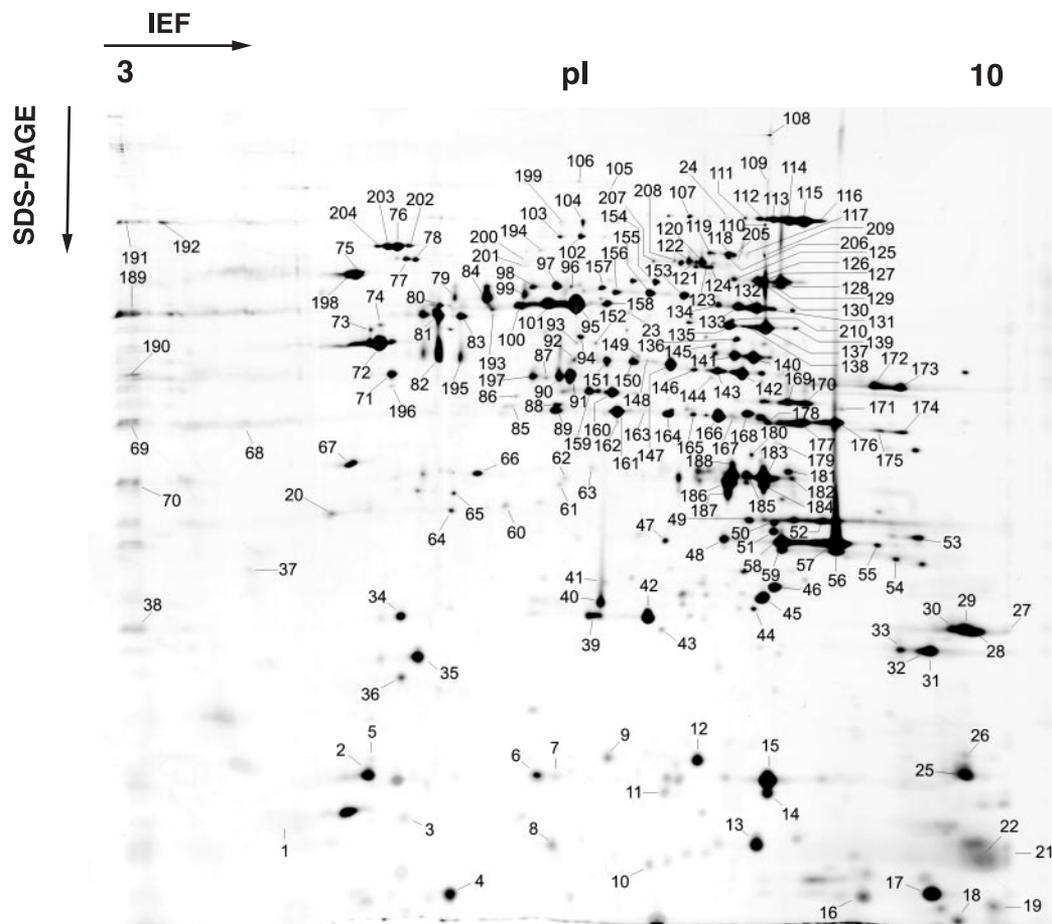
Fig. 2. Separation of mitochondrial proteins by 2D PAGE. Isolated yeast WT mitochondria were subjected to isoelectric focusing (IEF), followed by SDS/PAGE. The identified protein spots are numbered and listed in Table 2.

the isolation of highly pure yeast mitochondria from WT cells (27). Mitochondria were isolated from yeast lysates by differential centrifugation (P12; Fig. 1*B*, lane 2) and two subsequent sucrose gradients (Fig. 1*B*, lane 3). Western blotting demonstrated that the resulting mitochondria contained the mitochondrial marker proteins, whereas marker proteins for other cellular compartments were not detectable (Fig. 1*B*, lane 3).

We used several separation methods in parallel (Fig. 1*C*) to minimize the problem that a significant fraction of proteins can escape detection in individual separation approaches. The purified mitochondria were subjected to: (*i*) 2D PAGE and matrix-assisted laser desorption/ionization–time of flight-MS or nano-liquid chromatography-MS/MS (n-LC-MS/MS); (*ii*) multidimensional liquid chromatography and MS/MS; and (*iii*) 1D PAGE (in particular for identification of membrane proteins) and n-LC-MS/MS analysis. Moreover, two mitochondria-associated fractions were generated by treating mitochondria with salt or trypsin and analyzed by 1D PAGE or 1D HPLC and MS/MS. In total, we generated >20 million MS spectra that were analyzed by database searches. A total of 750 different proteins that were identified are listed in Table 1, which is published as supporting information on the PNAS web site.

Subcellular Localization of the Identified Proteins. From the 2D PAGE (Fig. 2), 209 spots were determined and listed in Table 2, which is published as supporting information on the PNAS web site, leading to the identification of 109 different proteins. Of these, the function of 13 proteins is unknown. A total of 102 proteins have previously been localized to mitochondria,

whereas 7 proteins have not been localized so far. None of the proteins had been localized to another cellular compartment, confirming the high purity of the mitochondria used.

Of the 750 proteins that were identified in total, 436 proteins (58%) are known mitochondrial proteins. A total of 208 proteins have not yet been localized so far, and 106 proteins have been reported to be located in other cellular compartments.

We analyzed the proteins that were previously found in other cellular compartments, but not in mitochondria, in detail. (*i*) A systematic screen of the original literature of the identified proteins revealed that half of the proteins had been localized only by a tagging approach (see above for the problem of tagging approaches for the exact localization of mitochondrial proteins). Fifty-two authentic, i.e., nontagged proteins (7% of total) had been localized to another compartment. (*ii*) Some proteins were contaminations of the purified mitochondria and detected because of the high sensitivity of MS. For example, the abundant plasma membrane proteins Pma1 and Pma2 are easily accessible to tryptic digest and MS analysis and can be identified even when present in tiny amounts (23). Pma1 and Pma2 were also detected in the fraction generated by trypsin treatment of the mitochondrial surface. (*iii*) The homologues of at least 13 proteins that had previously been located to other cellular compartments were also found in the recent proteome analysis of human heart mitochondria (13), including the Hsp90 homologue, Hsc82, and GTP-binding/activating proteins mainly of the Rho and Rab family, suggesting that they may be specifically associated with mitochondria. (*iv*) A dual localization in mitochondria and another cellular compartment has been reported for at least 30
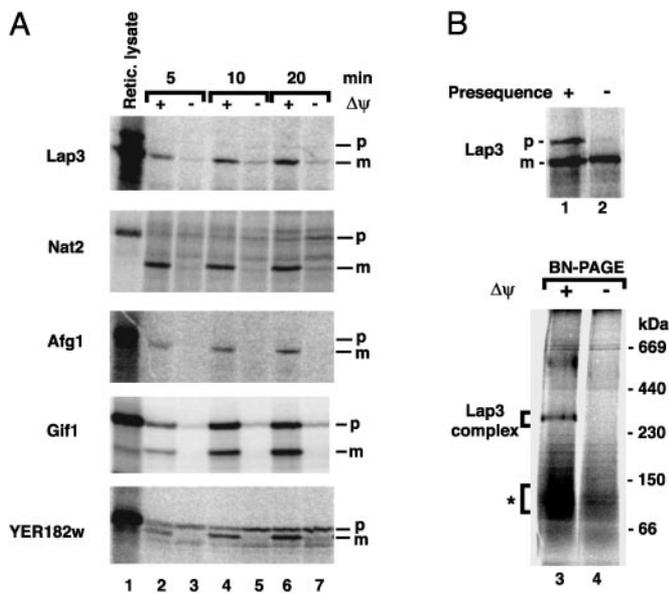
Fig. 3. Import of proteins into isolated yeast mitochondria. (*A*) ^{35}S-labeled precursor proteins were incubated with isolated yeast WT mitochondria in the presence or absence of a membrane potential (Δψ) for the indicated times. The samples were treated with proteinase K and separated by SDS/PAGE. Imported proteins were detected by digital autoradiography. Lane 1, nonimported labeled precursor protein in reticulocyte lysate (5% of material added to mitochondria). p, precursor form; m, mature form. (*B*) Lap3 forms a 300-kDa complex in mitochondria. ^{35}S-labeled Lap3 with or without a presequence (lanes 1 and 2). The precursor form of Lap3 was incubated with isolated mitochondria in the presence or absence of a Δψ (lanes 3 and 4). The mitochondria were solubilized by digitonin after the import reaction and separated by blue native PAGE (BN/PAGE). *, Putative dimer of Lap3.

of the known mitochondrial proteins. Several more proteins are likely candidates for a dual localization (36, 37). (*v*) We synthesized a number of the proteins, which had been localized to other compartments or had not been localized so far, in rabbit reticulocyte lysate in the presence of [35S]methionine/cysteine and incubated them with isolated yeast mitochondria. Many of them were found with the mitochondria after centrifugation and may thus be associated with the mitochondrial outer membrane. The most striking effect was observed, however, when the dependence on the inner membrane potential Δψ was determined. At least five of the proteins were specifically processed by mitochondria only in the presence of a Δψ (Fig. 3*A*): Lap3, an aminopeptidase of the cysteine protease family (38, 39); Nat2, an essential protein reported to be a protein *N*-acetyl transferase (40); Afg1, a member of the AAA family of ATPases (41); Gif1, a protein required for the normal G₁ phase of the cell cycle (33); and YER182w, a protein of unknown function. The processed forms were protected against proteinase K added to the mitochondria only when the import reaction was performed in the presence of a Δψ. These findings prove that the proteins are transported into or across the mitochondrial inner membrane (34) and are thus true mitochondrial proteins.

We noticed that Lap3 contained a presequence-like segment upstream of the starting methionine of the purified protein (42). *In vitro* synthesis of Lap3 from the amplified ORF including the upstream segment led to two translation products, designated p and m (Fig. 3*B*, lane 1). Without the upstream segment, only the m form of Lap3 was synthesized (Fig. 3*B*, lane 2). When incubated with mitochondria *in vitro*, the m form neither associated with nor was imported into mitochondria (data not shown). Purified Lap3 has been shown to form a ring-shaped hexamer of ≈300 kDa, probably consisting of three dimers (42).

We asked whether Lap3 imported into mitochondria in the presence of a Δψ formed a complex. Blue native electrophoresis indeed demonstrated that radiolabeled Lap3 was integrated into a complex of ≈300 kDa (Fig. 3*B*, lane 3). Additionally, a 100-kDa complex was observed that may correspond to the reported dimer (42). We suggest that Lap3 has a dual localization in the cytosol (as reported) and the mitochondria.

These findings taken together with the high purity of the mitochondria used indicate that several proteins that were previously found in other compartments are actually mitochondrial or mitochondria-associated proteins, including cases of dual localization.

Coverage of the Yeast Mitochondrial Proteome. We identified all known subunits of the pyruvate dehydrogenase complex, the tricarboxylic acid cycle, and the inner membrane complexes of the oxidative phosphorylation machinery, including the eight stable proteins that are encoded by the mitochondrial genome. Because most of the latter proteins are hydrophobic proteins, our approach was not biased against membrane proteins that are often derished in proteome studies based on single separation methods like 2D PAGE. Moreover, our approach was not biased against small or large proteins because we identified proteins from a broad size range. The molecular weight distribution of all identified proteins correlated well with that of the YPD database for mitochondrial proteins and the overall molecular weight distribution of the predicted proteins from the whole yeast genome (36) (data not shown). The proteins identified by us cover 92% of the known mitochondrial proteins listed in the MITOP yeast database (43). Taylor *et al.* (13) identified 35 subunits (45%) of the known 77 subunits of the human heart mitochondrial ribosome; this value is in good agreement with the assessed overall coverage of the mitochondrial proteome by this study (13, 14). We identified 65 subunits (89%) of the known 73 subunits of the yeast mitochondrial ribosome (36). Taken together, we assess that the yeast mitochondrial proteome reported here covers ≈90% of all putative yeast mitochondrial proteins.

It will be difficult to identify 100% of the proteins of an entire cell organelle by a proteome analysis. Because some proteins are expressed only under special growth conditions, there is probably no single growth condition under which all proteins are expressed simultaneously. Moreover, some proteins will escape detection by the available separation and identification methods. We observed that the identification of mitochondrial proteins by the combination of several approaches used here came close to saturation (Fig. 4*A*). More than 650 different proteins were identified with the first 5 million spectra and most of the remaining proteins with the next 10 million spectra.
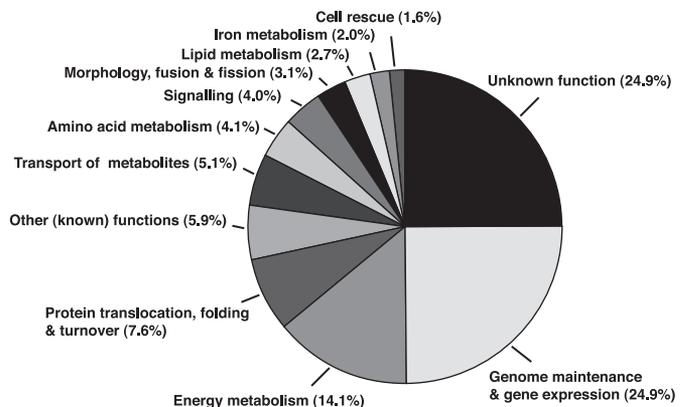
The high sensitivity of the approach is underscored by the unexpected discovery of presequences on the protein level. The fraction released by a trypsin treatment of the mitochondrial surface contained peptides that were entirely present in the presequence regions of mitochondrial proteins. Fig. 4*B* shows the identified peptides for three mitochondrial proteins: cytochrome b_2, the inner membrane protein D-lactate dehydrogenase (Dld1), and the matrix heat shock protein 70 (mtHsp70), also termed Ssc1 in yeast. It had generally been assumed that preproteins are rapidly imported *in vivo* and the presequences are removed. The precursor forms of preproteins could usually only be observed *in vivo* after radiolabeling or impairment of the mitochondrial import or processing machinery (44–47). Although we used WT cells grown under standard conditions, the precursor forms of preproteins remained associated with mitochondria even after extensive washing and long isolation procedures. This finding may explain previous surprising observations of proteins in a different mitochondrial subcompartment. For example, the presence of mtHsp70 in purified mitochondrial outer membranes was ascribed to a contamination of the mem-
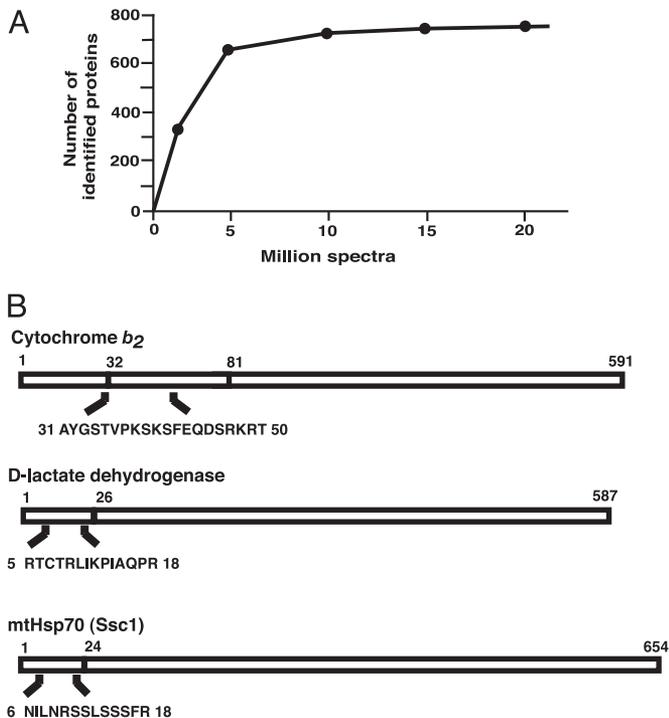
Fig. 4. Identification of mitochondrial proteins and presequences. (*A*) Number of identified different proteins per million MS spectra. (*B*) Identification of presequence segments in proteins of the mitochondria-associated fraction. The sequences of the identified peptides found closest to the N terminus are shown. Numbers refer to amino acid positions, starting with the first residue of the presequences. The first and last residues of the mature proteins are indicated. Cytochrome *b₂* is processed in two steps, after residues 31 and 80 (49).

Fig. 5. Classification of the identified proteins according to function. Protein functions were assigned according to the databases YPD (36), SGD (33), and MITOP (43).

brane preparation with matrix proteins (48), yet may now be explained by the presence of the precursor form of mtHsp70 at the outer membrane.

Functional Classification of the Identified Proteins. An assignment of the identified proteins according to their known or predicted functions yielded many different functional classes (Fig. 5). Only 14% of the proteins directly act in energy metabolism, including the oxidative phosphorylation machinery, the tricarboxylic acid cycle, and pyruvate dehydrogenase, whereas 25%

of all proteins are involved in maintaining and expressing the mitochondrial genome, including the protein synthesis machinery in the matrix. Thus, a quarter of all mitochondrial proteins are needed for the expression of only eight stable proteins encoded by the yeast mitochondrial genome. Fourteen percent of the proteins function in transport processes of metabolites or metabolism of amino acids, lipids, and iron. Approximately 8% of the mitochondrial and mitochondria-associated proteins are involved in protein translocation, folding, or turnover, including further cochaperones, proteases, and possibly components related to the ubiquitin system. Approximately 6% of the proteins function in processes of cell rescue and signaling, including GTP-binding (Rho- and Rab-related) proteins and regulators of GTPases. Three percent of the proteins are involved in maintenance of morphology or processes of fusion and fission. The function of 25% of the identified proteins is currently unknown.

The database of the yeast mitochondrial proteome now represents a comprehensive source for the characterization of novel mitochondrial and mitochondria-associated functions, signaling pathways, and proteolytic systems and for the molecular identification of the basis for mitochondrial diseases.

1. Schatz, G. (1995) *Biochim. Biophys. Acta* **1271,** 123–126.
2. Scheffler, I. E. (1999) *Mitochondria* (Wiley, New York).
3. Lill, R. & Kispal, G. (2000) *Trends Biochem. Sci.* **25,** 352–356.
4. Newmeyer, D. D. & Ferguson-Miller, S. (2003) *Cell* **112,** 481–490.
5. Wallace, D. C. (1999) *Science* **283,** 1482–1488.
6. Schapira, A. H. (2000) *Curr. Opin. Neurol.* **13,** 527–532.
7. Schon, E. A. (2000) *Trends Biochem. Sci.* **25,** 555–560.
8. Steinmetz, L. M., Scharfe, C., Deutschbauer, A. M., Mokranjac, D., Herman, Z. S., Jones, T., Chu, A. M., Giaever, G., Prokisch, H., Oefner, P. J., *et al.* (2002) *Nat. Genet.* **31,** 400–404.
9. Kruft, V., Eubel, H., Jansch, L., Werhahn, W. & Braun, H. P. (2001) *Plant Physiol.* **127,** 1694–1710.
10. Millar, A. H., Sweetlove, L. J., Giege, P. & Leaver, C. J. (2001) *Plant Physiol.* **127,** 1711–1727.
11. Grandier-Vazeille, X., Bathany, K., Chaignepain, S., Camougrand, N., Manon, S. & Schmitter, J. M. (2001) *Biochemistry* **40,** 9758–9769.
12. Pflieger, D., Le Caer, J. P., Lemaire, C., Bernard, B. A., Dujardin, G. & Rossier, J. (2002) *Anal. Chem.* **74,** 2400–2406.
13. Taylor, S. W., Fahy, E., Zhang, B., Glenn, G. M., Warnock, D. E., Wiley, S., Murphy, A. N., Gaucher, S. P., Capaldi, R. A., Gibson, B. W., *et al.* (2003) *Nat. Biotechnol.* **21,** 281–286.
14. Westermann, B. & Neupert, W. (2003) *Nat. Biotechnol.* **21,** 239–240.
15. Fountoulakis, M & Schlaeger, E. J. (2003) *Electrophoresis* **24,** 260–275.
16. Heazlewood, J. L., Millar, A. H., Day, D. A. & Whelan, J. (2003) *Genome Biol.* **4,** 218.
17. Martin, H., Eckerskorn, C., Gärtner, F., Rassow, J., Lottspeich, F. & Pfanner, N. (1998) *Anal. Biochem.* **265,** 123–128.
18. Da Cruz, S., Xenarios, I., Langridge, J., Vilbois, F., Parone, P. A. & Martinou, J. C. (2003) *J. Biol. Chem.*, in press.
19. Karlberg, O., Canback, B., Kurland, C. G. & Andersson, S. G. (2000) *Yeast* **17,** 170–187.
20. Marcotte, E. M., Xenarios, I., van Der Bliek, A. M. & Eisenberg, D. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 12115–12120.
21. Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., Galibert, F., Hoheisel, J. D., Jacq, C., Johnston, M., *et al.* (1996) *Science* **274,** 563–567.
22. Winzeler, E. A., Shoemaker, D. D., Astromoff, A., Liang, H., Anderson, K., Andre, B., Bangham, R., Benito, R., Boeke, J. D., Bussey, H., *et al.* (1999) *Science* **285,** 901–906.
23. Washburn, M. P., Wolters, D. & Yates, J. R., III (2001) *Nat. Biotechnol.* **19,** 242–247.
24. Giaever, G., Chu, A. M., Ni, L., Connelly, C., Riles, L., Veronneau, S., Dow, S., Lucau-Danila, A., Anderson, K., Andre, B., *et al.* (2002) *Nature* **418,** 387–391.
25. Sikorski, R. S. & Hieter, P. (1989) *Genetics* **122,** 19–27.

BIOCHEMISTRY

26. Meisinger, C., Ryan, M. T., Hill, K., Model, K., Lim, J. H., Sickmann, A., Müller, H., Meyer, H. E., Wagner, R. & Pfanner, N. (2001) *Mol. Cell. Biol.* **21,** 2337–2348.
27. Meisinger, C., Sommer, T. & Pfanner, N. (2000) *Anal. Biochem.* **287,** 339–342.
28. Sickmann, A., Dormeyer, W., Wortelkamp, S., Woitalla, D., Kuhn, W. & Meyer, H. E. (2000) *Electrophoresis* **21,** 2721–2728.
29. Sickmann, A., Dormeyer, W., Wortelkamp, S., Woitalla, D., Kuhn, W. & Meyer, H. E. (2002) *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **771,** 167–196.
30. Wagner, Y., Sickmann, A., Meyer, H. E. & Daum, G. (2003) *J. Am. Soc. Mass. Spectrom.* **14,** 1003–1011.
31. Simpson, R. J., Connolly, L. M., Eddes, J. S., Pereira, J. J., Moritz, R. L. & Reid, G. E. (2000) *Electrophoresis* **21,** 1707–1732.
32. Yates, J. R. & Eng, J. (1995) *Anal. Chem.* **67,** 1426–1436.
33. Issel-Tarver, L., Christie, K. R., Dolinski, K., Andrada, R., Balakrishnan, R., Ball, C. A., Binkley, G., Dong, S., Dwight, S. S., Fisk, D. G., *et al.* (2002) *Methods Enzymol.* **350,** 329–346.
34. Ryan, M. T., Voos, W. & Pfanner, N. (2001) *Methods Cell Biol.* **65,** 189–215.
35. Kumar, A., Agarwal, S., Heyman, J. A., Matson, S., Heidtman, M., Piccirillo, S., Umansky, L., Drawid, A., Jansen, R., Liu, Y., *et al.* (2002) *Genes Dev.* **16,** 707–719.
36. Hodges, P. E., McKee, A. H., Davis, B. P., Payne, W. E. & Garrels, J. I. (1999) *Nucleic Acids Res.* **27,** 69–73.
37. Rehling, P., Pfanner, N. & Meisinger, C. (2003) *J. Mol. Biol.* **326,** 639–657.
38. Kambouris, N. G., Burke, D. J. & Creutz, C. E. (1992) *J. Biol. Chem.* **267,** 21570–21576.
39. Enenkel, C. & Wolf, D. H. (1993) *J. Biol. Chem.* **268,** 7036–7043.
40. Kulkarni, M. S. & Sherman, F. (1994) *J. Biol. Chem.* **269,** 13141–13147.
41. Lee, Y. J. & Wickner, R. B. (1992) *Yeast* **8,** 787–790.
42. Joshua-Tor, L., Xu, H. E., Johnston, S. A. & Rees, D. C. (1995) *Science* **269,** 945–950.
43. Scharfe, C., Zaccaria, P., Hoertnagel, K., Jaksch, M., Klopstock, T., Lill, R., Prokisch, H., Gerbitz, K. D., Mewes, H. W. & Meitinger, T. (1999) *Nucleic Acids Res.* **27,** 153–155.
44. Hallermayer, G., Zimmermann, R. & Neupert, W. (1977) *Eur. J. Biochem.* **81,** 523–532.
45. Reid, G. A. & Schatz, G. (1982) *J. Biol. Chem.* **257,** 13062–13067.
46. Kang, P. J., Ostermann, J., Shilling, J., Neupert, W., Craig, E. A. & Pfanner, N. (1990) *Nature* **348,** 137–143.
47. Neupert, W. (1997) *Annu. Rev. Biochem.* **66,** 863–917.
48. Scherer, P. E., Krieg, U. C., Hwang, S. T., Vestweber, D. & Schatz, G. (1990) *EMBO J.* **9,** 4315–4322.
49. Beasley, E. M., Müller, S. & Schatz, G. (1993) *EMBO J.* **12,** 2303–2311.