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Profiling of Mitochondrial Associated Proteins From Rat Colon

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Abstract Mitochondrial dysfunction, damage and mutations of mitochondrial proteins give rise to a range of ill understood patterns of disease. Although there is significant general knowledge of the proteins and the functional processes of the mitochondria, there is little knowledge of difference about how mitochondria respond and how they are regulated in different organs and tissues. Proteomic profiling of mitochondria and associated proteins involved in mitochondrial regulation and trafficking within cells and tissues has the potential to provide insights into mitochondrial dysfunction associated with many human diseases. The rat colon mitoproteome analysis presented here provides a useful tool to assist in identification and interpretation of mitochondrial dysfunction implicated in colon pathogenesis. 2DPAGE followed by LC/MS/MS was used to identify 430 proteins from mitochondrial enriched fractions prepared from rat colon, resulting in 195 different proteins or approximately 50% of the resolved proteins being identified as multiple protein expression forms. Proteins associated with the colon mitoproteome were involved in calcium binding, cell cycle, energy metabolism and electron transport chain, protein folding, protein synthesis and degradation, redox regulation, structural proteins, signalling and transporter and channel proteins. The mitochondrial associated proteins identified in this study of colon tissue complement and are compared with other recently published mitoproteome analyses from other organ tissues, and will assist in revealing potentially organ specific roles of the mitochondria and organ specific disease associated with mitochondrial dysfunction. J. Cell. Biochem. 103: 78–97, 2008.

Key words: organelle proteomics; electron transport chain; mitochondrial dysfunction; flow cytometry; transmission electron microscopy

Mitochondria are intracellular double membrane-bound structures that regulate energy metabolism, cell division and cell death [Scheffler, 1999, 2001]. They utilize oxygen and produce ATP through carbohydrate and fatty acid metabolism, modulate ionic homeostasis and participate in numerous other catabolic and anabolic pathways. They play a central role in the cascade of events that lead to apoptosis [Mignotte and Vayssiere, 1998; van Loo et al., 2002]. Consequently mitochondrial dysfunction, damage and mutations of mitochondrial proteins gives rise to a range of ill understood patterns of disease including cancer, type 2 diabetes, cardiovascular disease, Alzheimer's and Parkinson's disease [Brandon et al., 2006; Schapira, 2006].

Proteomic techniques have been commonly used to investigate cellular and tissue extracts limiting analysis to only the most abundant proteins, at the expense of subproteomes and less abundant proteins. The potential importance of less abundant proteins in disease processes thus requires a targeted approach to overcome such limitations. The application of

Abbreviations used: 2DPAGE, two-dimensional polyacrylamide gel electrophoresis; MPEFs, multiple protein expression forms; LC/MS/MS, liquid chromatography tandem mass spectrometry; MALDI-TOF MS, matrix-assisted laser desorption/ionisation time of flight mass spectrometry.

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organelle proteomics is thus a potentially powerful method to discover proteins involved in specific cellular functions or disease processes and reduce sample complexity. Although there is significant general knowledge of the proteins and the functional processes of the mitochondria, there is little knowledge of differences in how the mitochondria respond and how they are regulated in different organs and tissues. Different mammalian organ tissues have distinct energy needs and the number of mitochondria per cell, structure and function vary widely independently of the tissues' respiratory needs [Fawcett, 1981; Scheffler, 1999; Mannella, 2006]. Much of our understanding of the eukaryotic mitochondrion and its proteome has been carried out on mitochondria extracted from heart [Taylor et al., 2003; Gaucher et al., 2004; Kiri et al., 2005; Forner et al., 2006; Hunzinger et al., 2006; Kim et al., 2006; Reifschneider et al., 2006], brain [Reifschneider et al., 2006], kidney [Forner et al., 2006; Reifschneider et al., 2006], liver [Forner et al., 2006; Miller et al., 2006; Reifschneider et al., 2006], skeletal muscle [Forner et al., 2006; Reifschneider et al., 2006] and neural chondrocytes [Ruiz-Romero et al., 2006].

Recent investigations into inflammatory bowel disease have found changes to colon and ileal epithelial mitochondrial ultra structure [Soderholm et al., 2002; Nazli et al., 2004; Farhadi et al., 2005]. Studies investigating colon pathologies using proteomics approaches have revealed changes to mitochondrial proteins in response to inflammation and disease [Cole et al., 2002; Drew et al., 2005a, 2006a; Mazzanti and Giulivi, 2006; Mazzanti et al., 2006]. Many in vitro and in vivo studies implicate mitochondria in colon cancer progression [Rana et al., 1980; Sun et al., 1981; Pleshkwych et al., 1983; Oseroff, 1986; Modica-Napolitano et al., 1989; Mancini et al., 1997; Tutton and Barkla, 1997; Heerdt et al., 1998; Li et al., 1999; Cuezva et al., 2002; Ruemmele et al., 2003; Isidoro et al., 2004; Lakshman et al., 2004; Wang and MacNaughton, 2005]. Despite these numerous studies implicating mitochondria in colon pathology, none have focused on characterising the colon mitochondrial proteome. Since rat models are commonly used to study colon pathologies [Corpet and Parnaud, 1999; Drew et al., 2005a,b, 2006a; Mazzon et al., 2005; Yuki et al., 2006] this study was initiated to profile mitochondrial enriched fractions extracted from rat colon.

METHODS

Preparation of Mitochondrial Enriched Fractions

Sprague Dawley male rats weighing between 515 and 580 g were fed ad libitum on Chow for Rat and Mouse, CRM (Special Diet Services Ltd., Witham, Essex, UK) prior to sacrifice with carbon dioxide and cervical dislocation. Colons were excised and flushed with ice-cold 250 mM sucrose/10mM Tris (pH 7.4) buffer. A 2 cm segment was dissected from the mid-point of the distal two thirds of the colon for mitochondrial extraction as described below.

Mitochondrial enriched extracts were prepared using a mitochondrial isolation kit (MITO-ISO1, Sigma, UK) according to the manufacturers instructions. Briefly, tissues were minced and disrupted using an ultra-turrax T25 (IKA) at 17,500 rpm, in buffer A (10 mM HEPES, pH 7.5, containing 200 mM mannitol, 70 mM sucrose, and 1 mM EGTA) supplied with the kit. Large cellular debris and nuclei were pelleted by centrifuging for 5 min at 600*g*, at 4°C. Mitochondria were pelleted by centrifuging the supernatant for 10 min at 11,000*g*. The pellet was resuspended in 500 μl of extraction buffer A and the centrifugation steps at 600 and 11,000*g* were repeated. The resulting mitochondrial enriched fraction was then resuspended in storage buffer (10 mM HEPES, pH 7.4, containing 250 mM sucrose, 1 mM ATP, 0.08 mM ADP, 5 mM sodium succinate, 2 mM K_2HPO_4, and 1 mM DTT). Aliquots were extracted for protein estimation by BioRad Bradford Protein Assay, 2D page analysis and flow cytometric analysis. Alternatively, pellets were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) for transmission electron microscopy (TEM). Aliquots for flow cytometry were used immediately to assess inner membrane integrity by JC-1 staining assay and aliquots for 2D page analysis were snap frozen and stored at −80°C until required.

Flow Cytometric Analysis of Mitochondrial Enriched Fractions

The enriched mitochondrial extracts were tested for inner membrane integrity using the JC-1 stain supplied with MITO-ISO1 kit according to the manufacturers instructions

(Sigma, UK). Briefly, 20 μg of protein was used per 1 ml of JC-1 assay buffer (20 mM MOPS, pH 7.5, containing 110 mM KCl, 10 mM ATP, 10 mM $MgCl_2$, 10 mM sodium succinate, and 1 mM EGTA) containing 1 μl of JC-1 stain in DMSO. Samples were analysed using a FACS Calibur Flowcytometer G4 (Becton Dickinson, NJ) at flow rate of 35 μl/min, measuring 10,000 events using FL-1 voltage 650 and FL-2 voltage 557, both in logarithmic mode. The green FL-1 and orange FL-2 filters were used to detect JC-1 aggregates. One micromole of carbonyl cyanide 3-chlorophenylhydrazone (CCCP) in DMSO, a powerful and selective mitochondrial uncoupling agent that destroys the inner membrane potential was added for comparison. Data was analysed using CELL-Quest software version 3.3 (Becton-Dickinson). During data analysis intact mitochondria were identified with a decrease in FL-2 and an increase in FL-1 signal.

Transmission Electron Microscopic Analysis of Mitochondrial Enriched Fractions

Mitochondrial enriched fractions were initially fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4. The fractions were then washed in the cacodylate buffer, post-fixed for 1 h at room temperature in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer pH 7.4, washed again in the buffer and embedded in 1% agar. The agar-embedded pellet was dehydrated in a 50%, 70%, 90% and 100% ethanol series, cleared in propylene oxide and embedded in araldite resin CY212 (Agar Scientific, Stansted, UK). Ultra thin sections were cut using a Reichert microtome (Leica, UK), stained with uranyl acetate and lead citrate and examined in a JEOL 1200 EXB electron microscope (JEOL, UK) operating at 80 kV.

2D PAGE of Mitochondrial Enriched Fractions

Mitochondrial enriched protein extracts (290 μg) were loaded onto BioRad IPG strips (17 cm, pH 3–10) in 340 μl of 7 M urea, 2 M thiourea, 4% Chaps, 2% biolyte (BioRad) and 3% DTT buffer to separate the proteins in the first dimension. A second dimension SDS-PAGE step was run on an 18 cm × 18 cm linear SDS polyacrylamide gradient as described previously [Drew et al., 2005a]. The gels were then stained with colloidal Coomassie Blue staining as described by Anderson [1991]. Gels ($n = 4$) were then rinsed in deionised water and brushed to remove particulate Coomassie Blue and imaged on a BioRad GS710 flat bed imager followed by image analysis using BioRad PD Quest Version 7.1.1. The gel with highest spot number and quality was selected as the match set standard. A total of 430 spots were matched on all four gels and were cut out for trypsin digestion and identification by LC/MS/MS.

Protein Identification by Nano-LC/MS/MS

Spots cut from 2D PAGE gels were analysed using a nano-LC system (LC Packings, Camberly, Surrey, UK) consisting of an 'Ultimate' nano-LC system, pumping at 0.187 ml/min with a 625 splitter giving a column flow rate of 0.3 μl/min, a 'Famos' autosampler set to an injection volume of 5 μl and a 'Switchos' microcolumn switching device. The nanocolumn was a C18 PepMap 100, 15 cm × 75 μm i.d., 3 μm, 100 Å (LC Packings). HPLC grade solvents were used, 2% acetonitrile and 0.1% formic acid (A) and 80% acetonitrile and 0.08% formic acid (B). The gradient started at 5% B, going to 50% B over 30 min, then ramping to 80% B over a further 2 min, and holding for 10 min. The system was equilibrated at 95% A for 9 min prior to injection of subsequent samples. The solvent used by the 'Switchos' is 0.1% formic acid. The switching device was switched on after 3 min and off after 58 min. The flow rate of the Switchos was 0.03 ml/min. Mass spectrometry was then performed using a Q-Trap (Applied Biosystems/MDS Sciex, Warrington, UK) triple quadrupole fitted with a nanospray ion source using parameters as described previously [Drew et al., 2005a]. Proteins were identified from the rat database, using Mascot, with individual ion scores >28 indicating identity or extensive homology ($P < 0.05$). The mouse database was searched where no significant match was made using the rat database. Identified proteins were then researched using SOURCE at BioInformatic Harvester (http://harvester.embl.de/), and PSORT II (http://psort.ims.u-tokyo.ac.jp/form2.html) databases to identify their origin. Index of hydophobicity was calculated using the protein grand average of hydropathy index [Kyte and Doolittle, 1982] (http://bioinformatics.org/sms2/protein_gravy.html). Proteins ID's were compared with published mitoproteomes of Reifschneider et al. [2006], Forner et al. [2006] and Taylor et al. [2003]. Protein ID's were BLAST searched to identify homologous proteins.

RESULTS

Flow Cytometric Analysis of Mitochondrial Enriched Fractions

Mitochondria enriched protein extracts were assessed for inner membrane integrity using JC-1 assay. Extracts were found to have intact inner membrane integrity (Fig. 1A) with a loss of FL-2 upon CCCP challenge (Fig. 1C), but not with DMSO (Fig. 1B). Cytosolic fractions (Fig. 1D) did not change in fluorescence with either DMSO (Fig. 1E) or CCCP (Fig. 1F) addition, indicating the absence of mitochondria.

Transmission Electron Microscopic Analysis of Mitochondrial Enriched Fractions

Transmission electron microscopy (TEM) was used to further confirm the presence of intact mitochondria in extract (Fig. 2), as characterised by a double membrane and cristae. Rough endoplasmic reticulum, as characterised by ribosome-rich membranes was also identified as a component of the mitochondrial enriched fractions indicative of the intimate relationship of mitochondria with these intracellular structures.

Proteomic Analysis of Mitochondrial Enriched Fractions

Four hundred and thirty spots (Mr 9.75–151.03, pI 4.03–9.6) were matched on all four gels (Fig. 3). A total of 430 proteins were identified by LC/MS/MS, yielding 195 different proteins categorised into 11 functional groups (Table I and Fig. 4). Proteins associated with the colon mitochondria were distributed over a pI range of 4.03–9.6 and a mass range between 12.35 and 151.03. Fifty-eight percent of the resolved and identified proteins were recognised as mitochondrial using SOURCE database in the BioInformatic Harvester search engine which uses updated gene and protein databases to attribute information on cellular localisation and function. This identified 101 different mitochondrial associated proteins (pI 4.22–9.6, Mr 13.58–126.76). Proteins not recognised as mitochondrial by BioInformatic Harvester were analysed by PSORT II, which predicts the sub-cellular localisation sites of

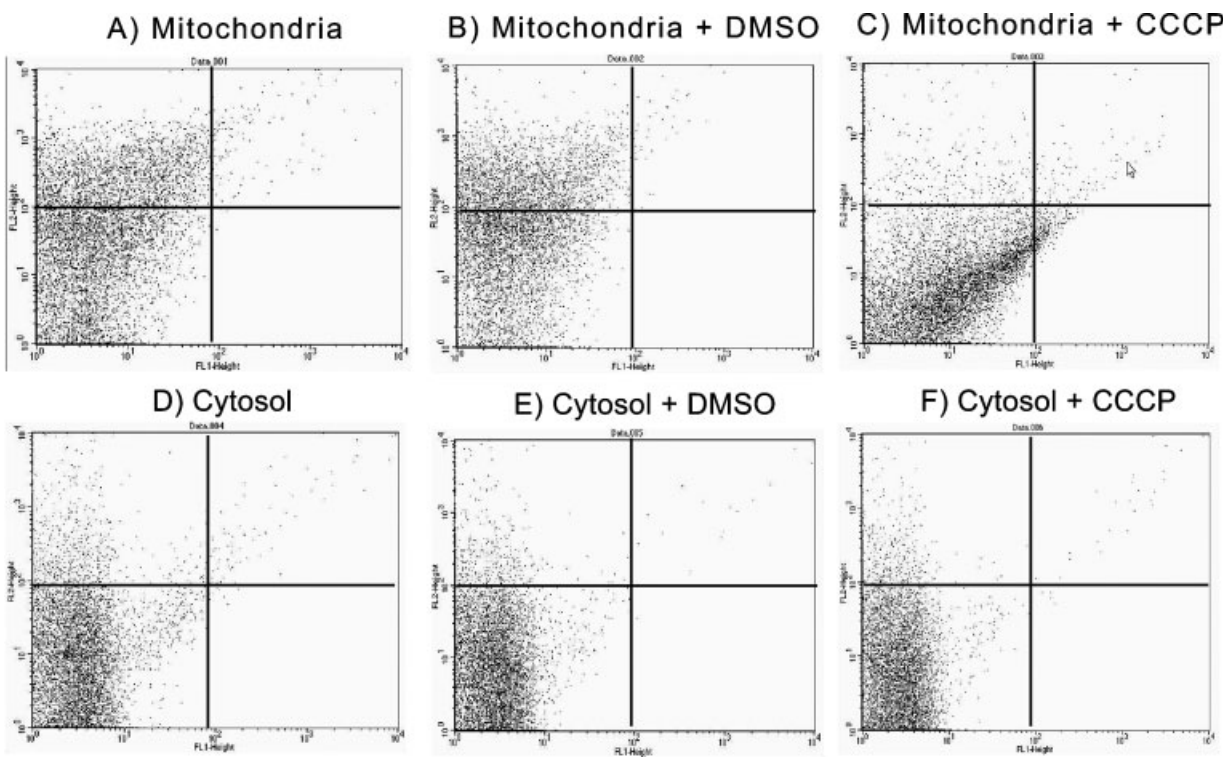

Fig. 1. Flow cytometric analysis of mitochondrial enriched fractions. **A,C**: Loss of FL-2 height and increased FL-1 height indicates loss of mitochondrial inner membrane integrity with CCCP (in DMSO) challenge, but not with DMSO (**B**). Cytosolic fractions do not change with either DMSO (**E**) or CCCP (**F**) addition, indicating the absence of mitochondria in the cytosolic fraction (**D**).

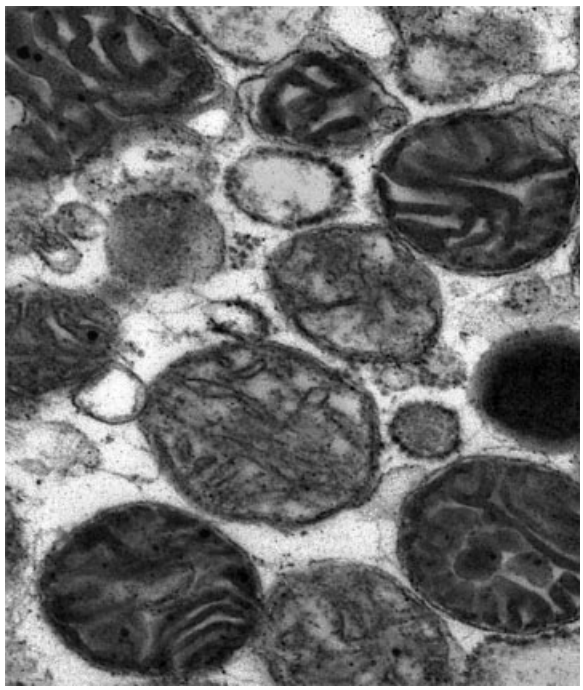

Fig. 2. Transmission electron microscopic image of mitochondrial enriched fractions. Mitochondria characterised by a double membrane and cristae.

proteins from recognition of mitochondrial targeting signals in the amino acid sequences [Nakai and Horton, 1999] by employing the discriminate analysis "MITDISC". Percentages depict the predicted percentage probability a protein is mitochondrial using the k-nearest neighbour (k-NN) algorithm [Horton and Nakai, 1997]. The probability assigned by PSORT II that a protein was localised to mitochondria is cited in Table I. Comparison of the proteins associated with the colon mitochondria with published mitoproteomes from other rat organs, kidney, liver, heart, brain and skeletal muscle [Forner et al., 2006; Reifschneider et al., 2006], revealed both common and unique proteins (Fig. 5). Ten proteins (5%) were common to other published rat mitoproteomes only (Fig. 5), 54 proteins (28%) were common with published human heart mitoproteome only [Taylor et al., 2003; Gaucher et al., 2004] and 62 proteins (32%) were common to both published rat organ and human heart mitoproteomes [Taylor et al., 2003; Gaucher et al., 2004; Forner et al., 2006; Reifschneider et al., 2006] resulting in 69 proteins novel to the colon mitoproteome (Fig. 5). Proteins common to previously profiled rat and human heart mitoproteomes were largely proteins involved in energy metabolism and oxidative phosphorylation, 61% and 50%, respectively. Novel mitochondrial associated proteins of the colon were mainly structural proteins such as cytokeratin 8 and those involved in protein synthesis and degradation, for example GM2 activator protein. These proteins although not classically associated with the mitochondria, still received high PSORT II predicted percentages, for example cytokeratin 8 at 78.3% (Table I). This protein was found to have 12 MPEFs in the colon mitoproteome and has been previously associated with the normal functioning of the colon [Toivola et al., 2004). Twenty-three proteins (mass 9.75–131.03 kDa and pI 3.7–9.34) were not identified by LC/MS/MS (Table I). Profile of the GRAVY values (Fig. 6) indicated the presence of many membrane proteins (high GRAVY index) but most proteins were not associated with membranes.

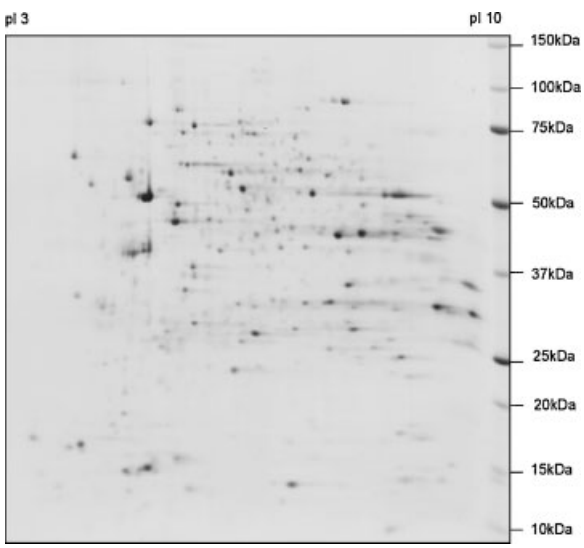

Fig. 3. Proteomic analysis of mitochondrial enriched fractions. 2D PAGE Coomassie Blue stained gel of colon mitochondrial enriched fractions generated over 430 spots common to four biological replicates (pI 4.03–9.6, Mr 9.75–151.03). Spots were trypsin digested and identified using LC/MS/MS.

DISCUSSION

There is already considerable information known about the important role of mitochondria in regulation of apoptotic responses, energy metabolism and electron transfer. However, advances in application of proteomic analysis is now revealing further insights into the mitoproteome yielding significant tissue and

TABLE I. Four Hundred and Thirty Proteins Identified From Two-Dimensional Gel Electrophoresis of Mitochondrial Enriched Protein Fractions Using LC/MS/MS

Protein name	Accession number	Mr	pI	Function	Organelle	GRAVY	PSORT II (%)
Annexin A2 = Lipocortin II = Calpactin I heavy chain	*ANX2_RAT*	*35.7*	*7.97*	*CB*		*−0.533*	*21.7*
Annexin A2 = Lipocortin II = Calpactin I heavy chain	*ANX2_RAT*	*36.02*	*7.34*	*CB*		*−0.533*	*21.7*
Annexin A2 = Lipocortin II = Calpactin I heavy chain	*ANX2_RAT*	*36.26*	*8.29*	*CB*		*−0.533*	*21.7*
Annexin A4 = Lipocortin IV	*ANX4_RAT*	*35.13*	*5.66*	*CB*		*−0.430*	*8.7*
Annexin v mutant = Lipocortin V	*LURT5*	*34.14*	*5.18*	*CB*		*−0.329*	*4.3*
Calcium binding protein	I55472	67.2	5.26	CB	Golgi	−1.022	22.2
Calcium binding protein	I55472	68.04	5.18	CB	Golgi	−1.022	22.2
Calpactin I light chain	S100_RAT	12.35	6.91	CB		−0.319	26.1
Calpactin I light chain	S100_RAT	13.86	4.92	CB		−0.319	26.1
Adenylate kinase 2 = ATP-AMP transphosphorylase	*AAH61727*	*30.17*	*7.92*	*Cell Cycle*	*M*	*−0.385*	
Histone H2A.1	*H2A1_RAT*	*15.69*	*7.9*	*Cell Cycle*		*−0.472*	*21.7*
Histone H2A.1	*H2A1_RAT*	*15.94*	*7.76*	*Cell Cycle*		*−0.472*	*21.7*
Histone H4	*S03427*	*15.57*	*5.32*	*Cell Cycle*		*−0.545*	*8.7*
Prohibitin	***A39682***	***30.53***	***6.37***	***Cell Cycle***	***M***	***0.009***	
Prohibitin	***A39682***	***30.59***	***6.97***	***Cell Cycle***	***M***	***0.009***	
Prohibitin	***A39682***	***30.69***	***6.28***	***Cell Cycle***	***M***	***0.009***	
Prohibitin	***A39682***	***30.86***	***5.96***	***Cell Cycle***	***M***	***0.009***	
Prohibitin	***A39682***	***31.19***	***6.17***	***Cell Cycle***	***M***	***0.009***	
Prohibitin	***A39682***	***31.4***	***5.76***	***Cell Cycle***	***M***	***0.009***	
Similar to SEPTIN6 type II	Q8C1B7	52.34	7.14	Cell Cycle		−0.703	8.7
2-Enoyl-coa hydratase chain A/B	***S06447***	***29.47***	***7.01***	***EM***	***M***	***−0.147***	
2-Enoyl-coa hydratase chain A/B	***S06447***	***29.63***	***7.16***	***EM***	***M***	***−0.147***	
2-Enoyl-coa hydratase chain A/B	***S06447***	***29.96***	***6.65***	***EM***	***M***	***−0.147***	
3-Hydroxybutyrate dehydrogenase precursor	*A42345*	*31.37*	*9.22*	*EM*	*M*	*−0.242*	
3-Hydroxyisobutyrate dehydrogenase, mitochondrial precursor	*A32867*	*32.78*	*6.56*	*EM*	*M*	*0.031*	
3-Oxoacid CoA transferase 1	*Q9CV92*	*60.05*	*7.3*	*EM*	*M*	*−0.106*	
4-Trimethylaminobutyraldehyde dehydrogenase	Q9JLJ3	53.95	6.88	EM		−0.065	8.7
Acetyl-CoA C-acetyltransferase precursor, mitochondrial	***XXRTAC***	***42.86***	***9.11***	***EM***	***M***	***0.086***	
Acetyl-CoA C-acetyltransferase precursor, mitochondrial	***XXRTAC***	***43.07***	***8.99***	***EM***	***M***	***0.086***	
Acetyl-CoA C-acetyltransferase precursor, mitochondrial	***XXRTAC***	***43.36***	***8.68***	***EM***	***M***	***0.086***	
Acetyl-CoA C-acyltransferase mitochondrial	***XXRTAC***	***44.22***	***9.18***	***EM***	***M***	***0.086***	
Acetyl-CoA C-acyltransferase mitochondrial	***XXRTAC***	***44.67***	***9.1***	***EM***	***M***	***0.086***	
Aconitase 2 = Mitochondrial aconitase precursor	***Q9ER34***	***88.97***	***7.15***	***EM***	***M***	***−0.355***	
Aconitase 2 = Mitochondrial aconitase precursor	***Q9ER34***	***93.6***	***7.58***	***EM***	***M***	***−0.355***	
Aconitase 2 = *Mitochondrial aconitase precursor*	***Q9ER34***	***93.9***	***7.75***	***EM***	***M***	***−0.355***	
Aconitase 2 = Mitochondrial aconitase precursor	***Q9ER34***	***88.93***	***7.25***	***EM***	***M***	***−0.355***	
Aconitase hydrase = aconitase 2	***AAH61999***	***88.57***	***7.38***	***EM***		***−0.154***	***30.4***
Aconitase hydrase = aconitase 2	***AAH61999***	***92.7***	***7.9***	***EM***		***−0.154***	***30.4***
Aconitase hydrase = aconitase 2	***AAH61999***	***92.86***	***7.95***	***EM***		***−0.154***	***30.4***
Aconitase hydrase = aconitase 2	***AAH61999***	***94.02***	***7.8***	***EM***		***−0.154***	***30.4***
Acyl-CoA dehydrogenase precursor, medium-chain-specific, mitochondrial	**DERTCM**	**43.74**	**7.12**	**EM**	**M**	**−0.297**	
Acyl-CoA dehydrogenase precursor, medium-chain-specific, mitochondrial	**DERTCM**	**44.13**	**7.97**	**EM**	**M**	**−0.297**	
Acyl-CoA dehydrogenase very-long-chain-specific precursor	***A54872***	***69.12***	***8.28***	***EM***	***M***	***−0.111***	
Acyl-CoA dehydrogenase, short-chain specific, mitochondrial precursor	***B34252***	***42.06***	***7.79***	***EM***	***M***	***−0.146***	
Acyl-CoA dehydrogenase, short-chain specific, mitochondrial precursor	***B34252***	***42.25***	***7.15***	***EM***	***M***	***−0.146***	
Acyl-CoA dehydrogenase, short-chain specific, mitochondrial precursor	***B34252***	***42.34***	***7.34***	***EM***	***M***	***−0.146***	
Acyl-CoA dehydrogenase, short-chain specific, mitochondrial precursor	***B34252***	***42.39***	***6.98***	***EM***	***M***	***−0.146***	
Acyl-CoA dehydrogenase, short-chain specific, mitochondrial precursor	***B34252***	***42.65***	***6.7***	***EM***	***M***	***−0.146***	
Acyl-CoA synthetase short-chain family member 1	BAC40232	84.9	6.58	EM	M	−0.120	

(*Continued*)

TABLE I. (*Continued*)

Protein name	Accession number	Mr	pI	Function	Organelle	GRAVY	PSORT II (%)
Aldehyde dehydrogenase 2	**Q91Zd7**	***55.73***	***6.84***	***EM***	***M***	***−0.111***	
Aldehyde dehydrogenase 2	***Q91Zd7***	***56.78***	***6.4***	***EM***	***M***	***−0.111***	
Aldehyde dehydrogenase 2	***Q91Zd7***	***56.87***	***6.23***	***EM***	***M***	***−0.111***	
Aldehyde dehydrogenase 2 (Mitochondrial)	***Q8K3V8***	***57.61***	***6.1***	***EM***	***M***	***−0.155***	
Aldehyde dehydrogenase 2, Mitochondrial (Fragment)	***Q6Q289_RAT***	***59.45***	***6.12***	***EM***	***M***	***−0.160***	
Alpha enolase	ENOA_RAT	53.74	6.59	EM		−0.198	13.0
Alpha glucosidase 2	Q8BHN3	117.82	6.08	EM	ER	−0.325	33.3
Carnitine *O*-palmitoyltransferase II precursor, mitochondrial	***A35447***	***55.97***	***5.04***	***EM***	***M***	***−0.295***	
Carnitine O-palmitoyltransferase II precursor, mitochondrial	***A35447***	***73.79***	***7.19***	***EM***	***M***	***−0.295***	
Coenzyme Q5 homolog, methyltransferase	Q9D6Y6	30.3	8.35	EM		−0.382	69.6
Creatine kinase precursor, mitochondrial	***Q5BJT9_RAT***	***45.66***	***7.58***	***EM***	***M***	***−0.411***	
Creatine kinase precursor, mitochondrial	***S17189***	***44.08***	***7.79***	***EM***	***M***	***−0.422***	
Creatine kinase precursor, mitochondrial	***S17189***	***44.51***	***8.09***	***EM***	***M***	***−0.422***	
Creatine kinase precursor, mitochondrial	***S17189***	***44.55***	***7.55***	***EM***	***M***	***−0.422***	
Creatine kinase precursor, mitochondrial	***S17189***	***46.3***	***8.07***	***EM***	***M***	***−0.422***	
Dienoyl-coa isomerase probable peroxisomal enoyl-coa hydratase	*1DCIA*	*33.85*	*6.84*	*EM*	*M*	*−0.059*	
D-Lactate dehydrogenase	Q7TNG8	51.67	6.83	EM	M	−0.127	
Electron transfer flavoprotein alpha chain precursor	*A31568*	*33.13*	*7.61*	*EM*	*M*	*0.120*	
Electron transferring flavoprotein, beta polypeptide	*Q810V3*	*29.36*	*8.03*	*EM*	*M*	*−0.091*	
Electron-transferring-flavoprotein dehydrogenase	*AAQ67364*	*68.86*	*7.17*	*EM*	*M*	*−0.311*	
Electron-transferring-flavoprotein dehydrogenase	*AAQ67364*	*69.11*	*7.06*	*EM*	*M*	*−0.311*	
Flavoprotein subunit of succinate-ubiquinone reductase	***Q920L2***	***76.24***	***6.42***	***EM***	***M***	***−0.254***	
Flavoprotein subunit of succinate-ubiquinone reductase	***Q920L2***	***76.33***	***6.59***	***EM***	***M***	***−0.254***	
Flavoprotein subunit of succinate-ubiquinone reductase	***Q920L2***	***76.36***	***6.51***	***EM***	***M***	***−0.254***	
Fructose-bisphosphate aldolase A	***ADRTA***	***41.33***	***8.42***	**EM**	***M***	***−0.279***	
Fructose-bisphosphate aldolase A	***ADRTA***	***39.24***	***9.17***	***EM***	***M***	***−0.279***	
Fructose-bisphosphate aldolase A	***ADRTA***	***39.36***	***8.9***	***EM***	***M***	***−0.279***	
Fructose-bisphosphate aldolase A	***ADRTA***	***40.37***	***8.91***	***EM***	***M***	***−0.279***	
Fumarate hydratase precursor, mitochondrial	***UFRT***	***46.36***	***8.94***	***EM***	***M***	***−0.090***	
Fumarate hydratase precursor, mitochondrial	***UFRT***	***46.8***	***8.55***	***EM***	***M***	***−0.090***	
Fumarate hydratase precursor, mitochondrial	UFRT	***47.34***	***8.16***	***EM***	***M***	***−0.090***	
Glutamate dehydrogenase [NAD(P)] precursor	***S03707***	***48.76***	***8.05***	***EM***	***M***	***−0.306***	
Glutamate dehydrogenase [NAD(P)] precursor	***S03707***	***54.33***	***7.44***	***EM***	***M***	***−0.306***	
Glutamate dehydrogenase [NAD(P)] precursor	***S03707***	***54.64***	***7.23***	***EM***	***M***	***−0.306***	
Glutamate dehydrogenase [NAD(P)] precursor	***S03707***	***54.64***	***7.71***	***EM***	***M***	***−0.306***	
Glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)	***DERTG***	***36.17***	***8.76***	***EM***		***−0.084***	***8.7***
Glycerol-3-phosphate dehydrogenase mitochondrial precursor	A54051	78.36	6.42	EM	M	−0.195	
Hydroxymethylglutaryl-CoA synthase precursor	***A35865***	***25.9***	***4.63***	***EM***	***M***	***−0.360***	
Hydroxymethylglutaryl-CoA synthase precursor	***A35865***	***47.62***	***8.7***	***EM***	***M***	***−0.360***	
Hydroxymethylglutaryl-CoA synthase precursor	***A35865***	***47.63***	***8.78***	***EM***	***M***	***−0.360***	
Hydroxymethylglutaryl-CoA synthase precursor	***A35865***	***47.95***	***8.5***	***EM***	***M***	***−0.360***	
Hydroxymethylglutaryl-CoA synthase precursor	***A35865***	***48.07***	***8.4***	***EM***	***M***	***−0.360***	
Isocitrate dehydrogenase [NAD] subunit alpha mitochondrial precursor = isocitrate dehydrogenase 3 alpha	***Q99NA5***	***42.74***	***6.13***	***EM***	***M***	***−0.397***	
Isocitrate dehydrogenase [NAD] subunit alpha mitochondrial precursor = isocitrate dehydrogenase 3 alpha	***Q99NA5***	***41.92***	***6.71***	***EM***	***M***	***−0.397***	
Isocitrate dehydrogenase [NAD] subunit alpha mitochondrial precursor = isocitrate dehydrogenase 3 alpha	***Q99NA5***	***42.65***	***6.7***	***EM***	***M***	***−0.397***	
Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial precursor = isocitrate dehydrogenase 3 alpha	Q99NA5	***43.69***	***5.98***	***EM***	***M***	***−0.073***	

Isocitrate dehydrogenase 2	***Q8C2R9***	***44.9***	***8.88***	***EM***	***M***	***−0.398***	
Isocitrate dehydrogenase 2	***Q8C2R9***	***45.22***	***8.63***	***EM***	***M***	***−0.398***	
isocitrate dehydrogenase 2 (NADP + specific)	Q9EQK1	***26.14***	***7.85***	***EM***	***M***	***−0.400***	
Isocitrate dehydrogenase 3 (NAD+) beta Tumor-related protein	*Q91VA7*	*42.92*	*8.51*	*EM*	*M*	*−0.132*	
Isovaleryl-CoA dehydrogenase precursor	*C34252*	*44.91*	*6.6*	*EM*	*M*	*−0.113*	
Isovaleryl-CoA dehydrogenase precursor	*C34252*	*45.45*	*6.4*	*EM*	*M*	*−0.113*	
L-Lactate dehydrogenase chain A	**A23083**	**34.37**	**8.96**	**EM**		**0.064**	**22.2**
L-Lactate dehydrogenase chain A	**A23083**	**34.43**	**8.87**	**EM**		**0.064**	**22.2**
Long-chain-acyl-CoA dehydrogenase precursor	**A34252**	**44.59**	**6.96**	**EM**	**M**	**−0.223**	
Long-chain-acyl-CoA dehydrogenase precursor	**A34252**	**44.89**	**7.28**	**EM**	**M**	**−0.223**	
Long-chain-acyl-CoA dehydrogenase precursor	**A34252**	**45.51**	**6.92**	**EM**	**M**	**−0.223**	
Long-chain-acyl-CoA dehydrogenase precursor	**A34252**	**46.1**	**6.78**	**EM**	**M**	**−0.223**	
Long-chain-acyl-CoA dehydrogenase precursor	**A34252**	**46.15**	**6.93**	**EM**	**M**	**−0.223**	
Malate dehydrogenase 2	***AAH63165***	***35.07***	***9.54***	***EM***	***M***	***0.119***	
Malate dehydrogenase 2 (precursor, mitochondrial)	***DERTMM***	***36.07***	***9.17***	***EM***	***M***	***0.121***	
Methylmalonate-semialdehyde dehydrogenase, family 6	A44097	58	8.13	EM	M	−0.048	
Methylmalonate-semialdehyde dehydrogenase, family 6	A44097	59.58	7.76	EM	M	−0.048	
Methylmalonyl CoA epimerase	*Q9D1I5*	*16.15*	*7.41*	*EM*	*M*	*0.055*	
Mitochondrial acyl-CoA thioesterase 1	*CAA70513*	*46.21*	*7.12*	*EM*	*M*	*−0.172*	
Mitochondrial acyl-CoA thioesterase 1	*CAA70513*	*47.2*	*7.12*	*EM*	*M*	*−0.172*	
NADH-ubiquinone oxidoreductase 75 kDa subunit	***Q8BM16***	***92.7***	***5.67***	***EM***	***M***	***−0.120***	
NADH-ubiquinone oxidoreductase 75 kDa subunit	***Q8BM16***	***92.73***	***5.62***	***EM***	***M***	***−0.120***	
Nucleoside-diphosphate kinase precursor	***A38369***	***19.14***	***7.37***	***EM***	***M***	***−0.270***	
Ornithine-oxo-acid transaminase precursor	**XNRTO**	**49.98**	**6.65**	**EM**	**M**	**−0.113**	
Ornithine-oxo-acid transaminase precursor	**XNRTO**	**50.29**	**6.48**	**EM**	**M**	**−0.113**	
Oxoglutarate dehydrogenase (lipoamide) = Ogdh protein	***Q91WP2***	***109.33***	***6.68***	***EM***	***M***	***−0.347***	
Oxoglutarate dehydrogenase (lipoamide) = Ogdh protein	***Q91WP2***	***115.7***	***6.75***	***EM***	***M***	***−0.347***	
Oxoglutarate dehydrogenase (lipoamide) = Ogdh protein	***Q91WP2***	***116.33***	***6.72***	***EM***	***M***	***−0.347***	
Oxoglutarate dehydrogenase (lipoamide) = Ogdh protein	***Q91WP2***	***117.34***	***6.61***	***EM***	***M***	***−0.347***	
Pancreatic lipase	AAA79888	50.14	7.02	EM		−0.284	11.1
Propionyl-CoA carboxylase alpha chain precursor	***PCCA_RAT***	***80.1***	***7.11***	***EM***	***M***	***−0.217***	
Propionyl-CoA carboxylase alpha chain precursor	***PCCA_RAT***	***81.85***	***6.53***	***EM***	***M***	***−0.217***	
Propionyl-CoA carboxylase alpha chain precursor	***PCCA_RAT***	***83.2***	***6.39***	***EM***	***M***	***−0.217***	
Propionyl-CoA carboxylase beta chain precursor	***A25516***	***58.24***	***7.02***	***EM***	***M***	***0.022***	
Propionyl-CoA carboxylase beta chain precursor	***A25516***	***58.47***	***6.89***	***EM***	***M***	***0.022***	
Propionyl-CoA carboxylase beta chain precursor	***A25516***	***58.77***	***6.75***	***EM***	***M***	***0.022***	
Pyruvate carboxylase precursor	JC4391	126.76	6.76	EM	M	−0.169	
Pyruvate dehydrogenase (lipoamide) (E1) beta chain	***S15892***	***36.31***	***5.66***	***EM***	***M***	***−0.304***	
Pyruvate dehydrogenase (lipoamide) (E1) beta chain	***S15892***	***36.53***	***5.58***	***EM***	***M***	***−0.304***	
Pyruvate dehydrogenase (lipoamide) alpha chain 1 precursor	***DERTPA***	***45.94***	***7.17***	***EM***	***M***	***−0.304***	
Pyruvate dehydrogenase (lipoamide) alpha chain 1 precursor	***DERTPA***	***46.04***	***7.44***	***EM***	***M***	***−0.304***	
Pyruvate dehydrogenase (lipoamide) alpha chain 1 precursor	***DERTPA***	***46.17***	***7.34***	***EM***	***M***	***−0.304***	
Pyruvate dehydrogenase (lipoamide) alpha chain 1 precursor	***DERTPA***	***46.21***	***7.03***	***EM***	***M***	***−0.304***	
Pyruvate dehydrogenase (lipoamide) alpha chain 1 precursor	***DERTPA***	***33.39***	***7.72***	***EM***	***M***	***−0.304***	
Pyruvate kinase isozyme M2	***A26186***	***63.42***	***8.03***	***EM***	***M***	***−0.096***	
Similar to 3-hydroxyisobutyryl-coenzyme A hydrolase	*Q8QZS1*	*40.22*	*7.63*	*EM*	*M*	*−0.214*	
Similar to hypothetical protein FLJ20920—Blast search: hypothetical protein LOC264895	Q8VCW8	65.08	7.39	EM		−0.154	34.8
Similar to hypothetical protein FLJ20920—Blast search: hypothetical protein LOC264895	Q8VCW8	65.28	7.2	EM		−0.154	34.8
Succinate-coenzyme A ligase	*Q8BGS6*	*48.64*	*6.15*	*EM*	*M*	*−0.023*	
Succinate-semialdehyde dehydrogenase = aldehyde dehydrogenase 5	***I61704***	***52.3***	***6.94***	***EM***	***M***	***−0.019***	
Transketolase	AAA18026	70.9	7.95	EM	Non M	−0.132	
Ubiquinone biosynthesis protein = demethyl-Q 7	T10806	20.45	6.29	EM		−0.183	13.0
Malic enzyme 2, NAD(+)-dependent, mitochondrial	Q99KE1	*64.55*	*7.14*	*EM*	*M*	*−0.138*	
AE017189 – Blast search: similar to capping protein muscle Z-line, a 2	AAR16292	37.9	5.94	Other	Unknown		
Carbonic anhydrase II	CAH2_RAT	17.9	6.43	Other		−0.532	4.3

(*Continued*)

TABLE I. (*Continued*)

Protein name	Accession number	Mr	pI	Function	Organelle	GRAVY	PSORT II (%)
Coiled-coil-helix-coiled-coil-helix domain containing 3	Q9CRB9	23.76	8.42	Other	M	−1.030	
Complement component 1, q subcomponent binding protein	CAA04531	35.33	4.32	Other	M	−0.447	73.9
DNA segment, Chr 10, Johns Hopkins University 81 expressed Blast search: es1 protein	***Q9D172***	***27.72***	***8.42***	Other	***M***	***−0.022***	
GOB-4 protein	O88312	15.54	9.23	Other		−0.394	11.1
IgE-dependent histamine-releasing factor	S00775	26.3	4.78	Other	Unknown	−0.361	
Lactose-binding lectin L-36	A46631	21.29	7.58	Other		−0.250	4.3
Mucosal pentraxin	AAr04681	27.87	5.41	Other	Unknown	−0.090	
Nitrilase family, member 2	Q9JHW2	31.63	7.12	Other	M	−0.224	
Nitrilase family, member 2	Q9JHW2	32.13	7.19	Other	M	−0.224	
Polymerase delta-interacting protein 2	Q91VA6	37.84	7.31	Other		−0.526	95.7
Purine-nucleoside phosphorylase	Q9D8C9	31.82	6.78	Other		−0.121	13.0
Stomatin (Epb7.2)-like 2	***Q9DCG8***	***43.51***	***6.2***	***Other***	***M***	***−0.193***	
Thiosulfate sulfurtransferase	S15081	***36.02***	***8.47***	***Other***	***M***	***−0.447***	
Unknown	Unknown	17.9	3.7	Other	Unknown		
Unknown	Unknown	15.52	3.8	Other	Unknown		
Unknown	Unknown	15.69	4.16	Other	Unknown		
Unknown	Unknown	31.06	4.58	Other	Unknown		
Unknown	Unknown	24.45	5.1	Other	Unknown		
Unknown	Unknown	74.01	5.26	Other	Unknown		
Unknown	Unknown	107.03	5.63	Other	Unknown		
Unknown	Unknown	131.03	5.68	Other	Unknown		
Unknown	Unknown	103.9	5.98	Other	Unknown		
Unknown	Unknown	53.43	6.1	Other	Unknown		
Unknown	Unknown	77.13	6.17	Other	Unknown		
Unknown	Unknown	72.47	6.29	Other	Unknown		
Unknown	Unknown	61.72	7.08	Other	Unknown		
Unknown	Unknown	67.04	7.09	Other	Unknown		
Unknown	Unknown	40.82	7.09	Other	Unknown		
Unknown	Unknown	39.05	7.47	Other	Unknown		
Unknown	Unknown	39.05	7.47	Other	Unknown		
Unknown	Unknown	19.91	7.58	Other	Unknown		
Unknown	Unknown	59.78	7.69	Other	Unknown		
Unknown	Unknown	15.08	7.84	Other	Unknown		
Unknown	Unknown	16.45	7.95	Other	Unknown		
Unknown	Unknown	9.75	8.31	Other	Unknown		
Unknown	Unknown	27.34	9.34	Other	Unknown		
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***14.22***	***5.63***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***26.62***	***4.9***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***29.63***	***5.97***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***31.39***	***5.43***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***32.69***	***4.72***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***54.78***	***5.28***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***57.31***	***5.44***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***59.15***	***5.07***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***63.53***	***5.16***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***54.79***	***5.2***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***55.59***	***5.3***	***OXPHOS***	***M***	***0.034***	
ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***55.61***	***5.13***	***OXPHOS***	***M***	***0.034***	

ATP synthase beta chain mitochondrial precursor	***ATPB_RAT***	***31.28***	***5.59***	***OXPHOS***	***M***	***0.034***	
ATP synthase D chain, mitochondrial F0 complex	*ATPQ_RAT*	*24.29*	*6.58*	*OXPHOS*	*M*	***−0.718***	
ATP synthase D chain, mitochondrial F0 complex	*ATPQ_RAT*	*24.61*	*6.22*	*OXPHOS*	*M*	*−0.718*	
ATP synthase, H+transporting, mitochondrial F0 complex, subunit F6	A44861	12.89	8.38	OXPHOS	M	−0.465	
ATP synthase, H+transporting, mitochondrial F1 complex, δ-subunit	*AAC28872*	*17.75*	*4.35*	OXPHOS	*M*	*0.197*	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***53.12***	***8.61***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***50.36***	***9.15***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***52.99***	***8.76***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***15.38***	***5.04***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***15.6***	***4.87***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***15.6***	***4.87***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit r	***A35730***	***15.67***	***4.16***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***16.73***	***4.91***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***17.37***	***7.65***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***53.6***	***8.42***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***53.6***	***8.42***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***53.68***	***8.13***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***53.81***	***7.91***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***55.73***	***8.12***	***OXPHOS***	***M***	***−0.090***	
ATP synthase, H+transporting, mitochondrial F1 complex, alpha subunit	***A35730***	***61.84***	***7.86***	***OXPHOS***	***M***	***−0.090***	
Cytochrome b5 microsomal splice form	***CBRT5***	***18.62***	***4.77***	***OXPHOS***	***M***	***−0.598***	
Cytochrome C oxidase chain Va precursor	***S04592***	***15.81***	***5.2***	***OXPHOS***	***M***	***−0.142***	
Cytochrome C oxidase subunit Vb	*BAA01743*	*16.55*	*5.54*	*OXPHOS*	*M*	*−0.281*	
Cytochrome c-1	***BAB22380***	***32.32***	***6.94***	***OXPHOS***	***M***	***−0.247***	
DLST dihydrolipoamide succinyltransferase component (E2)	***S21766***	***50.94***	***6.24***	OXPHOS	***M***	***0.034***	
DLST Dihydrolipoamide succinyltransferase component (E2)	***S21766***	***51.89***	***6.23***	***OXPHOS***	***M***	***0.034***	
DLST Dihydrolipoamide succinyltransferase component (E2)	S21766	***70.9***	***6.02***	***OXPHOS***	***M***	***0.034***	
DLST Dihydrolipoamide succinyltransferase component (E2)	***S21766***	***73.69***	***6.02***	**OXPHOS**	***M***	***0.034***	
E3 Dihydrolipoamide dehydrogenase	***AAH62069***	***59.11***	***8.13***	OXPHOS	***M***	***−0.010***	
E3 Dihydrolipoamide dehydrogenase	***AAH62069***	***61.07***	***7.53***	***OXPHOS***	***M***	***−0.010***	
E3 Dihydrolipoamide dehydrogenase	***AAH62069***	***61.49***	***7.25***	***OXPHOS***	***M***	***−0.010***	
E3 Dihydrolipoamide dehydrogenase	***AAH62069***	***61.94***	***6.93***	***OXPHOS***	***M***	***−0.010***	
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 5	*NUFM_RAT*	*13.99*	*7.03*	*OXPHOS*	*M*	*−0.369*	
NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 5	NUFM_RAT	*14.2*	*6.79*	*OXPHOS*	*M*	*−0.369*	
NADH dehydrogenase 1 alpha subcomplex 10-like protein	***Q80WE0***	***41.17***	***6.54***	***OXPHOS***	***M***	***−0.442***	
NADH dehydrogenase 1 alpha subcomplex 10-like protein	***Q80WE0***	***41.76***	***6.54***	***OXPHOS***	***M***	***−0.442***	
NADH dehydrogenase 1 alpha subcomplex 10-like protein	***Q80WE0***	***44.34***	***6.37***	***OXPHOS***	***M***	***−0.442***	
NADH dehydrogenase 1 alpha subcomplex 11	Q80W89	15.25	8.47	OXPHOS	M	0.157	
NADH2 dehydrogenase (ubiquinone) 24K chain precursor	*A31868*	*30.04*	*5.48*	*OXPHOS*	*M*	*−0.316*	
Similar to NADH dehydrogenase (Ubiquinone) Fe-S protein 2	***Q91WD5***	***48.1***	***6.41***	***OXPHOS***	***M***	***−0.283***	
Similar to NADH dehydrogenase Ubiquinone flavoprotein 1 51 kDa	**Q91YT0**	**50.36**	**8.13**	**OXPHOS**	**M**	**−0.253**	
Similar to NADH dehydrogenase Ubiquinone flavoprotein 1 51 kDa	**Q91YT0**	**50.57**	**7.83**	**OXPHOS**	**M**	**−0.253**	
Similar to NADH dehydrogenase Ubiquinone flavoprotein 1 51 kDa	**Q91YT0**	**67.72**	**6.09**	**OXPHOS**	**M**	**−0.253**	
Ubiquinol-cytochrome c reductase core protein 1	***BAB27022***	***53.42***	***5.68***	***OXPHOS***	***M***	***−0.189***	
Ubiquinol-cytochrome-c reductase core protein II precursor	***S29510***	***44.24***	***8.51***	***OXPHOS***	***M***	***−0.067***	
Ubiquinol-cytochrome-c reductase core protein II precursor	***S29510***	***44.58***	***8.36***	***OXPHOS***	***M***	***−0.067***	
Ubiquinol-cytochrome-c reductase Rieske iron-sulfur protein precursor	***A32296***	***26.95***	***7.64***	***OXPHOS***	***M***	***−0.108***	
Calreticulin precursor	JH0819	59.56	4.5	PF	ER	−1.099	
Calreticulin precursor	JH0819	69.01	4.3	PF	ER	−1.099	
Calreticulin precursor	JH0819	71.24	4.31	PF	ER	−1.099	
Chaperonin groEL precursor=heat shock protein 60	***HHRT60***	***65.71***	***5.96***	***PF***	***M***	***−0.085***	
Chaperonin groEL precursor=heat shock protein 60	***HHRT60***	***66.76***	***5.65***	***PF***	***M***	***−0.085***	
Chaperonin groEL precursor=heat shock protein 60	***HHRT60***	***66.87***	***5.7***	***PF***	***M***	***−0.085***	
Chaperonin groEL precursor=heat shock protein 60	***HHRT60***	***67.72***	***6.09***	***PF***	***M***	***−0.085***	
Chaperonin subunit 3 (gamma)	AAH63178	71.4	6.84	PF		−0.271	17.4
Chaperonin subunit 3 (gamma)	AAH63178	71.56	6.7	PF		−0.271	17.4
Chaperonin subunit 3 (gamma)	AAH63178	71.56	6.78	PF		−0.271	17.4

(*Continued*)

TABLE I. (*Continued*)

Protein name	Accession number	Mr	pI	Function	Organelle	GRAVY	PSORT II (%)
Clathrin-associated protein complex 2, beta chain minor component	B32105	29.08	6.89	PF	Golgi	−0.061	17.4
Clathrin-associated protein complex 2, beta chain minor component	B32105	29.59	6.87	PF	Golgi	−0.061	17.4
DnaJ (Hsp40) homolog, subfamily B, member 11	AAQ91040	43.83	6.56	PF	ER	−0.556	
Dnak-type molecular chaperone grp75 precursor = Mortalin	**AAb33049**	**81.75**	**6.71**	**PF**		**−0.420**	**65.2**
Dnak-type molecular chaperone grp75 precursor = Mortalin	**AAb33049**	**93.31**	**5.58**	**PF**		**−0.420**	**65.2**
Dnak-type molecular chaperone grp75 precursor = Mortalin	**AAb33049**	**72.34**	**5.53**	**PF**		**−0.420**	**65.2**
DnaK-type molecular chaperone grp75 precursor = Mortalin	**AAb33049**	**82.91**	**5.81**	***PF***		**−0.420**	**65.2**
DnaK-type molecular chaperone grp75 precursort = Mortalin	**AAb33049**	**83.2**	**5.96**	**PF**		**−0.420**	**65.2**
DnaK-type molecular chaperone hsc73, heat shock protein 8	***S07197***	***79.39***	***5.68***	***PF***	***Non M***	***−0.452***	
DnaK-type molecular chaperone hsc73, heat shock protein 8	***S07197***	***79.42***	***5.81***	***PF***	***Non M***	***−0.452***	
DnaK-type molecular chaperone hsc73, heat shock protein 8	***S07197***	***80.81***	***5.67***	***PF***	***Non M***	***−0.452***	
DnaK-type molecular chaperone precursor = heat shock 70 kDa protein 5	*HHRTGB*	*17.75*	*6.86*	*PF*	*ER*	*−0.481*	
DnaK-type molecular chaperone precursor = heat shock 70 kDa protein 5	*HHRTGB*	*84.86*	*5.29*	*PF*	*ER*	*−0.481*	
DnaK-type molecular chaperone precursor = heat shock 70 kDa protein 5	*HHRTGB*	*85.31*	*5.19*	*PF*	*ER*	*−0.481*	
DnaK-type molecular chaperone precursor = heat shock 70 kDa protein 5	*HHRTGB*	*84.85*	*5.24*	*PF*	*ER*	*−0.481*	
DnaK-type molecular chaperone precursor = heat shock 70 kDa protein 5	*HHRTGB*	*73.2*	*5.49*	*PF*	*ER*	*−0.481*	
Heat shock 70 kDa protein 1A	**AAA17441**	**76.36**	**6.3**	**PF**	**Non M**	**−0.395**	
Heat shock 70 kDa protein 1A	**AAA17441**	**78.4**	**5.98**	**PF**	**Non M**	**−0.395**	
Peptidylprolyl isomerase A	*CSRTA*	*17.13*	*8.27*	*PF*		*−0.340*	*4.3*
Peptidylprolyl isomerase A	*CSRTA*	*17.24*	*8.78*	*PF*		*−0.340*	*4.3*
Protein disulfide isomerase associated 3	*BAA09695*	*58.77*	*6.6*	*PF*	*ER*	*−0.455*	
Protein disulfide isomerase associated 3	*BAA09695*	*61.39*	*6.72*	*PF*	*ER*	*−0.455*	
Protein disulfide isomerase associated 3	*BAA09695*	*62.05*	*6.38*	*PF*	*ER*	*−0.455*	
Protein disulfide isomerase associated 3	*BAA09695*	*62.08*	*6.27*	*PF*	*ER*	*−0.455*	
Protein disulfide isomerase associated 3	*BAA09695*	*62.55*	*6.49*	*PF*	*ER*	*−0.455*	
Protein disulfide isomerase associated 3	*BAA09695*	*62.65*	*6.19*	*PF*	*ER*	*−0.455*	
Protein disulfide isomerase associated 3	*BAA09695*	*63.03*	*6.11*	*PF*	*ER*	*−0.455*	
Protein disulfide-isomerase precursor	*ISRTSS*	*61.01*	*4.97*	*PF*	*ER*	*−0.382*	
Similar to for protein disulfide isomerase-related	Q921X9	66.13	7.66	PF	ER	−1.099	
3-Mercaptopyruvate sulfurtransferase	THTM_RAT	33.34	6.45	PSD	M	−0.299	
3-Mercaptopyruvate sulfurtransferase	THTM_RAT	33.5	6.3	PSD	M	−0.299	
Acidic ribosomal protein P0, cytosolic (similarity)	R5RT10	37.84	7.31	PSD	Non M	0.050	
Alpha-amylase (EC 3.2.1.1) precursor, pancreatic	ALRTP	56.39	7.72	PSD	Non M	−0.447	
Beta-*N*-acetylhexosaminidase beta chain	B54745	52.8	6.78	PSD		−0.237	
Branched chain aminotransferase 2	*AAH61790*	*41.16*	*7.94*	*PSD*	*M*	*−0.115*	
Branched chain aminotransferase 2	*AAH61790*	*42.06*	*7.57*	*PSD*	*M*	*−0.115*	
Cathepsin D precursor	*KHRTD*	*46.5*	*5.89*	*PSD*	*M*	*0.008*	
Cathepsin E precursor	S66465	50.81	4.64	PSD	ER	0.092	
Cathepsin S	A45087	28.85	5.6	PSD		−0.519	4.3
Cathepsin Z	BAA82844	38.75	5.64	PSD	Non M	−0.429	
Dipeptidyl-peptidase II	JC7668	60.2	4.79	PSD		−0.102	11.1
Elongation factor 1-beta	EF1B_MOUSE	34.73	4.57	PSD		−0.250	11.1
Elongation factor TU	***Q8BFR5***	***36.3***	***6.41***	***PSD***	***M***	***−0.179***	
Elongation factor TU	***Q8BFR5***	***48.08***	***6.87***	***PSD***	***M***	***−0.179***	
GM2 activator protein	Q8CJH4	25.27	4.89	PSD		0.108	26.1
Mast cell proteinase II precursor	PRRTG	26.73	9.6	PSD		0.075	11.1
Methylcrotonoyl-Coenzyme A carboxylase 1 (alpha)	*AAH21382*	*82.45*	*6.94*	*PSD*	*M*	*−0.288*	
Methylcrotonoyl-Coenzyme A carboxylase 2 (beta)	*AAH58987*	*56.09*	*7.89*	*PSD*	*M*	*−0.078*	
Mitochondrial Lon protease homolog	*Q924s5*	*115.82*	*6.27*	*PSD*	*M*	*−0.287*	
Mitochondrial ribomsomal protein L12	*BAB23955*	*22.72*	*5.48*	*PSD*	*M*	*−0.165*	

Mitochondrial ribosomal protein L38	Q8BTZ1	41.43	7.68	PSD	M	−0.801	
Neurolysin (metallopeptidase M3 family)	CAA60630	84.2	6.3	PSD	M	−0.363	
Nitrogen fixation gene 1	AAK12337	47.83	7.74	PSD	M	−0.222	
Peptidase (mitochondrial processing) beta	*BAA03007*	*52.41*	*6.51*	*PSD*	*M*	*−0.178*	
Plasma glutamate carboxypeptidase	Q9Z1Y1	59.84	5.97	PSD		−0.089	17.4
Ribosomal protein S12	Q6pdw1	15.82	6.83	PSD		−0.187	21.7
Translation elongation factor EF-G, mitochondrial	S40780	95.19	6.3	PSD	M	−0.280	
Catalase	**CATA_RAT**	**65.45**	**7.78**	**Redox**	**M**	**−0.643**	
Coproporphyrinogen oxidase	Q8VD08	38.65	7.82	Redox	M	−0.514	
CuZn Superoxide dismutase	*SODC_RAT*	*18.01*	*6.16*	*Redox*	*M*	*−0.419*	
Peroxiredoxin 3	*Q9Z0v6*	*27.94*	*6.66*	*Redox*	*M*	*0.013*	
Peroxiredoxin 3	*Q9Z0V6*	*28.13*	*6.5*	*Redox*	*M*	*0.013*	
Peroxiredoxin 3	*Q9Z0V6*	*28.29*	*6.34*	*Redox*	*M*	*0.013*	
Peroxiredoxin 3	*Q9Z0V6*	*28.52*	*6.15*	*Redox*	*M*	*0.013*	
Peroxiredoxin 3	*Q9Z0V6*	*29.04*	*6.16*	*Redox*	*M*	*0.013*	
Peroxiredoxin 4	*Q9Z0V5*	*29.43*	*6.36*	*Redox*	*M*	*−0.249*	
Peroxiredoxin 5	*AAF03751*	*18.21*	*7.55*	*Redox*	*M*	*0.191*	
Superoxide dismutase Mn precursor	*DSRTN*	*25.61*	*8.56*	*Redox*	*M*	*−0.422*	
Superoxide dismutase Mn precursor	*DSRTN*	*25.85*	*8.00*	*Redox*	*M*	*−0.422*	
Superoxide dismutase Mn precursor	*DSRTN*	*25.51*	*8.45*	*Redox*	*M*	*−0.422*	
Thiol-specific antioxidant Peroxiredoxin 2 (Prdx2)	*A57716*	*24.71*	*5.23*	*Redox*		*−0.175*	*8.7*
Thioredoxin peroxidase 2 mutant YES dimmer structure (Prdx1)	*I52425*	*24.9*	*8.05*	*Redox*		*−0.222*	*8.7*
Thioredoxin peroxidase 2 mutant YES dimmer structure (Prdx1)	*I52425*	*24.91*	*6.58*	*Redox*		*−0.222*	*8.7*
Thioredoxin peroxidase 2 mutant YES dimmer structure (Prdx1)	*I52425*	*25.27*	*8.84*	*Redox*		*−0.222*	*8.7*
Thioredoxin peroxidase 2 mutant YES dimmer structure (Prdx1)	*I52425*	*25.42*	*7.17*	*Redox*		*−0.222*	*8.7*
Annexin A1	*ANX1_RAT*	*38.29*	*7.19*	*Signalling*		*−0.437*	*17.4*
Guanine nucleotide binding protein (G protein), beta polypeptide 2 like 1 activated protein kinase C receptor RACK1	A36986	32.98	8.03	Signalling		−0.258	21.7
Guanine nucleotide binding protein (G protein), beta polypeptide 2 like 1 activated protein kinase C receptor RACK1	A36986	32.27	8.01	Signalling		−0.258	21.7
Guanine nucleotide binding protein, beta 1	AAC72249	38.74	5.77	Signalling	Non M	−0.237	
LIM protein	JC4385	36.67	7.26	Signalling		−0.487	4.3
Actin alpha 2, vascular smooth muscle	***A22224***	***39.6***	***6.16***	***Structural***	***Non M***	***−0.233***	
Actin beta	ATRTC	29.02	5.36	Structural		−0.005	8.7
Actin beta	ATRTC	29.75	7.63	Structural		−0.005	8.7
Actin beta	ATRTC	32.52	5.74	Structural		−0.005	8.7
Actin beta	ATRTC	39.87	5.92	Structural		−0.005	8.7
Actin beta	ATRTC	40.15	5.75	Structural		−0.005	8.7
Actin beta	ATRTC	49.12	5.6	Structural		−0.005	8.7
Actin beta	ATRTC	49.22	6	Structural		−0.005	8.7
Actin beta	ATRTC	53.57	5.62	Structural		−0.005	8.7
Actin beta	ATRTC	72.57	7.2	Structural		−0.005	8.7
Alpha-spectrin 2	*AAC33127*	*151.03*	*5.72*	*Structural*	*Unknown*	*−0.789*	
Coronin, actin-binding protein, 1B	AAH61558	78.54	5.9	Structural		−0.147	26.1
Desmin	*DESM_RAT*	*38.39*	*5.06*	*Structural*		*−0.714*	*21.7*
Desmin	*DESM_RAT*	*41.1*	*4.95*	*Structural*		*−0.714*	*21.7*
Desmin	*DESM_RAT*	*42.76*	*4.98*	*Structural*		*−0.714*	*21.7*
Desmin	*DESM_RAT*	*42.81*	*4.9*	*Structural*		*−0.714*	*21.7*
Desmin	*DESM_RAT*	*49.08*	*5.49*	*Structural*		*−0.714*	*21.7*
Destrin	*JE0223*	*17.73*	*8.51*	*Structural*		*−0.234*	*4.3*
Ezrin	*Q8VHK3*	*63.96*	*6.92*	*Structural*		*−0.971*	*8.7*
Ezrin	*Q8VHK3*	*76.69*	*6.23*	*Structural*		*−0.971*	*8.7*
Ezrin	*Q8VHK3*	*89.94*	*6.39*	*Structural*		*−0.971*	*8.7*
F-actin capping protein beta subunit	B54819	32.43	5.91	Structural		−0.568	4.3
Keratin 18	Q63278	44.51	5.02	Structural		−0.654	87.0
Keratin 2 epidermis	Q61869	27.8	8.11	Structural		−0.597	4.3

(*Continued*)

TABLE I. (*Continued*)

Protein name	Accession number	Mr	pI	Function	Organelle	GRAVY	PSORT II (%)
Keratin 2 epidermis	Q61869	67.67	6.71	Structural		−0.597	4.3
Keratin 21, type I, cytoskeletal	A40452	48.01	4.89	Structural		−0.727	78.3
Keratin 21, type I, cytoskeletal	A40452	48.13	4.96	Structural		−0.727	78.3
Keratin 59K type I cytoskeletal (cytokeratin 10)	KRMSE1	19.07	5.37	Structural		−0.695	43.5
Keratin 59K type I cytoskeletal (cytokeratin 10)	KRMSE1	23.2	8.66	Structural		−0.695	43.5
Keratin 59K type I cytoskeletal (cytokeratin 10)	KRMSE1	39.2	6.61	Structural		−0.695	43.5
Keratin, type II cytoskeletal 2 (cytokeratin 8) (cytokeratin endo A)	K2C8_RAT	42.85	5.18	Structural		−0.672	78.3
Keratin, type II cytoskeletal 2 (cytokeratin 8) (cytokeratin endo A)	K2C8_RAT	18.84	4.92	Structural		−0.672	78.3
Keratin, type II cytoskeletal 2 (cytokeratin 8) (cytokeratin endo A)	K2C8_RAT	22.44	4.89	Structural		−0.672	78.3
Keratin, type II cytoskeletal 2 (cytokeratin 8) (cytokeratin endo A)	K2C8_RAT	24.02	4.89	Structural		−0.672	78.3
Keratin, type II cytoskeletal 2 (cytokeratin 8) (cytokeratin endo A)	K2C8_RAT	27.55	5.48	Structural		−0.672	78.3
Keratin, type II cytoskeletal 2 (cytokeratin 8) (cytokeratin endo A)	K2C8_RAT	43.47	5.26	Structural		−0.672	78.3
Keratin, type II cytoskeletal 2 (cytokeratin 8) (cytokeratin endo A)	K2C8_RAT	42.16	5.04	Structural		−0.672	78.3
Keratin, type II cytoskeletal 8	K2C8_RAT	42.64	5.08	Structural		−0.672	78.3
Keratin, type II cytoskeletal 8	K2C8_RAT	43.81	5.19	Structural		−0.672	78.3
Keratin, type II cytoskeletal 8	K2C8_RAT	51.76	5.82	Structural		−0.672	78.3
Keratin, type II cytoskeletal 8	K2C8_RAT	59.23	6.24	Structural		−0.672	78.3
Keratin, type II cytoskeletal 8 (Cytokeratin-8)	K2C8_RAT	41.75	5.26	Structural		−0.672	78.3
LASP-1.	Q99MZ8	39.44	6.99	Structural		−1.035	8.7
Myosin light chain 6, alkali, smooth muscle isoform MLC3SM	MLES_RAT	17.16	4.38	Structural		−0.421	4.3
Myosin light chain 6, alkali, smooth muscle isoform MLC3SM	MLES_RAT	17.44	4.22	Structural		−0.421	4.3
Myosin regulatory light chain 2-A, smooth muscle isoform (Myosin RLC-A)	***MLRA_RAT***	***19.22***	***4.71***	***Structural***		***−0.809***	***4.3***
Myosin regulatory light chain 2-A, smooth muscle isoform (Myosin RLC-A)	***MLRA_RAT***	***20.17***	***4.87***	***Structural***		***−0.809***	***4.3***
Myosin regulatory light chain 2-A, smooth muscle isoform (Myosin RLC-A)	***MLRA_RAT***	***20.24***	***4.65***	***Structural***		***−0.809***	***4.3***
Plectin 1	A39638	115.67	5.63	Structural		−0.687	8.7
Profilin I	*Pro1_MOUSE*	*15.11*	*8.85*	*Structural*		*0.018*	*8.7*
Saposin precursor	A28716	14.44	4.03	Structural	M	−0.034	
Similar to intermediate filament-like protein MGC:2625 isoform 2; HOM-TES-103 tumor antigen-like	**Q7TP27**	**66.79**	**7.28**	*Structural*	**Non M**	**−0.191**	
Similar to transgelin 2 (SM22 beta)	*Q91VU2*	*23.22*	*8.83*	*Structural*		*−0.637*	*21.7*
Transgelin (Smooth muscle protein 22-alpha)	TAGL_RAT	79.27	5.41	Structural		−0.634	4.3
Transgelin (Smooth muscle protein 22-alpha) (SM22-alpha	TAGL_RAT	39.1	6.76	Structural		−0.634	4.3
Transgelin SM22-alpha	TAGL_RAT	16.74	6.6	Structural		−0.634	4.3
Transgelin SM22-alpha	TAGL_RAT	17.31	7.04	Structural		−0.634	4.3
Transgelin SM22-alpha	TAGL_RAT	17.48	6.58	Structural		−0.634	4.3
Tropomyosin alpha isoform 1	Q923Z2	44.74	4.62	Structural		−0.992	4.3
Tropomyosin alpha isoform 6	S34124	33.6	4.75	Structural		−1.018	17.4
Tubulin alpha-6 chain (Alpha-tubulin 6)	Q6AYZ1	63.78	5.45	Structural		−0.234	4.3
Tubulin, beta 2c	AAH60597	59.62	5.18	Structural	Non M	−0.357	
Vinculin	Q922D9	126.34	6.38	Structural		−0.421	17.4
14-3-3 protein epsilon (Mitochondrial import stimulation factor L subunit) = tyrosine 3-monooxygenase	***AAC52676***	***32.69***	***4.61***	***TC***	***Non M***	***−0.540***	
14-3-3 protein epsilon (Mitochondrial import stimulation factor L subunit) = tyrosine 3-monooxygenase	***AAC52676***	***33.69***	***4.61***	***TC***	***Non M***	***−0.540***	
Chloride intracellular channel 1	*BAC40585*	*33.48*	*5.45*	*TC*		*−0.305*	*8.7*
Cofilin-1	*S49101*	*18.37*	*8.66*	*TC*		*−0.388*	*4.3*
Ethylmalonic encephalopathy 1	Q9DCM0	28.85	7.13	TC	M	−0.096	

Ethylmalonic encephalopathy 1	Q9DCM0	29.14	6.54	TC	M	−0.096	
Flotillin 1	AAC98705	49.48	7.29	TC		−0.355	21.7
Hemoglobin alpha-1 and alpha-2 chains	*HBA_RAT*	*14.14*	*9.08*	*TC*	*Non M*	*−0.130*	
Hemoglobin beta chain, major-form	*HBB1_RAT*	*14.07*	*8.55*	*TC*		*−0.055*	*4.3*
Hemoglobin beta chain, major-form	*HBB1_RAT*	*14.09*	*8.77*	*TC*		*−0.055*	*4.3*
Hemoglobin beta chain, major-form	*HBB1_RAT*	*14.11*	*8.45*	*TC*		*−0.055*	*4.3*
Neonatal fc receptor chain B	2FRTB	13.58	7.76	TC	Non M	−0.142	
Protein CGI-51 homolog	***Q8BGH2***	***57.03***	***7***	***TC***	***M***	***−0.206***	
Ribosome binding protein 1	BAC98159	38.83	4.88	TC	ER	−0.923	
Similar to golgi phosphoprotein 3 (Coat-protein)	Q8R088	35.16	6.05	TC	Golgi	−0.625	39.1
Transitional endoplasmic reticulum ATPase	***A55190***	***108.62***	***5.55***	***TC***	***ER***	***−0.354***	
Translocase of inner mitochondrial membrane 50 homolog	Q9D880	36.57	6.11	TC	M	−0.383	
Translocase of inner mitochondrial membrane 50 homolog	Q9D880	37.17	6.77	TC	M	−0.383	
Tumor rejection antigen gp96 = heat shock protein 90 kDa, Grp 94	*Q91V38*	*110.97*	*4.92*	*TC*	*ER*	*−0.720*	
Voltage-dependent anion channel 2	***Q9JI32***	***32.42***	***8.5***	***TC***	***M***	***−0.221***	
Voltage-dependent anion channel 2	***Q9JI32***	***32.9***	***7.03***	***TC***	***M***	***−0.221***	
Voltage-dependent anion channel 2	***Q9JI32***	***33.11***	***8.2***	***TC***	***M***	***−0.221***	
Voltage-dependent anion channel 2	***Q9JI32***	***33.14***	***7.32***	***TC***	***M***	***−0.221***	
Voltage-dependent anion channel 2	***Q9JI32***	***33.47***	***7.15***	***TC***	***M***	***−0.221***	
Voltage-dependent anion channel 2	***Q9JI32***	***33.57***	***7.01***	***TC***	***M***	***−0.221***	
Voltage-dependent anion channel 2	***Q9JI32***	***34.2***	***6.5***	***TC***	***M***	***−0.221***	
Voltage-dependent anion channel 2	***Q9JI32***	***34.27***	***7.07***	***TC***	***M***	***−0.221***	
Voltage-dependent anion channel 2	***Q9JI32***	***34.93***	***6.54***	***TC***	***M***	***−0.221***	
Voltage-dependent anion channel 2	***Q9JI32***	***35.46***	***7.04***	***TC***	***M***	***−0.221***	
Voltage-dependent anion-selective channel protein 1	***POR1_RAT***	***28.03***	***6.8***	***TC***	***M***	***−0.374***	
Voltage-dependent anion-selective channel protein 1	***POR1_RAT***	***31***	***9.6***	***TC***	***M***	***−0.374***	
Voltage-dependent anion-selective channel protein 1	***POR1_RAT***	***32.05***	***9.07***	***TC***	***M***	***−0.374***	

Proteins are ordered by functional groups. Mr and pI values estimated from 2D gel. ER = endoplasmic reticulum and M = mitochondrial, as according to SOURCE from BioInformatic Harvester (http://harvester.embl.de/), where no subcellular localisation is stated a PSORT II percentages depicting the predicted percentage probability a protein is mitochondrial using the k-nearest neighbour (k-NN) algorithm [Horton and Nakai, 1997] is stated instead. CB = calcium binding, EM = energy metabolism, OXPHOS = oxidative phosphorylation, PF = protein folding, PSD = protein synthesis and degradation, TC = Transporters and channels. *Italics* represents proteins found in human heart mitoproteomes [Taylor et al., 2003; Gaucher et al., 2004], **bold** represents proteins found in mitoproteomes of other rat tissues [Forner et al., 2006; Reifschneider et al., 2006], ***bold italics*** represents proteins found in both human heart and other rat tissue mitoproteomes [Taylor et al., 2003; Gaucher et al., 2004; Forner et al., 2006; Reifschneider et al., 2006].

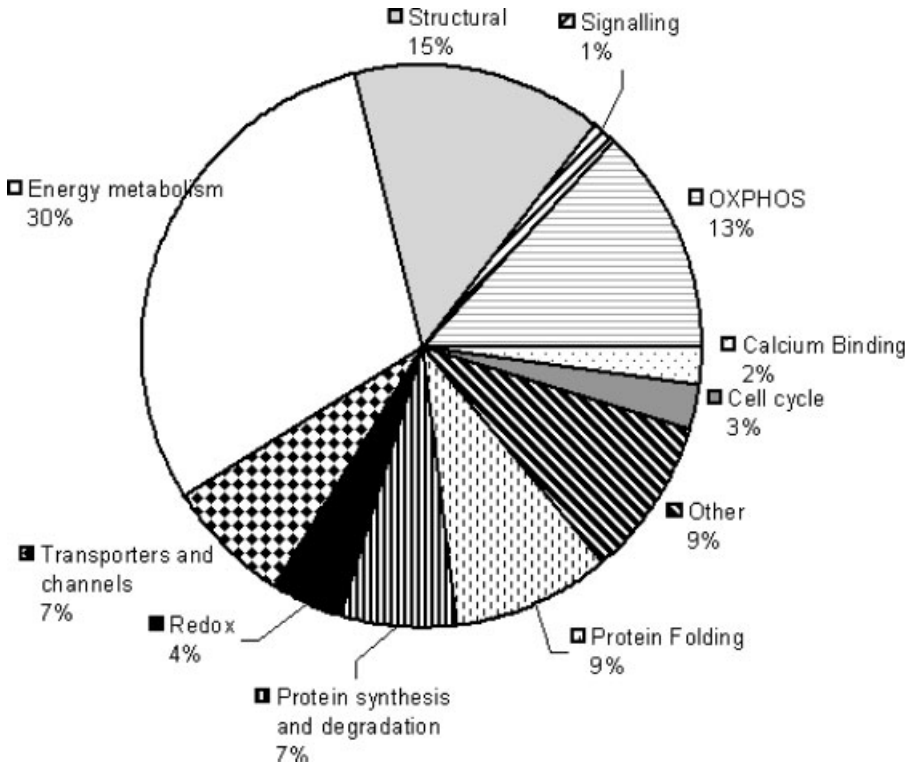

Fig. 4. Functional classification of the 430 proteins identified from rat colon mitochondrial enriched fractions.

organ specific differences in mitoproteomes [Taylor et al., 2003; Gaucher et al., 2004; Kiri et al., 2005; Lovell et al., 2005; Forner et al., 2006; Hunzinger et al., 2006; Kim et al., 2006; Miller et al., 2006; Reifschneider et al., 2006; Ruiz-Romero et al., 2006]. This implies that mitochondria may be regulated differently in specific cells and tissues. Studies of proteins associated with mitochondria from the liver [Forner et al., 2006; Reifschneider et al., 2006], kidney [Reifschneider et al., 2006], brain [Reifschneider et al., 2006], heart [Taylor et al., 2003; Gaucher et al., 2004; Forner et al., 2006; Reifschneider et al., 2006] and skeletal muscle [Forner et al., 2006; Reifschneider et al., 2006] have previously been reported. These studies provide insights into the optimal functioning and regulation of mitochondria in these tissues and the subsequent determination of altered regulation associated with dysfunction and disease. This study is the first to profile the proteins associated with mitochondria extracted from rat colon and is significant considering that rat models are used extensively to study human colon pathologies, such as inflammatory bowel disease and cancer [Corpet and Parnaud, 1999; Drew et al., 2005ab, 2006a; Mazzon et al., 2005; Yuki et al., 2006].

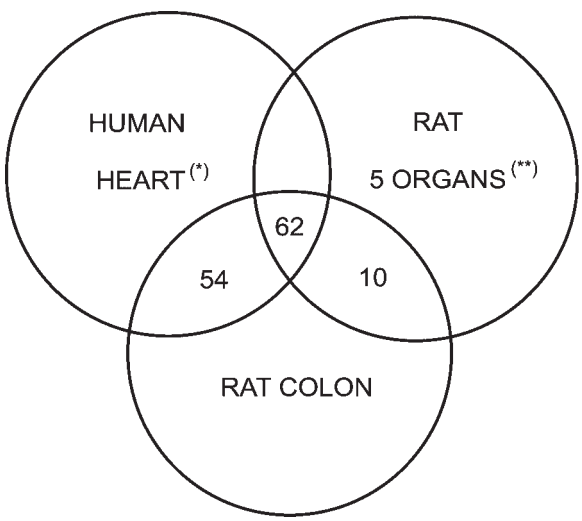

Fig. 5. Common multiple protein expression forms of mitochondrial associated proteins identified in colon by LC/MS/MS compared with published mitoproteomes. * Taylor et al. [2003] and Gaucher et al. [2004]; ** Reifschneider et al. [2006] and Forner et al. [2006].

This study of mitochondria enriched fractions from rat colon revealed resolution of

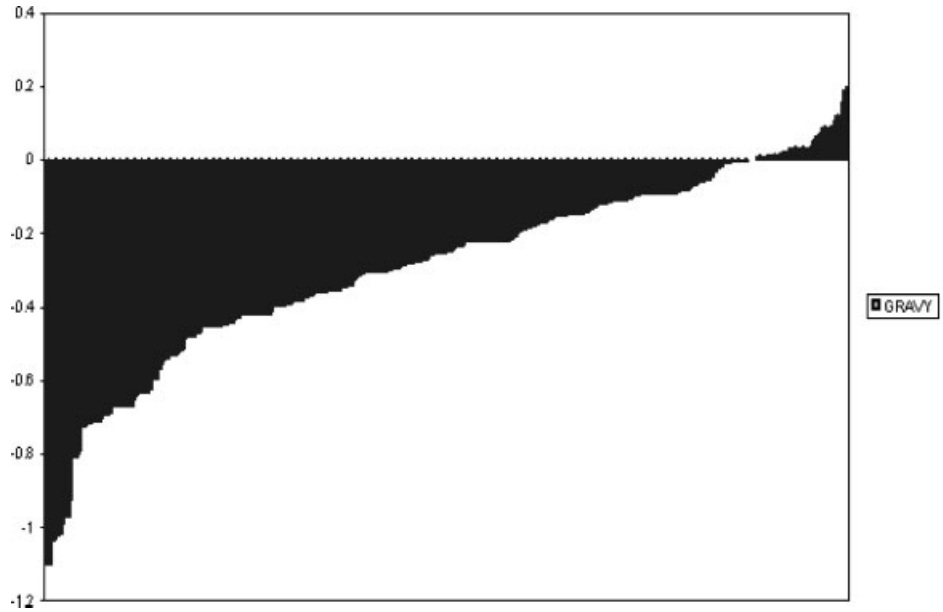

Fig. 6. Profile of GRAVY values of mitochondrial associated proteins of rat colon. The plot shows the index of hydrophobicity for the identified mitochondrial associated proteins calculated using Kyte and Doolittle [1982] (http://bioinformatics.org/sms2/protein_gravy.html).

430 proteins consisting of 195 different proteins and 23 unknown proteins that were distributed into 11 functional groups (see Table I and Fig. 4). Hence, around 50% of the resolved proteins from the colon mitochondrial enriched fractions are multiple protein expression forms (MPEFs) (Table II). The number of identified proteins is greater than other published studies using 2D PAGE [Hunzinger et al., 2006; McDonald et al., 2006; Reifschneider et al., 2006]. However, Coomassie blue staining of proteins used in this study ensures sufficient quantities of protein are available for identification, while LC/MS/MS maximises positive identification.

The presence of multiple protein expression forms (MPEFs) with several spots being identified as the same protein, potentially representing splice variants, truncated products or co- and post-translational modifications has been highlighted by Yang et al. [2005] and Hunzinger et al. [2006]. Comparative proteomic studies often fail to identify the entire complement of MPEFs for a specific protein present on a 2D gel. The current analysis of MPEFs associated with the colon mitochondria will assist interpretation and analysis of proteomic data from future studies employing comparative proteomics of colon mitochondria. MPEFs present a significant problem in

TABLE II. Multiple Protein Expression Forms of Identified Proteins, Categorised by Functional Groups, From Mitochondrial Enriched Fractions of the Rat Colon

Functional group	No. of different proteins	No. of resolved proteins	% MPEFs
Calcium binding	5	9	44
Cell cycle	5	11	55
Energy metabolism	58	128	55
Other[a]	14	15	7
OXPHOS	20	59	66
Protein folding	13	39	67
Protein synthesis and degradation	25	28	10
Redox	9	18	50
Signalling	4	5	20
Structural	27	63	57
Transporters and channels	16	32	50

[a]Table excludes unidentified proteins.

verifying comparative proteomic data since western blotting is often not suitable for verification of these MPEFs. The two-dimensional patterns resolved by 2D PAGE cannot be replicated using a one-dimensional format as the proteins often have similar mass and pI values. Antibodies often do not discriminate between the observed MPEFs due to the extensive regions of shared amino acid sequence. Furthermore, it is often not possible to properly interpret the biological significance of changes in protein expression patterns revealed by 2D gel analysis without an awareness of the complement and identification of MPEFs present.

Proteins involved in energy metabolism (EM) and oxidative phosphorylation (OXPHOS) represented the major group accounting for 43% of the resolved proteins (see Fig. 4), reflecting a significant function of the mitochondria. These groups included 58 and 20 different proteins respectively (Tables I and II) involved in the oxidative phosphorylation machinery, for example complex I–V subunits, proteins involved in the TCA cycle, for example fumarate hydratase mitochondrial precursor and those involved in fatty acid metabolism, for example carnitine *O*-palmitoyltransferase II precursor.

Seven percent of the proteins were involved in protein synthesis and degradation (Fig. 4). These 25 proteins (Tables I and II) are required to activate, synthesise and process precursor nuclear-encoded mitochondrial proteins that are imported into the mitochondria via transporters and channels, the latter accounting for 7% of the proteins associated with the colon mitochondria (Fig. 4). These precursor proteins require folding and 9% of the colon mitoproteome are involved in protein folding (Fig. 4). Redox proteins, such as manganese superoxide dismutase contribute a further 4% of the resolved proteins. Mitochondrial antioxidant defence systems play an important role in protecting mitochondria from reactive oxygen species produced from oxidative phosphorylation in the electron transport chain [Jezek and Hlavata, 2005].

Fifteen percent of the proteins were structural (see Fig. 4). This included a number of MPEFs, that is, 63 resolved spots identified as 27 different proteins. Structural proteins such as cytoskeletal proteins (actins, intermediate filaments and microtubules) play a central role in many cell functions such as the maintenance of cell shape, cell division, adhesion, signal transduction, protein sorting, mitosis, cell and intracellular organelle anchorage, gene regulation, motility during migration, differentiation and wound repair [Ku et al., 1999]. Studies have shown that mitochondria are closely associated, transported and positioned within cells via interaction with microtubules and actin filaments [Morris and Hollenbeck, 1995; Ligon and Steward, 2000; Carre et al., 2002]. This implies that the proteins associated with mitochondrial-enriched fractions may be indicative of processes linked to normal functioning of mitochondria in the colon. Proteins involved in calcium binding which were not previously associated with mitochondria, accounted for 2% for the colon mitoproteome. The presence of these proteins reflects the intimate relationship between mitochondria and other vesicular membranes such as the endoplasmic reticulum for calcium homeostasis [Breckenridge et al., 2003]. These proteins may also work closely with the structural proteins such as actins in exo- and endocytosis [Weinman et al., 1994; Merrifield et al., 2001]. Proteins involved in the cell cycle such as the mitochondrial inner membrane protein prohibitin made up 2% of the colon mitoproteome (Fig. 4). Other proteins in this group such as the histones, although not typically associated with mitochondria have also been identified by Taylor et al. [2003] and may be present due to the intimate association between mitochondria and the nucleus coupled with electrostatic interactions as suggested by Taylor et al. [2003].

The remaining 9% of proteins resolved were categorised as other proteins (Fig. 4). These include unidentified proteins and proteins with no currently established function, for example mucosal pentraxin [Van Der Meer-Van Kraaij et al., 2003; Drew et al., 2006b], as well as those whose functions could not be easily categorised into the other groups such as Stomatin (Epb7.2)-like 2.

Analysis of the proteins associated with the colon mitochondria revealed 72 proteins common to the mitoproteomes of rat liver, heart and skeletal muscle [Forner et al., 2006; Reifschneider et al., 2006]; and kidney and brain [Reifschneider et al., 2006] (Table I). Sixty-one percent of these common proteins are proteins involved in energy metabolism and OXPHOS again reflecting the major role of mitochondria in activities associated with

respiration and possibly the abundance of these proteins in the mitoproteome. Comparison of the mitochondrial associated proteins of the rat colon with the previously published human heart mitoproteomes [Taylor et al., 2003; Gaucher et al., 2004] revealed 116 proteins common with the colon mitoproteome, with 58 of these proteins involved in energy metabolism and OXPHOS. Although the human heart mitoproteome had more proteins in common with the rat colon mitoproteome compared to other rat tissues [Forner et al., 2006; Reifschneider et al., 2006], this may be indicative of the methodologies used. Reifschneider et al. [2006] used 2D blue native/SDS PAGE and identified their resolved proteins using MALDI-TOF MS. Forner et al. [2006] and Taylor et al. [2003] used a 1D gel electrophoresis followed by liquid chromatography separation and LC/MS/MS that provides a greater degree of positive identification due to greater confidence of the peptide matching scores from MASCOT compared with MALDI-TOF MS. Using MALDI-TOF MS we found a 10–15% identification rate compared with 94% with LC/MS/MS (unpublished data). Hunzinger et al. [2006] recently demonstrated the 2D-IEF-SDS-PAGE resolved more spots and better separated protein isoforms compared with blue native SDS, benzyldimethyl-*n*-hexadecylammonium chloride PAGE and tricine-urea/tricine SDS-PAGE. In comparative studies [Hunzinger et al., 2006; McDonald et al., 2006] LC/MS/MS provides better identification hydrophobic proteins. Membrane associated proteins, such as those of the mitochondria, have a hydrophobic protein distribution bias, that is, a greater degree of hydrophobic amino acids. This enables a better association with the hydrophobic 'tails' of fatty acids in the membranes themselves, particularly in protein transmembrane regions [Ho et al., 2006]. The degree of hydrophobicity can be depicted by GRAVY values [Kyte and Doolittle, 1982]. This study has a similar GRAVY value profile (Fig. 6) compared with other published mitoproteomes [Hunzinger et al., 2006; McDonald et al., 2006; Reifschneider et al., 2006] with the presence of membrane associated proteins such as malate dehydrogenase mitochondrial precursor (0.121) and tropomyosin alpha isoform 6 (−1.018). Furthermore, differences in sample preparation in these studies also potentially contribute to differences in the mitochondrial proteins analysed. Forner et al. [2006] also excluded proteins such as actin, keratins and haemoglobin from their result set as they were considered as contaminants. However, the current study and Taylor et al. [2003] both identified these proteins and have included them in the dataset. Further study is required to establish the association of these proteins in mitochondrial functions. There were 69 proteins not previously identified as associated with mitoproteome analysis of other organs studied [Taylor et al., 2003; Forner et al., 2006; Reifschneider et al., 2006]. Over half of these novel proteins were structural proteins and those involved in protein synthesis and degradation and may be indicative of the differences in the sample processing as described above, as well as variations between mitochondrial proteins of differing organs.

Proteomic profiling of mitochondria and associated proteins involved in mitochondrial regulation and trafficking within cells and tissues has the potential to provide insights into mitochondrial dysfunction associated with many human diseases. Furthermore, profiling of organ specific mitoproteomes will potentially reveal organ specific roles of the mitochondria and assist in study of organ specific disease associated with mitochondrial dysfunction. Thus the colon mitoproteome analysis presented here provides a useful tool to assist in identification and interpretation mitochondrial dysfunction implicated in colon pathogenesis.

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