Supplementary Figure S1

Sequence alignment of the Cox11 protein family. Sequences representing Cox11 from a diversity of organisms were aligned using ClustalW version 1.81 with default parameters (http://www.ebi.ac.uk/clustalw/). Amino acid residues are colored to show chemically-similar residues (pink, basic; blue, acidic; green, polar; red, non-polar), with the predicted transmembrane segment shaded grey. Across the alignment, asterisks denote absolutely conserved residues; colons represent residues highly-conserved across species. The positively-charged residues (+) and putative stop-transfer enhancement signal (underlined) in the predicted mitochondrial targeting sequence of *Sc*Cox11 are shown.

Footnotes:

1 – While it lacks the N-terminal extension seen in the nuclear-encoded Cox11 from *Saccharomyces*, the N-terminus of *Ra*Cox11 could form a short basic, amphipathic helix (with residues K_3, R_5 and K_6) and is followed by a helical transmembrane segment (grey shaded) of amphipathic character (S_7, S_{16}, T_{18}, S_{23} and S_{26}) that could provide for the observed "minimalist importability". Note that the N-terminal sequence of *Ra*Cox11 does not conform to sequence motifs defined for the outer membrane proteins Tom20 and Tom70 (Chan et al 2006; Hulett et al 2008).

References:

Chan NC, Likić VA, Waller RF, Mulhern TD and Lithgow T. 2006. The C-terminal TPR domain of Tom70 defines a family of mitochondrial protein import receptors found only in animals and fungi. J Mol Biol. **358**:1010-22

Hulett JM, Lueder F, Chan NC, Perry AJ, Wolynec P, Likić VA, Gooley PR and Lithgow T. 2008. The transmembrane segment of Tom20 is recognized by Mim1 for docking to the mitochondrial TOM complex. J Mol Biol **376**:694-704

Table S1. Membrane proteins encoded on the mitochondrial genome of *Reclinomonas americana*.

Gene	***Protein function***	***Hydrophobicity*[1]**		***Signal Peptide*[2]**	***TMs*[3]**
		H17	***mesoH***		
atp1	F_1F_0-ATP synthase, peripheral membrane protein	*0.8*	*-0.1*	*N*	*-*
atp3	F_1F_0-ATP synthase, integral membrane protein	*0.7*	*-0.9*	*N*	*-*
atp4	F_1F_0-ATP synthase, integral membrane protein	*2.5*	*-0.1*	*Y*	*2*
atp6	F_1F_0-ATP synthase, integral membrane protein	*2.5*	*1.5*	*N*	*-*
atp8	F_1F_0-ATP synthase, integral membrane protein	*2.2*	*-0.5*	*N*	*1*
atp9	F_1F_0-ATP synthase, integral membrane protein	*2.8*	*0.2*	*Y*	*1-2*
cob	Cytochrome *c* reductase (Complex III)	*2.8*	*1.2*	*N*	*8*
cox1	Cytochrome *c* oxidase (Complex IV)	*2.5*	*1.3*	*N*	*5*
cox2	Cytochrome *c* oxidase (Complex IV)	*2.8*	*0.8*	*N*	*3*
cox3	Cytochrome *c* oxidase (Complex IV)	*2.4*	*1.0*	*N*	*6*
cox11	Cytochrome oxidase assembly factor	*2.2*	*-0.5*	*Y*	*1*
nad1	NADH dehydrogenase (Complex I)	*2.6*	*1.4*	*N*	*7-8*
nad2	NADH dehydrogenase (Complex I)	*2.5*	*1.2*	*N*	*11*
nad3	NADH dehydrogenase (Complex I)	*2.7*	*0.6*	*Y*	*3*
nad4	NADH dehydrogenase (Complex I)	*2.5*	*1.3*	*N*	*6*
nad4L	NADH dehydrogenase (Complex I)	*2.3*	*1.0*	*N*	*3-4*
nad5	NADH dehydrogenase (Complex I)	*2.6*	*1.1*	*N*	*9*
nad6	NADH dehydrogenase (Complex I)	*2.6*	*1.8*	*Y*	*5*
nad7	NADH dehydrogenase (Complex I)	*1.1*	*-0.5*	*N*	*-*
nad8	NADH dehydrogenase (Complex I)	*0.6*	*-1.4*	*N*	*-*
nad9	NADH dehydrogenase (Complex I)	*1.5*	*-1.1*	*N*	*-*
nad10	NADH dehydrogenase (Complex I)	*1.5*	*-0.6*	*N*	*1*
nad11	NADH dehydrogenase (Complex I)	*0.8*	*-0.6*	*N*	*-*
orf169	Putative protein of unknown function	*2.2*	*0.75*	*N*	*1-2*
orf717	Putative protein of unknown function	*1.6*	*0.5*	*N*	*3*
sdh2	Succinate dehydrogenase (Complex II)	*1.1*	*-1.2*	*N*	*-*
sdh3	Succinate dehydrogenase (Complex II)	*2.7*	*1.3*	*N*	*3*
sdh4	Succinate dehydrogenase (Complex II)	*1.7*	*0.4*	*N*	*3*
secy	SecY subunit of translocon.	*2.2*	*0.9*	*N*	*10*
tatA	TatA subunit of TAT protein transporter.	*2.4*	*-4.4*	*N*	*1*
tatC	TatC subunit of TAT protein transporter.	*2.1*	*1.1*	*N*	*6*
ccmF/ yejR	CcmF subunit of Ccm complex. Integral membrane protein[4].	*3*	*1.2*	*N*	*12*
ccmC/ yejU	CcmC subunit of Ccm complex. Integral membrane protein[4].	*2.5*	*1.3*	*N*	*5-6*
ccmB/ yejV	CcmB subunit of Ccm complex. Integral membrane protein channel subunit[4].	*2.5*	*1.6*	*N*	*6*
ccmA/	CcmA subunit of Ccm complex. Peripheral	*0.5*	*-0.6*	*N*	*-*

yejW	membrane protein, ATP-binding subunit[4].

Footnotes:

1 - Hydropathy scores were predicted according to Claros *et al* (1995).

2 - SignalP3.0 (Bendtsen *et al* 2004) suggests several proteins have signal sequences that would direct them to the Sec translocon for integration into the mitochondrial inner membrane.

3 - Prediction of membrane proteins made use of (i) DAS (Cserzo *et al.* 1997) to detect putative transmembrane domains – TMs - the number predicted is shown, and (ii) functional annotations for peripheral membrane proteins.

4 - The mitochondrial genome of *R. americana* encodes the CcmA, CcmB, CcmC and CcmF subunits of a Type II cytochrome *c* assembly pathway. This system is used to present and conjugate heme to apocytochrome *c* in the intermembrane space and is found in various protists as well as plants (Allen *et al.* 2008; Hamel *et al.* 2009). The multiple transmembrane segments predicted in CcmB (6 transmembrane segments), CcmC (6 transmembrane segments) and CcmF (11-12 transmembrane segments) make them putative substrates for the Sec translocon and both CcmC and CcmF predict strongly for a signal-anchor targeting sequence that would initiate their assembly by the Sec translocon.

References:

Allen JW, Jackson AP, Rigden DJ, Willis AC, Ferguson SJ, Ginger ML. 2008. Order within a mosaic distribution of mitochondrial c-type cytochrome biogenesis systems? FEBS J. **275**:2385-2402

Bendtsen JD, Nielsen H, von Heijne G and Brunak S. 2004. Improved prediction of signal peptides: SignalP 3.0. J Mol Biol. **340**:783-795.

Claros MG, Perea J, Shu Y, Samatey FA, Popot JL and Jacq C. 1995. Limitations to *in vivo* import of hydrophobic proteins into yeast mitochondria. The case of a cytoplasmically synthesized apocytochrome b. Eur J Biochem **228**:762-771.

Cserzö M, Wallin E, Simon I, von Heijne G and Elofsson A. 1997. Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: the dense alignment surface method. Protein Eng. **10**:673-676.

Hamel P, Corvest V, Giegé P, Bonnard G. 2009. Biochemical requirements for the maturation of mitochondrial c-type cytochromes. Biochim Biophys Acta **1793**:125-138.

Table S2. Matrix proteins encoded on the mitochondrial genome of *Reclinomonas americana*.

Gene name	***Protein function***	***Hydrophobicity***[1]	
		H17	***mesoH***
rp11	50S ribosomal subunit L1	*0.4*	*-1.0*
rp12	50S ribosomal subunit L2	*0.2*	*-0.8*
rp15	50S ribosomal subunit L5	*0.6*	*-0.7*
rp16	50S ribosomal subunit L6	*0.3*	*-0.8*
rp110	50S ribosomal subunit L10	*0.8*	*-1.0*
rp111	50S ribosomal subunit L11	*0.4*	*-1.0*
rp114	50S ribosomal subunit L14	*-0.1*	*-1.8*
rp116	50S ribosomal subunit L16	*0.7*	*-1.1*
rp118	50S ribosomal subunit L18	*-0.1*	*-1.6*
rp119	50S ribosomal subunit L19	*0.1*	*-1.5*
rp120	50S ribosomal subunit L20	*0.9*	*-1.1*
rp127	50S ribosomal subunit L27	*0.6*	*-1.7*
rp131	50S ribosomal subunit L31	*0.9*	*-2.6*
rp132	50S ribosomal subunit L32	*-1*	*-7.1*
rp134	50S ribosomal subunit L34	*-1.8*	*-3.3*
rps1	30S ribosomal subunit S1	*1.6*	*-0.8*
rps2	30S ribosomal subunit S2	*0.9*	*-0.3*
rps3	30S ribosomal subunit S3	*0.3*	*-0.9*
rps4	30S ribosomal subunit S4	*0.3*	*-1.1*
rps7	30S ribosomal subunit S7	*0*	*-1.4*
rps8	30S ribosomal subunit S8	*1.1*	*-1.1*
rps10	30S ribosomal subunit S10	*0.3*	*-1.7*
rps11	30S ribosomal subunit S11	*0.8*	*-0.7*
rps12	30S ribosomal subunit S12	*-0.4*	*-1.7*
rps13	30S ribosomal subunit S13	*-0.4*	*-1.5*
rps14	30S ribosomal subunit S14	*-1.1*	*-2.6*
rps19	30S ribosomal subunit S19	*0.8*	*-1.5*
rpoA	RNA polymerase subunit RNA polymerase subunit alpha	*1.1*	*-0.5*
rpoB	RNA polymerase subunit RNA polymerase subunit beta	*1.5*	*-0.4*
rpoc	RNA polymerase subunit RNA polymerase subunit beta'	*1.6*	*0.4*
rpod	RNA polymerase, transcription initiation factor sigma	*0.8*	*-0.8*
tufA	Elongation factor Tu	*1.1*	*-0.7*

Footnotes:

1 - Hydropathy scores were predicted according to Claros *et al* (1995).

References:

Claros MG, Perea J, Shu Y, Samatey FA, Popot JL and Jacq C. 1995. Limitations to *in vivo* import of hydrophobic proteins into yeast mitochondria. The case of a cytoplasmically synthesized apocytochrome b. Eur J Biochem **228**:762-771.

Table S3. EST analysis.

Cluster ID	No. of ESTs	AutoFACT Annotation[1]	Rapid Annotation Name[1]	Rapid Annotation Score
RAL00003938	3	Mitochondrial import inner membrane translocase subunit TIM10 related cluster	Mitochondrial import inner membrane translocase subunit TIM10 related cluster	1.00e-08
RAL00003758	2	Mitochondrial intermembrane space translocase subunit Tim9, putative related cluster	Translocase of inner membrane TIM9 related cluster	4.00e-05
RAL00006751	1	Mitochondrial intermembrane space translocase subunit Tim9, putative related cluster	Translocase of inner membrane TIM9 related cluster	4.00e-05
RAL00003642	3	Probable mitochondrial processing peptidase alpha subunit, mitochondrial precursor related cluster	Mitochondrial processing peptidase alpha subunit, mitochondrial precursor related cluster	2.00e-41
RAL00006561	2	Putative mitochondrial processing peptidase related cluster	F1C9.12 mitochondrial processing peptidase beta subunit, putative	4.00e-94
RAL00006644	1	Tim17 domain containing protein		2.00e-18
RAL00003686	1	Similar to sp|Q12328 Saccharomyces cerevisiae YDL217c TIM22 related cluster	T22K18.6 mitochondrial import inner membrane translocase subunit Tim17/Tim22/Tim23 family protein	2.00e-16
RAL00001769	1	T24P15.12 mitochondrial import inner membrane translocase subunit Tim17/Tim22/Tim23 family protein	T24P15.12 mitochondrial import inner membrane translocase subunit Tim17/Tim22/Tim23 family protein	2.00e-06
RAL00001943	1	Inner mitochondrial membrane peptidase 2 related cluster	Inner mitochondrial membrane peptidase 2 related cluster	5.00e-19

RAL00004675	1	Inner mitochondrial membrane peptidase 2 related cluster	Inner mitochondrial membrane peptidase 2 related cluster	1.00e-20

Footnotes:

1 - Annotations in the Taxonomically Broad expressed sequence tag Database (TBestDB; http://tbestdb.bcm.umontreal.ca/) were used to identify candidate TOMs and TIMs. The candidate sequences were extracted and analyzed for conserved sequence features using hidden Markov models previously constructed for each of the TOM and TIM components (Dolezal et al. 2006; Likic et al. 2010).

References:

Dolezal P, Likic V, Tachezy J and Lithgow T. 2006. Evolution of the molecular machines for protein import into mitochondria. Science **313**:314-318.

Likic VA, Dolezal P, Celik N, Dagley M and Lithgow T. 2010. Using hidden markov models to discover new protein transport machines. Methods Mol Biol. **619**: 271-284.