place the root of the eukaryotic tree within the superior group of Euglenozoa or between Euglenozoa and other eukaryotes [9].

Our analyses show that ATOM represents the missing Tom40 protein in the mitochondria of *T. brucei* and of other trypanosomatids with no clear link to the bacterial proteins. Given that all eukaryotes analysed to date contain a Tom40 homologue, we propose that all mitochondria of current eukaryotes descended from an ancestral Tom40-containing mitochondrial compartment (Figure 1).

#### Supplemental Information

Supplemental Information includes one figure and can be found with this article online at doi: 10.1016/j.cub.2012.03.057.

#### Acknowledgements

This work was supported by grants from the Czech Science Foundation (P305-10-0651 (P.D.) and LC07032 (J.T.)) and by a grant from the Ministry of Education of the Czech Republic (MSM 0021620858 (J.T.)).

#### References

1. Hewitt, V., Alcock, F., and Lithgow, T. (2011). Minor modifications and major adaptations: the evolution of molecular machines driving mitochondrial protein import. Biochim. Biophys. Acta *1808,* 947–954.
2. Pusnik, M., Charriebre, F., Mäser, P., Waller, R.F., Dagley, M.J., Lithgow, T., and Schneider, A. (2009). The single mitochondrial porin of Trypanosoma brucei is the main metabolite transporter in the outer mitochondrial membrane. Mol. Biol. Evol. *26,* 671–680.
3. Pusnik, M., Schmidt, O., Perry, A.J., Oeljeklaus, S., Niemann, M., Warscheid, B., Lithgow, T., Meisinger, C., and Schneider, A. (2011). Mitochondrial preprotein translocase of trypanosomatids has a bacterial origin. Curr. Biol. *21,* 1738–1743.
4. Zeth, K. (2010). Structure and evolution of mitochondrial outer membrane proteins of beta-barrel topology. Biochim. Biophys. Acta *1797,* 1292–1299.
5. Dagley, M.J., Dolezal, P., Likic, V.A., Smid, O., Purcell, A.W., Buchanan, S.K., Tachezy, J., and Lithgow, T. (2009). The protein import channel in the outer mitosomal membrane of Giardia intestinalis. Mol. Biol. Evol. *26,* 1941–1947.
6. Dolezal, P., Dagley, M.J., Kono, M., Wolynec, P., Likicb, V.A., Foo, J.H., Sedinovab, M., Tachezy, J., Bachmann, A., Bruchhaus, I., *et al.* (2010). The essentials of protein import in the degenerate mitochondrion of Entamoeba histolytica. PLoS Pathog. *6,* e1000812.
7. Likic, V.A., Dolezal, P., Celik, N., Dagley, M., and Lithgow, T. (2010). Using hidden markov models to discover new protein transport machines. Meth. Mol. Biol. *619,* 271–284.
8. Söding, J., Biegert, A., and Lupas, A.N. (2005). The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Res. *33,* W244–W248.
9. Cavalier-Smith, T. (2010). Kingdoms Protozoa and Chromista and the eozoan root of the eukaryotic tree. Biol. Lett. *6,* 342–345.

Charles University in Prague, Faculty of Science, Department of Parasitology, Vinicna 7, 128 44, Prague 2, Czech Republic.
*E-mail: paveldolezal@yahoo.com

# Response to Zarsky *et al.*

**Mascha Pusnik[1], Oliver Schmidt[2], Andrew J. Perry[3], Silke Oeljeklaus[4], Moritz Niemann[1], Bettina Warscheid[4], Chris Meisinger[2], Trevor Lithgow[3], and André Schneider[1,*]**

Mitochondria evolved from an α-proteobacterial endosymbiont and recent phylogenetic and function-based research has demonstrated that the major pieces of the protein transport machinery were inherited from the symbiont. This includes the SAM machinery for assembly of outer membrane proteins and the TIM machinery for protein transport across, and assembly into, the mitochondrial inner membrane [1–3]. Hidden Markov model (HMM) analysis, which enables a broad, all-encompassing approach for identifying protein homologies, has been very important in detecting members of protein families that are not easily recognized by simple BLAST-based comparisons [1]; HMM searches initially failed to find a Tom40 protein in one group of eukaryotes, the kinetoplastids. These organisms, which include the experimentally-tractable *Trypanosoma brucei*, have highly developed mitochondria that have evolved from the same ancestor as mitochondria in other eukaryotes. The initial failure to identify a Tom40 homolog in *T. brucei* was both surprising and exciting.

In our paper in *Current Biology* [4] we directly assayed for protein transport function and thereby discovered the archaic protein translocase in the outer mitochondrial membrane (ATOM). In seeking related protein sequences, using E < 0.005 the PSI-BLAST search identifies only the kinetoplastid ATOM sequences. But, at a lower significance, a sub-class of Omp85 protein sequences, referred to as the YtfM/TamA group (but not Tom40 sequences) are found and the top-scoring one was manually added into the first-round outcome from the PSI-BLAST. Multiple sequence alignments using the ATOM from *T. brucei* and related organisms suggested, albeit not at statistically significant levels, an affinity to a sub-class of Omp85 proteins referred to as the YtfM group, and the putative relationship between trypanosomatid ATOMs and YtfMs was further visualized using CLANS [4]. Since YtfM is found in the α-proteobacteria, from which mitochondria evolved, one prospect was that the ATOM evolved from the YtfM in the endosymbiont's outer membrane. A second model for the evolution of the ATOM allowed for the possibility of a lateral gene transfer (LGT) early in the evolution of mitochondrial protein transport. We also raised a third model that holds Tom40 and ATOM evolved from a common ancestor. These models proposed in the original paper [4], are summarized in Figure 1. We remain open-minded on which model best explains the evolution of the pathway for protein translocation across the outer mitochondrial membrane.

In their correspondence, Zarsky *et al.* [5] argue that the ATOM is not related to YtfM-type Omp85 proteins, but is exclusively similar to the Tom40 family of proteins and that the ATOM evolved from a Tom40 progenitor. This is an attractive idea in the sense that it would be a unifying theory, with the implication being that all eukaryotes simply have a Tom40 translocase in their outer mitochondrial membrane, with some more easily recognized than others. However, two important observations need also be kept in mind.

Firstly, using HMMs based on the broad diversity of Tom40 sequences, ATOM was not initially detected in *T. brucei* [6]. This gives a context to just how divergent the ATOM and other Tom40 proteins are, given that this same type of HMM approach has succeeded in finding highly diverse Tom40 sequences in *Entamoeba* [7] and *Giardia* [8]. By broadening the search criteria with a goal to capture all members of the mitochondrial porin protein family (i.e. isoforms of Tom40 and VDAC), Flinner *et al*. [9] recently showed that *T. brucei* has two further prospective mitochondrial porins that might play a role in ion transport: their analysis did not detect ATOM.

Secondly, the ATOM protein sequence has predicted secondary structural features that seem to be consistent with a POTRA-type amino-terminal domain (data not shown) and a predicted β-barrel domain of comparable size to other members of the YtfM/TamA-family of proteins. POTRA domains are not found in Tom40 (or other mitochondrial porins), which have instead a simple amino-terminal helix [10]. With the size and characteristics of the β-barrel

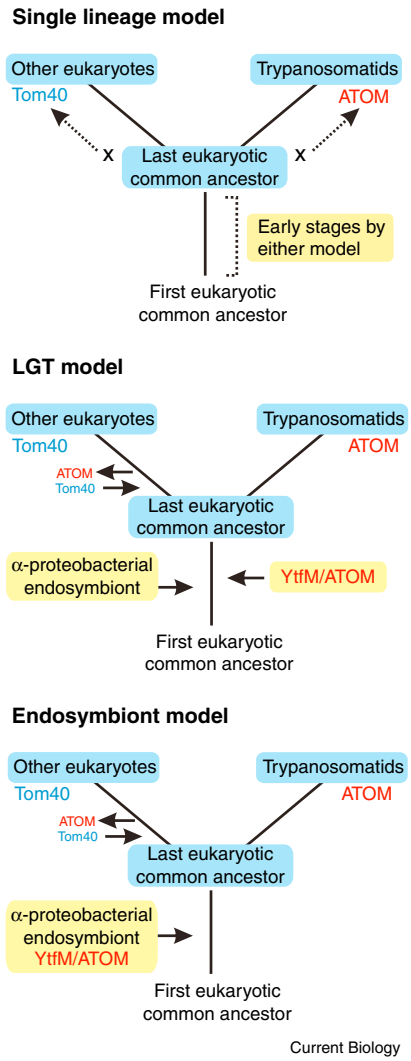


Figure 1. The three models for the evolution of ATOM and Tom40 [6], see text for details. "X" refers to a putative common ancestor to both Tom40 and ATOM.

domain and the structure of the amino-terminal domain being distinguishing features of the mitochondrial porins and the Omp85 family, biophysical and structural analyses of ATOM will be important in moving beyond sequence analysis and to reach a better understanding of the evolution of Tom40 and ATOM.

It is possible that both Tom40 and ATOM were derived from a common ancestor (Figure 1, 'single lineage model'). In our paper [4] we proposed this and two other models, and we believe that it would be premature at this stage to reject the other two possibilities from consideration. Each model in Figure 1 is built on a common scenario for the early evolutionary history of eukaryotes: that the last common ancestor lacked a bacterial protein that would be recognizable as a member of the mitochondrial porin protein family, but had a protein translocase that served to import proteins across the outer mitochondrial membrane. We propose that this protein translocase was of bacterial origin, and had an amino-terminal domain with features common to POTRA domains. In the 'endosymbiont model', ATOM evolved from the bacterial translocase present in the original endosymbiont. A variation of this model would allow that the ancestor of ATOM was derived by lateral gene transfer from other bacterial sources (Figure 1, 'LGT model'). In these first two models, the ATOM function is subsequently replaced by Tom40, which then serves as the common core in the TOM complex of all eukaryotes bar the trypanosomes [1]. In the 'single lineage model', Tom40 evolved from an ancestral ATOM. The Tom40-type sequence features detected in ATOM by Zarsky *et al*. [5] would be fully explained by this evolutionary path.

### References

1. Dolezal, P., Likic, V., Tachezy, J., and Lithgow, T. (2006). Evolution of the molecular machines for protein import into mitochondria. Science *313*, 314–318.
2. Alcock, F., Clements, A., Webb, C., and Lithgow, T. (2010). Evolution. Tinkering inside the organelle. Science *327*, 649–650.
3. Lithgow, T., and Schneider, A. (2010). Evolution of macromolecular import pathways in mitochondria, hydrogenosomes and mitosomes. Philos. Trans. R. Soc. Lond. B Biol. Sci. *365*, 799–817.
4. Pusnik, M., Schmidt, O., Perry, A.J., Oeljeklaus, S., Niemann, M., Warscheid, B., Lithgow, T., Meisinger, C., and Schneider, A. (2011). Mitochondrial preprotein translocase of trypanosomatids has a bacterial origin. Curr. Biol. *21*, 1738–1743.
5. Zarsky, V., Tachezy, J., and Dolezal, P. (2012). Tom40 is common to all mitochondria. Curr. Biol. *22*, R479–R481.
6. Pusnik, M., Charrière, F., Mäser, P., Waller, R.F., Dagley, M.J., Lithgow, T., and Schneider, A. (2009). The single mitochondrial porin of *Trypanosoma brucei* is the main metabolite transporter in the outer mitochondrial membrane. Mol. Biol. Evol. *26*, 671–680.
7. Dolezal, P., Dagley, M.J., Kono, M., Wolynec, P., Likić, V.A., Foo, J.H., Sedinová, M., Tachezy, J., Bachmann, A., Bruchhaus, I., and Lithgow, T. (2010). The essentials of protein import in the degenerate mitochondrion of *Entamoeba histolytica*. PLoS Pathog. *6*, e1000812.
8. Dagley, M.J., Dolezal, P., Likic, V.A., Smid, O., Purcell, A.W., Buchanan, S.K., Tachezy, J., and Lithgow, T. (2009). The protein import channel in the outer mitosomal membrane of *Giardia intestinalis*. Mol. Biol. Evol. *26*, 1941–1947.
9. Flinner, N., Schleiff, E., and Mirus, O. (2012). Identification of two voltage-dependent anion channel-like protein sequences conserved in Kinetoplastida. Biol. Lett. Jan 4, epub ahead of print, PMID: 22219392.
10. Zeth, K. (2010). Structure and evolution of mitochondrial outer membrane proteins of beta-barrel topology. Biochim. Biophys. Acta *1797*, 1292–1299.

[1]Department of Chemistry and Biochemistry, University of Bern, CH-3012 Bern, Switzerland. [2]Institut für Biochemie und Molekularbiologie, ZBMZ and BIOSS (Centre for Biological Signalling Studies), Universität Freiburg, Freiburg, Germany. [3]Department of Biochemistry & Molecular Biology, Monash University, Clayton 3800, Australia. [4]Faculty of Biology and BIOSS (Centre for Biological Signalling Studies), University of Freiburg, 79104 Freiburg, Germany.
*E-mail: andre.schneider@ibc.unibe.ch

# Lawrence's book review unfair to Hoffmann

**Alan Ezekowitz[1], Jean-Luc Dimarcq[2], Fotis Kafatos[3], Elena Levashina[4], Dominique Ferrandon[5], Charles Hetru[5], Jean-Luc Imler[5], and Jean Marc Reichhart[5]**

Peter Lawrence [1], in his otherwise scholarly review of a book by Peter Pringle "*Experiment Eleven*", is incorrect in asserting that Jules Hoffmann took unfair credit for discoveries made in his laboratory, like exemplars described in the book. As close witnesses to events we know that Hoffmann assembled and animated a group of researchers from various scientific backgrounds to decipher the mechanisms of innate immunity in insects, and has been impeccable in his assignment of credit and support for his co-workers both while they served in his laboratory and in their future independent careers.

All signatories are either long-standing collaborators or co-workers of Professor Jules Hoffmann.

### Reference

1. Lawrence, P. (2012). Rank, reinvention and the Nobel Prize. Curr. Biol. *22*, R214–R216.

[1]Abide Therapeutics, 230 Nassau Street Princeton, NJ 08540, USA. [2]Scientific Foundation IHU Strasbourg, 1, place de l'Hôpital, 67091 Strasbourg Cedex, France. [3]Imperial College London, South Kensington Campus, London SW7 2AZ, UK. [4]Vector Biology Laboratory, Max Plank Institute für Infektionsbiologie, Charitéplatz 1, Campus Charite Mitte; D–10117 Berlin, Germany. [5]CNRS UPR9022, Institut de Biologie Moléculaire et Cellulaire, Université de Strasbourg, 15 rue Descartes, 67084 Strasbourg cedex, France.
E-mail: alan@abidetx.com