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Results

ICAT-MS data summary

Proteins identified by each ICAT scheme were analyzed using the Gene Ontology term finder (http://db.yeastgenome.org/cgi-bin/SGD/GO/goTermFinder), which calculates the probability of identifying proteins annotated with a particular term (in this case, localization) by chance from the entire yeast proteome (data summarized in Tables S1 and S2). In the case of ICAT I, 346 proteins were identified and 23 were annotated in SGD as peroxisomal. The likelihood of identifying 23 proteins as peroxisomal by chance is reflected in $p = 3.37 \times 10^{-15}$. By comparison, 134 proteins were identified as mitochondrial ($p = 8.78 \times 10^{-47}$). The large number of mitochondrial proteins was expected because, as discussed above, organelles were collected for ICAT I from both the peroxisome and mitochondrial fractions and mixed before MS. However, when considering only the 57 proteins in this data set with P_E scores >0.65, 18 were annotated in SGD as being peroxisomal ($p = 8.09 \times 10^{-25}$) and none was mitochondrial. These results indicate that ICAT I provided an excellent mechanism by which to identify mitochondrial contaminants within the peroxisome fraction.

ICAT II identified 365 proteins, 38 of which were annotated in SGD as peroxisomal ($p = 3.65 \times 10^{-31}$). 98 proteins had P_E scores >0.65, and 28 of these were annotated as peroxisomal ($p = 2.26 \times 10^{-36}$). Clearly, ICAT II was capable of identifying more peroxisomal proteins than ICAT I. Moreover, ICAT II identified many peroxisomal proteins of low abundance that were not identified by ICAT I, including seven additional peroxins.

Materials and methods

ICAT-based MS approach

Enriched and crude fractions were differentially labeled with isotopically heavy ($^2H_8$ or $^{13}C_9$) or light ($^1H_8$ or $^{12}C_9$) ICAT reagent, respectively. The ICAT reagent forms a covalent adduct with the side chains of reduced cysteine amino acyl residues and contains a biotin moiety for affinity purification on an avidin resin. After adduct formation, the enriched and crude peroxisome fractions were combined and digested with trypsin. The peptides were separated by cation exchange chromatography, and ICAT-labeled peptides were isolated by avidin affinity chromatography. This procedure serves to reduce sample complexity by spreading complex samples over several ion exchange eluate fractions and by selectively retaining only those peptides containing cysteinyl residues (present in ~90% of proteins in the yeast proteome; Gygi et al., 1999). Each eluate fraction was then analyzed by automated gas-phase fractionation μLC/ESI-MS/MS, and proteins were identified by searches of sequence databases (Goodlett et al., 2000). The relative abundances of chemically identical ICAT-labeled peptide pairs, whose differences in mass were due to isotopic labeling, were calculated and expressed as the ratios of the signal intensities ($^2H_8/^1H_8$ or $^{13}C_9/^{12}C_9$), as described previously (Li et al., 2003; Ranish et al., 2003). This ratio represents the relative enrichment of proteins in enriched versus crude peroxisomal membrane fractions.

Subcellular fractionation and sample preparation for quantitative MS

Subcellular fractionation and organelle extractions were performed as previously described (Smith et al., 2002). Peak peroxisome (Fig. 1, fractions 8–10) and mitochondrial fractions (Fig. 1, fractions 2 and 3) were isolated from oleic acid-induced *BY4743* cells, and organelles were extracted with Ti8 buffer (10 mM Tris, pH 8.0, and 1 mM EDTA) containing PINS (0.2 mM PMSF, 2 μg leupeptin/ml, 2 μg aprotinin/ml, and 0.4 μg pepstatin A/ml). Membrane fractions from peroxisomal ($Ti8P_P$) and mitochondrial ($Ti8P_M$) fractions were collected by centrifugation, and the pellets were solubilized in 0.1% SDS. For the affinity purification of peroxisomal membranes, peroxisomes were isolated from a heterozygous diploid yeast strain synthesizing Pex11p fused to protein A (Pex11p-pA) by isopycnic density gradient centrifugation. Fractions enriched for peroxisomes were diluted fivefold in MS buffer (0.65 M sorbitol and 5 mM MES, pH 5.5) containing PINS, and organelles were collected by centrifugation at 20,000 *g* at 4°C. The resulting pellet was resuspended in Ti8 buffer containing 50 mM KP_i and PINS. Pex11p-pA–containing membranes were isolated from 500 μg of $Ti8P_P$ by affinity chromatography using IgG-coupled magnetic beads (Dynal) for 8 h at 4°C. Bound material was washed with Ti8 buffer containing 50 mM KP_i and PINS, and eluted with 0.1% SDS to generate a fraction of affinity-purified peroxisomal membranes.

ICAT labeling

The proteins in sample pairs consisting of a fraction enriched for peroxisomes and a control fraction of equal or lesser total protein were differently labeled with ICAT, and the relative abundances of labeled proteins in each sample were determined by quantitative MS (Ranish et al., 2003). Two independent experiments were performed for both ICAT I and ICAT II. For ICAT I, proteins derived from $Ti8P_P$ and $Ti8P_M$ fractions were labeled with heavy ($^2H_8$) and light ($^1H_8$) ICAT reagents, respectively. For ICAT II, $Ti8P_P$ and affinity-purified peroxisomal membrane samples were labeled with $^1H_8$ (or cleavable $^{12}C_9$) and $^2H_8$ (or cleavable $^{13}C_9$) ICAT reagents (ABI), respectively. The cleavable version of the ICAT reagent became available during the course of these studies, and the use of this reagent led to better recovery of peptides after affinity purification and better coelution of differentially labeled peptide pairs from reverse phase columns (Yi et al., 2004). In each experiment, sample pairs each consisting of 500–800 μg of protein in 0.1% SDS were combined with an equal volume of 40 mM Tris-HCl, pH 8.3, and 10 mM EDTA. Proteins were denatured with 6 M urea at 37°C for 30 min and reduced in 5 mM Tris (2-carboxyethyl)-phosphine-HCl for 45 min at 23°C. ICAT reagent was added to a final concentration of 1.2 mM, and the mixture was incubated at 37°C for 2 h. Labeling reactions were quenched with 12 mM DTT for 5 min at 23°C. Labeled sample pairs were combined and diluted 10-fold with 20 mM Tris-HCl, pH 8.3, and 5 mM EDTA, and proteins were digested with 10 ng trypsin/μL for 12 h at 37°C. Samples were acidified with 85% phosphoric acid to approximately pH 3, and peptides were fractionated by cation exchange chromatography using a Polysulphoethyl A column (2.1 ×200 mm; PolyLC) using the following program: 0–25% buffer B (5 mM KH_2PO_4, pH 3, 600 mM KCl, and 25% CH_3CN) over 30 min, followed by 25–100% buffer B over 20 min at 0.44 ml/min. 36 0.4-ml fractions were collected. ICAT-derivitized peptides were purified from each fraction by binding to a monomeric avidin column (ABI). Bound material was eluted and dissolved in 0.1% formic acid for MS/MS analyses. Where cleavable ICAT regent was used, ICAT-derivitized peptides were recovered by acid cleavage following the manufacturer's directions (ABI).

Quantitative MS

ICAT-labeled peptides were analyzed by μLC-ESI-MS/MS. Peptides were separated by on-line reversed-phase chromatography using a 75 μm × 10 cm self-packed Magic C18AQ column (Michrom BioResources) at a flow rate of 300 nL/min. Peptide fragmentation by collision-induced dissociation was performed in an automated fashion using the dynamic-exclusion option from the full-range MS spectra or by $GPF_{m/z}$ using an ion trap mass spectrometer (Thermofinnigan; Yi et al., 2002). Peptides were identified using SEQUEST (Eng et al., 1994) and organized using INTERACT (Han et al., 2001). PeptideProphet and Protein-Prophet softwares (Keller et al., 2002; Nesvizhskii et al., 2003) were used for statistical validation of the database search results. Relative abundances of peptide pairs were determined using ASAPRatio and INTERACT software, as described previously (Li et al., 2003; Ranish et al., 2003), and expressed as a heavy/light-ICAT ratio. Each ratio was manually inspected to ensure accuracy.

Expectation-maximization algorithm

The distribution of ICAT ratios observed for the entire data set in each experiment was used to calculate, for each protein, the probability of being enriched in the peroxisome fraction (expressed as a P_E value). Statistical analysis was performed using the mixture model expectation-maximization algorithm, which has been applied previously to detect differentially expressed genes in microarray studies (Lee, 2000; Pan et al., 2002). The underlying model assumption is that all identified proteins can be divided into two subpopulations of proteins, those that are enriched (denoted as E) and not enriched (denoted as U) in fractions of peroxisomal membranes relative to control samples. For each quantified protein, its ICAT ratio (r = heavy/light) was calculated and $f(r)$ modeled as $f(r) = p(E)p(r|E) + p(U)p(r|U)$, where $p(r|E)$ and $p(r|U)$ are the distributions of ICAT ratios among enriched and not enriched proteins, respectively. $p(E)$ and $p(U)$ are the mixing proportions and represent the overall distribution of the enriched and not enriched proteins in the data set. $p(r|E)$ and $p(r|U)$ were modeled using Gaussian distributions, and the mixture model was fitted to the data using the expectation-maximization algorithm. Because of the nature of the data in ICAT I (dominance by mitochondrial proteins with low ICAT ratios and relatively few peroxisomal proteins with high ratios), the ratios in this experiment were transformed to their square root for modeling. The mixture model distributions were initialized with parameters obtained using a k-mean clustering algorithm. On each iteration, current parameter estimates were used to probabilistically label the data by computing, for each protein in the data set, the probability of it belonging to the enriched or not enriched subpopulations, $p(E|r) = p(E)p(r|E)/(p(E)p(r|E) + p(U)p(r|U))$ and $p(U|r) = 1 - p(E|r)$, respectively (E-step). The parameter estimates were updated using all proteins in the data set, with the contribution from each of the proteins going toward the distributions $p(r|E)$ and $p(r|U)$, and proportions $p(E)$ and $p(U)$, being weighted according to its current probabilistic labels, $p(E|r)$ and $p(U|r)$ (M-step). At convergence, the learned distributions of ICAT scores among enriched and not enriched proteins were used to compute, for each protein in the data set, the probability of being enriched in the peroxisomal fraction as a function of its ICAT ratio r, $P_E = p(E|r)$. To speed up the convergence of the algorithm and improve the accuracy of the learned distributions (Nigam et al., 2000), the model was adjusted to take advantage of the knowledge of known peroxisomal proteins. For those proteins, their probabilistic labels, $p(E|r)$ and $p(U|r)$, were fixed at 1 and 0, respectively, because such proteins are expected to be enriched in the peroxisomal fraction. However, we note that the method presented here is general and, when no relevant prior information is available, it can be applied in a completely unsupervised manner. 52 proteins, whose ICAT II scores were >0.65 and were also identified in ICAT I, were organized using Cluster and Treeview software (Eisen et al., 1998) using Spearman similarity metric with single linkage clustering.

Yeast strains

All yeast strains used in this study were derived from BY4743 (MATa/MATα his3D1/his3D1 leu2D0/leu2D0 lys2D0/+ met15D0/+ ura3D0/ura3D0) and the corresponding deletion strain library (Winzeler et al., 1999; Resgen), unless otherwise indicated. Yeast strains expressing genomically encoded fusions of *Staphylococcus aureus* protein A or *Aequoria victoria* GFP were constructed by homologous recombination (Dilworth et al., 2001; Smith et al., 2002). A *rho1* (*MATa his3 ura3 leu2 rho1-104*) strain was constructed by mating *HNY21* (containing a *rho1-104* allele; Yamochi et al., 1994) cells with *BY4742* (*MATα his3D1 leu2D0 lys2D0 ura3D0*) cells. A temperature-sensitive segregant derived from this cross was backcrossed to *BY4743*-derived haploids four times. *rho1-2A* (*MATa ura3 leu2 rho1-104 pot1::POT1-GFP (HIS5)*) was generated by crossing *rho1* with *POT1-G*, a *BY4743* haploid segregant containing a genomically encoded *POT1-GFP* chimera, followed by sporulation. *rho1-2A* containing pRHO1 was designated *RHO1-2A*. *vps1*Δ *POT1-G*, *pex11*Δ *POT1-G*, *pex25*Δ *POT1-G* containing a genomically encoded *POT1-GFP* chimera were generated by crossing *POT1-G* with the corresponding yeast deletion mutants and haploid double mutants identified by marker selection. *pex11*Δ *pex25*Δ *POT1G* was generated by crossing *pex11*Δ *POT1-G* and *pex25*Δ *POT1-G* and sporulation of diploids. Haploid strains containing a triple mutation were identified by marker selection. *vps1*Δ *rho1 POT1-G* was generated by crossing *rho1-2A* with *vps1*Δ and selecting G418-resistant temperature-sensitive haploid segregants.

Plasmids

The following plasmids were used in this study: pRS315 *CEN/LEU*, pRS316 *CEN/URA*, pDsRed-PTS1 (Smith et al., 2002), pRHO1, containing a PCR product corresponding to nucleotides −202 to +1397 of *RHO1* (where +1 is the A of the start codon) inserted into EcoRI site of pRS315, pGFP-RHO1 containing the *RHO1* ORF (nucleotides +1 to +676) inserted between the HindIII–XbaI sites of pGFP-N-FUS (Niedenthal et al., 1996) in-frame with the coding region for GFP; pPOT1-RFP, generated by ligating the PCR products of *POT1* (−526 to +1251 up to, but not including, the stop codon) and of the coding sequence for RFP amplified from pRSETB-mRFP (Campbell et al., 2002) between the SalI and XbaI sites of pRS315. pGEX-RHO1, the *RHO1* ORF (+1 to +777), was amplified by PCR and inserted between the SalI and BamHI sites of pGEX-4T1 (Amersham Biosciences).

Yeast media and growth conditions

Strains were grown in YEPD (1% yeast extract, 2% peptone, and 2% glucose), YPB (0.5% KP_i, pH 6.0, 0.3% yeast extract, and 0.5% peptone) containing 2% glucose (YPBD), 2% glycerol (YPBG), 2% acetate (YPBA), 0.2% Tween 40, 0.15% oleic acid (YPBO), or 0.15% oleic acid, 0.075 g lauric acid /L (YPBO/L), and synthetic minimal medium (SM) supplemented with the necessary amino acids or nucleotides at 30°C unless otherwise stated. For peroxisome isolation, yeast cells were induced for 16 h in SCIM medium (0.7% yeast nitrogen base, 0.5% yeast extract, 0.5% peptone, 0.5% Tween 40, 0.79 g of complete synthetic medium [Qbiogene]/L, 0.5% $(NH_4)_2SO_4$, 0.1% glucose, and 0.15% oleic acid). For growth assays, yeast mutants were grown in YEPD at 25°C and spotted onto YPBD, YPBG, YPBA, and YPBO/L. Cells were incubated at 25°C and images captured after 2–3 d in YPBD, 4 d on the nonfermentable carbon sources (YPBA and YPBG), and after 7 d in YPBO/L. For slowly growing strains, cells were incubated for extended periods in nonfermentable carbon sources (8 d) and 20 d in YPBO/L.

Microscopy

The subcellular distribution of Pot1p-GFP was determined by direct fluorescence microscopy after induction in YPBO for 16 h. Yeast strains containing pPOT1-RFP and pDsRed-PTS1 were grown to mid-log phase in SM-Leu medium and induced in selective medium containing 0.15% oleic acid, 0.1% Tween-40 for 16 h at 30°C. RFP and DsRed fusions were visualized by direct fluorescence microscopy. To determine the localization of Rho1p, cells containing pGFP-RHO1 were grown in SM-Ura-Met for 4 h to induce the expression of GFP-Rho1p and transferred to SM-Ura-Met containing 2% glucose or 2% glycerol or 0.15% oleic acid, 0.1% Tween 40 for 16 h at 30°C. Actin patches were stained with phalloidin-RITC (Sigma-Aldrich) in oleic acid-induced cells as described by Amberg (1998) and modified by Hoepfner et al. (2001). The distribution of peroxisomes and actin patches was visualized by confocal microscopy. Cells were visualized using a microscope (model Axiophot II; Carl Zeiss MicroImaging, Inc.) equipped with a PlanNeofluar 100×/1.30 oil objective. Images were captured using a CCD camera (model CoolSnap HQ; Photometrics) and Image software (Roper Scientific). Confocal images were collected on an inverted microscope (model DM IRBE; Leica) with a TCS SP2 confocal system equipped with a HCX PL APO 100×/1.40 oil objective (Leica).

Ultrastructural analyses of *rho1-2A* and *RHO1-2A* were performed on cells induced for 16 h at 27°C in SM-Leu medium and processed as described previously (Nuttley et al., 1994). Thin sections were observed on an electron microscope (model 10; Phillips), and images were recorded with a digital camera (Soft Imaging System). Peroxisome size was quantified by determining the area of peroxisomes in 105 cells from each strain using ImageJ software (http://www.nih.gov).

In vitro binding assay

The plasmids pGEX-4T1 and pGEX-RHO1 were introduced into *Escherichia coli* BLR cells (Novagen). Recombinant expression and isolation of GST and the GST-Rho1p fusion were conducted according to the manufacturer's instructions (Amersham Biosciences). Whole cell lysates derived from yeast strains expressing genomically encoded protein A–tagged peroxins were generated by glass bead lysis of oleate-induced cells in lysis buffer (50 mM Hepes-KOH, pH 7.5, 100 mM KOAc, 5 mM MgOAc, and PINS) containing 1% digitonin. 150 μl of each lysate (5–7 mg of protein/ml) was diluted with 350 μl of lysis buffer containing 0.1% digitonin and incubated for 1 h at 4°C with 10 μl of GST or GST-Rho1p immobilized on glutathione-Sepharose beads. Bound material was extensively washed in lysis buffer containing 1% Triton X-100 and eluted with SDS sample buffer. Load samples were prepared by precipitating proteins from 100 μl of each lysate with 10% TCA. 25% of bound and 10% of each load sample were resolved by SDS-PAGE, and protein A–tagged fusions were detected by Western blotting.

Western blotting

Western blots were blocked in PBS. Protein A chimeras were detected using rabbit IgG (Cappel), and Fox2p, Mls1p, Cta1p, and Mdh3p were detected using an anti-SKL polyclonal antibody (Szilard et al., 1995). Polyclonal anti-Sdh2p (Dibrov et al., 1998; provided by B. Lemire, University of Alberta, Edmonton, Canada), anti-Rho1p (provided by G. Eitzen, University of Alberta, Edmonton, Canada), and anti-Pot1p antibodies were used to detect Sdh2p, Rho1p, and Pot1p, respectively. Antibody binding was visualized using antibodies conjugated to HRP (Pierce Chemical Co.) and chemiluminescence.

References

Amberg, D.C. 1998. Three-dimensional imaging of the yeast actin cytoskeleton through the budding cell cycle. *Mol. Biol. Cell.* 9:3259–3262.

Campbell, R.E., T. Oded, A.E. Palmer, P.A. Steinbach, G.S. Baird, D.A. Zacharias, and R.Y. Tsien. 2002. A monomeric red fluorescent protein. *Proc. Natl. Acad. Sci. USA.* 99:7877–7882.

Dibrov, E., S. Fu, and B.D. Lemire. 1998. The *Saccharomyces cerevisiae* TCM62 gene encodes a chaperone necessary for the assembly of the mitochondrial succinate dehydrogenase (complex II). *J. Biol. Chem.* 273:32042–32048.

Dilworth, D.J., A. Suprapto, B.T. Chait, R.W. Wozniak, M.P. Rout, and J.D. Aitchison. 2001. Nup2p dynamically associates with the distal regions of the yeast nuclear pore complex. *J. Cell Biol.* 153:1465–1478.

Eisen, M.B., P.T. Spellman, P.O. Brown, and D. Botstein. 1998. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA.* 95: 14863–14868.

Eng, J.K., A.L. McCormack, and J.R. Yates III. 1994. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J. Am. Soc. Mass Spectrom.* 5:976–989.

Goodlett, D.R., J.E. Bruce, G.A. Anderson, B. Rist, L. Pasa-Tolic, O. Fiehn, R.D. Smith, and R. Aebersold. 2000. Protein identification with a single accurate mass of a cysteine-containing peptide and constrained database searching. *Anal. Chem.* 72:1112–1118.

Gygi, S.P., B. Rist, S.A. Gerber, F. Turecek, M.H. Gelb, and R. Aebersold. 1999. Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat. Biotechnol.* 17:994–999.

Han, D.K., J. Eng, H. Zhou, and R. Aebersold. 2001. Quantitative profiling of differentiation-induced microsomal proteins using isotope-coded affinity tags and mass spectrometry. *Nat. Biotechnol.* 19:946–951.

Hoepfner, D., M. van den Berg, P. Philippsen, H.F. Tabak, and E.H. Hettema. 2001. A role for Vps1p, actin, and the Myo2p motor in peroxisome abundance and inheritance in *Saccharomyces cerevisiae*. *J. Cell Biol.* 155:979–990.

Keller, A., A.I. Nesvizhskii, E. Kolker, and R. Aebersold. 2002. Empirical statistical model to estimate the accuracy of peptide identifications made by MS/MS and database search. *Anal. Chem.* 74:5383–5392.

Lee, R.T. 2000. Use of microarrays to identify targets in cardiovascular disease. *Drug News Perspect.* 13:403–406.

Li, X.J., H. Zhang, J.A. Ranish, and R. Aebersold. 2003. Automated statistical analysis of protein abundance ratios from data generated by stable-isotope dilution and tandem mass spectrometry. *Anal. Chem.* 75:6648–6657.

Nesvizhskii, A.I., A. Keller, E. Kolker, and R. Aebersold. 2003. A statistical model for identifying proteins by tandem mass spectrometry. *Anal. Chem.* 75:4646–4658.

Niedenthal, R.K., L. Riles, M. Johnston, and J.H. Hegemann. 1996. Green fluorescent protein as a marker for gene expression and subcellular localization in budding yeast. *Yeast.* 12:773–786.

Nigam, K., A. McCallum, S. Thrun, and T. Mitchell. 2000. Text classification from labeled and unlabeled documents using EM. *Machine Learning.* 39:103–134.

Nuttley, W.M., A.M. Brade, G.A. Eitzen, M. Veenhuis, J.D. Aitchison, R.K. Szilard, J.R. Glover, and R.A. Rachubinski. 1994. PAY4, a gene required for peroxisome assembly in the yeast Yarrowia lipolytica, encodes a novel member of a family of putative ATPases. *J. Biol. Chem.* 269:556–566.

Pan, W., J. Lin, and C.T. Le. 2002. How many replicates of arrays are required to detect gene expression changes in microarray experiments? A mixture model approach. *Genome Biol.* 3:research0022.

Ranish, J.A., E.C. Yi, D.M. Leslie, S.O. Purvine, D.R. Goodlett, J. Eng, and R. Aebersold. 2003. The study of macromolecular complexes by quantitative proteomics. *Nat. Genet.* 33:349–355.

Smith, J.J., M. Marelli, R.H. Christmas, F.J. Vizeacoumar, D.J. Dilworth, T. Ideker, T. Galitski, K. Dimitrov, R.A. Rachubinski, and J.D. Aitchison. 2002. Transcriptome profiling to identify genes involved in peroxisome assembly and function. *J. Cell Biol.* 158:259–271.

Szilard, R.K., V.I. Titorenko, M. Veenhuis, and R.A. Rachubinski. 1995. Pay32p of the yeast Yarrowia lipolytica is an intraperoxisomal component of the matrix protein translocation machinery. *J. Cell Biol.* 131:1453–1469.

Winzeler, E.A., D.D. Shoemaker, A. Astromoff, H. Liang, K. Anderson, B. Andre, R. Bangham, R. Benito, J.D. Boeke, H. Bussey, et al. 1999. Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science.* 285:901–906.

Yamochi, W., K. Tanaka, H. Nonaka, A. Maeda, T. Musha, and Y. Takai. 1994. Growth site localization of Rho1 small GTP-binding protein and its involvement in bud formation in *Saccharomyces cerevisiae*. *J. Cell Biol.* 125:1077–1093.

Yi, E.C., X.J. Li, K. Vaughn, H. Lee, B. Raught, A. Page, P. Hieter, D.R. Goodlett, and R. Aebersold. 2004. Increased quantitative proteome coverage with a 13C/12C-based, acid cleavable ICAT reagent and modified data acquisition scheme. *Proteomics.*

Yi, E.C., M. Marelli, H. Lee, S.O. Purvine, R. Aebersold, J.D. Aitchison, and D.R. Goodlett. 2002. Approaching complete peroxisome characterization by gas-phase fractionation. *Electrophoresis.* 23:3205–3216.