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Supplemental Information

Mitochondrial Preprotein Translocase

of Trypanosomatids Has a Bacterial Origin

Mascha Pusnik, Oliver Schmidt, Andrew J. Perry, Silke Oeljeklaus, Moritz Niemann, Bettina Warscheid, Trevor Lithgow, Chris Meisinger, and André Schneider

Supplemental Inventory

Figure S1, related to Figure 1 Figure S2, related to Figure 2 Figure S3, related to Figure 3 Figure S4, related to Conclusion

	1 120
Trypanosoma brucei	
Trypanosoma cruzi	
Leishmania braziliensis	
Leishmania major	
Serratia proteamaculans	MPRY
Fecherichia coli	MLRY
Salmonella enterica	MX1
Dickeva zeae	nerii
	121 240
Trypanosoma brucei	
Trypanosoma cruzi	-MLMEWLRGKSEEVSAAPEKAVRRKLLDVDPETPLEPSPAEA-ASQREEKFRRKLAASAKEGPTSPAKL
Leishmania braziliensis	MLKEWLRGSSSEAEKPVE-KKPVSRMVVDAEKPLE-THESA-A <mark>S</mark> KRR <mark>ELY</mark> EKKLAESAKEGPLHPSKT
Leishmania major	MLKEWLRGSSSEAEKPAEEKKPVSRMVVDAEKPLE-THESA-A <mark>S</mark> KRREA¥EKKLAESAKEGPPHPSKM
Serratia proteamaculans	RIAGANIVLEGGAKTDDDVLALVKKGKFTIGBILNHGKYDSFKGSLTGLALRKGYFDADLTKSQLGVABELHKAYWDIDFNSGQRYRFGQVKFTGSQIREDYLQNLVPFHQGDY
ersinia pseudotuberculosis	LIAGVDIVLQGGAKTOPDIVALVRADTPKIGSVINHGDPDNPTSSLTGLALRRGYPDANHIKSQLGVAAQLHEAFWDIDPDSGRYRFGKVIFGGSQIREDTLQNUV
Escherichia coli	
Salmonella enterica	LIGGISVILKGGARTUKDILALIAL - KYALGIVINGGU DUKAKO LIGVOLKAGIVOLGI IKQUSILALGRUKA NU DIDIOGRI KYGYVIEGO UKULI UKUL
Dickeya zeae	YTKOPOTYTKOPAĞÖDDIĞĞIYAKĞYKLAYODILMBOYIDDIYMPTGILDALLAMAĞTAYHLƏLIĞAHMATDIDDƏLYIKLAKYADAĞIĞDALDQAL
	241 360
Trypanosoma brucei	VAYDTLLRPLORILLDGNAAETQEGLTISVARNAQNVMMSTKTMLVSPQMSNWELSLQMNGFSDIVVATYNTLSRWSLMYQRVSSTGALLFAQCMAQRQQMTQGTVVGHIQYPW
Trypanosoma cruzi	VSYESHFRGAORLLMEGNFGEINEGLTLNVARNAONVHISSKCVLLSPOMSHWEVSFOMNGFSDIVAASYNTLSRWSLMYORVSSTGALLLAOCMAOROOGMTOGTVVGHLOYFW
Leishmania braziliensis	VTYDGIPRKTQGVLLEGNNNEIQEGITLNIARNAQNTHISTKWALVNPQNSHWEVNLQMNGFSDIVAASWNTLNRYQLHYQRVFSTGAMLVTQFMAQKQGGLSQGTVFAKMQYPW
Leishmania major	ITYDGIFRKAQGVLLEGNNNEIQEGITLNIARNAQ <mark>NAMISTKWTLVNPQNSHWEVNLQNNGFSDIVAASWNTLNRYQLNYQRVSSTGAMLVTQFMAQKQGGMSQ</mark> GTVFAMTQYPW
Serratia proteamaculans	YSSEDLAELNRRLSATNWFNSVVVSPDFKDAKENKILPLDALVSPRTRN-TIETGVGYSTDVGPRIKGTWKKPWLNDRGHSLETSASVSAPEQQLDLTYKIPLLKNPLEQYYL
ersinia pseudotuberculosis	YTSDELAELNRRLAATNWPNSVVVSPDPQDAKESKILPLDAVVTPRTEN-TVELGGGYATDVGPRLTASWRRPWMNSFGHSITTTTALSAPEQTLDPSYRIPLERNPLEGYL
Escherichia coli	ISSKULABLENKLSATGWPRSVVVARQFDKARETKVLPLTGVVSKRTEN-TIETGVQGISTDVQFRVKATWKRPWRDSGGISTTSTSISAFEQTLDFSIKMPLLKNPLEQTLL
Saimonella enterica	ISSAULASLINKALGALUNG NOVVARSESSAGALIKISLAGVORTISKALGULGULGULGULGULGULGULGULGULGULGULGULGUL
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	161 480
Trypanosoma brucei	VQGGCTQVQYVKDQSFSVSHAQRLIRGFYLGSNLSWDALTKGTSLSY-AGCITNPSKTGSLAAEWSPSKGEWKVGLTRSDWASDVEFAMQLEYTKKGQSGLMGLLSFGT
Trypanosoma cruzi	VQ <mark>GG</mark> CTAVQYYKNQSFTLSHAQRIIRGLHVGSSLSWDPMTKGTSLSH-GFTAVNSLKKGSLSGDWTPSKGEWKLAITKKDWAHDAEFAMQLDHTQKDQD-MTSQLSFGL
Leishmania braziliensis	RFGGCTQVQYVKDQSLSLSHVQRLIRGVHIGTNLTFEPATHSSYLSH-AFTAVNSLKKGSLSGDWTPSKGEWKLAITKKDWAHDAEFAMQLDHTQKDQD-MTSQLSFGL
Leishmania major	RFGGCTQVQYVKDQSLGLSHVQRLIRGVHVGTNLTVEPTSHNSYLSH-AISLMTAKRDAGFMAEMTPSKGTWKIAATAFDWAVNMDAAIELEYME-GRESMKSALNVGC
Serratia proteamaculans	LQGGFRNVDLMDTKSVTSKAVVSRNWDLSSGWQRALNLTWRLDHFTQGNVTNTTMLLYPGVSVNRTRSGGLMPTWGDSQ-RYSIDVSDSTWGSDVDPALNQAQNVWIRTLAD
ersinia pseudotuberculosis	IQGGFRATDLADTNSDTTTLAVAR#WDLSSGWQRAINLKWSLOHFTQGKVTDTTMLLTPGVSINNTKQRGGAMPWGDSQ-RYSIDVSDTTWGSDVDFGIPQAQVV
Escherichia coli	VQCOTACT DIMOTEODCTTLANGAL HDLOGGYGAL ALANGDDET VQCLTAL ALAD TYGVALGAL AGAGGAL MENGAGAL AGAGAV DE SVEVYAQAV
Dickeva zeae	LOGGFKERDLNDTOSDSTSFNLARVWELSSGWORAVNLEWTLDHFTOANVTNTTMLVYPGVSFNRTRORGELMPDWGDTO-RYSVDVSNTLWGSDIDFAVPGAONVWIRTLAE
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Trypanosoma brucei	KKQFVGGGSVSTAMLG-FSQLKAVVEVPFGGDRSGFNQFMCMYNALYDIHSGGLKHGVVFTA
Trypanosoma cruzi	KKQFIGGSSMSAVLSG-FSVVKAAVGLPFGGERIGLNQMHCKYNVHYNIRSGALKHGLVFTA
Leishmania braziliensis	RKSPYGGAQLTFSLLG-FNIAKVNLDLPPGGEVP GTAQLRLTPNCQYDINSGALKQGLIFTA
Leishmania major	RKSFYGGAQUTTELLG-FSMARVNLDUPFGEVP VUBPUDGAQUTELLG-FSMARVNLDUPFGEVP
ersinia pseudotuberculosis	AND TARGET AND ANT TEAM TO AND ANT AND
Escherichia coli	RHRFVTRGTLGWIETGDFDKVPPDLRFPAGGDRSIRGYKYKSIAPKYANGDLKGASKL-ITGSLEYQYNVTGKWWGAVFVDSGEAVSDIRRSDFKTGTGVGVKWESPVGFIKLDFAVFVA
Salmonella enterica	RHRFVMRANLGWIETGDFDKVPPDLRFFAGGDRSIRGYKYKSISPKDSDGNLKGASKL-ATGSLEYQYNVTGKWWGAVFVDSGEAVSDIRRSDFKTGTGVGVRWASPVGPIKLDFAVPVG
Dickeya zeae	${\tt khr} {\tt vtranl} {\tt gwiet} {\tt gs} {\tt rvps} {\tt rfp} {\tt aggdrs} {\tt irgykyksvsprdsdgklt} {\tt gaskl-atgs} {\tt rvgvnltgkwwgavf} {\tt ids} {\tt gasklvdlvrtnlktgagvgvrwaspvgpvkldiarpig}$
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Trypanosoma brucei	
Trypanosoma cruzi	
Leishmania braziliensis	
Leisomania maior	

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Leishmania major Serratia proteamaculans DKDTHGVQFYIGLGPEL Tersinia pseudotuberculosis DNETHGVQFYIGLGPEL Escherichia coli DKDEHGLQFYIGLGPEL Salmonella enterica DKDEHGLQFYIGLGPEL Dickeya zeae DDDKHGVQFYIGLGPEL

Figure S1.

Figure S1. Multiple Sequence Alignment of ATOM Homologs from Trypanosomatides and YtfM-like Proteins from Bacteria

Analysis was performed using CLUSTAL W (1.83). GI of sequences are 70831434 (*Trypanosoma brucei*), 71659630 (*Trypanosoma cruzi* strain CL Brener), 154345277 (*Leishmania braziliensis*), 72549827 (*Leishmania major* strain Friedlin), 157368706 (*Serratia proteamaculans*), 153948759 (*Yersinia pseudotuberculosis*), 209398307 (*Escherichia coli*), 198243763 (*Salmonella enterica*), 251788425 (*Dickeya zeae*). Colours indicate regions of high (red) and low (blue) homology.



Figure S2. In Vivo Assembly of ATOM Requires Sam50

(A) Growth curve of uninduced and tet-induced Sam50 RNAi cell line. Northern blot indicates efficient ablation of Sam50 mRNA in induced cells. The ethidium bromide (EtBr) stained region of the gel depicting the rRNAs serves as a loading control.

(B) Levels of HA-tagged ATOM (ATOM-HA) and VDAC in total cellular extracts rapidly decline during tet-induction of Sam50 RNAi cells, whereas the levels of elongation factor 1a (EF-1a) are not affected.



Figure S3. RNAi of ATOM Does Not Induce a General Mitochondrial Dysfunction

(A) Mitotracker staining shows that ATOM RNAi cells (+Tet, 48 hours) have an intact membrane potential. CCCP, carbonyl cyanide m-chlorophenylhydrazone; DIC, differential interference contrast microscopy.

(B) Mitochondrial oxidative (OXPHOS) and mitochondrial substrate level phosporylation (SUBPHOS) is not affected in induced ATOM RNAi cells (+Tet, 48 hours).



Figure S4. Models for the Evolution of the Mitochondrial Outer Membrane Protein Translocase

(A) YtfM/ATOM-type bacterial protein exporter but not Tom40 were present in the original endosymbiont. The bacterial exporter, adapted to function in reverse, was comandeered for mitochondrial protein import in the last eukaryotic common ancestor and has been retained in the line leading to the trypanosomatids. Tom40 evolved after the ancestor of trypanosomatids diverged from the rest of the eukaryotes. The ATOM in the main branch of eukaryotes was subsequently lost.

(B) As A but ATOM was aquired by horizontal gene transfer and not by the original endosymbiontic event.

(C) ATOM, either acquired by the endosymbiontic event or by horizontal gene transfer, evolved into Tom40 after the trypanosomatids split from all other eukaryotes. Yellow and blue indicate bacterial and eukaryotic evolutionary origins, respectively.