RESEARCH NOTE

REVISED The ubiquitous and ancient ER membrane protein complex (EMC): tether or not? [version 2; referees: 2 approved, 1 approved with reservations]

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Abstract

The recently discovered endoplasmic reticulum (ER) membrane protein complex (EMC) has been implicated in ER-associated degradation (ERAD), lipid transport and tethering between the ER and mitochondrial outer membranes, and assembly of multipass ER-membrane proteins. The EMC has been studied in both animals and fungi but its presence outside the Opisthokont clade (animals + fungi + related protists) has not been demonstrated. Here, using homology-searching algorithms, I show that the EMC is truly an ancient and conserved protein complex, present in every major eukaryotic lineage. Very few organisms have completely lost the EMC, and most, even over 2 billion years of eukaryote evolution, have retained a majority of the complex members. I identify Sop4 and YDR056C in *Saccharomyces cerevisiae* as Emc7 and Emc10, respectively, subunits previously thought to be specific to animals. This study demonstrates that the EMC was present in the last eukaryote common ancestor (LECA) and is an extremely important component of eukaryotic cells even though its primary function remains elusive.

Open Peer Review

Referee Status: ✓ ✓ ?

	Invited Referees 1	2	3
REVISED version 2 published 05 Oct 2015	✓ report	✓ report	? report
version 1 published 25 Aug 2015	? report	✓ report	

1 **Sujoy Lahiri**, University of Virginia USA

2 **Courtney Stairs**, Dalhousie University Canada

3 **Christopher Loewen**, University of British Columbia Canada

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Comments (0)

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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: No competing interests were disclosed.

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REVISED **Amendments from Version 1**

In response to reviewer comments, the results and discussion have been expanded to include a short section on Emc8 and Emc9 and a supplementary phylogenetic tree (Figure S1). The analysis demonstrates that Emc8 and Emc9 are vertebrate-specific paralogues resulting from a duplication of an ancestral protein in the lineage leading to vertebrates (Phylogenetic methods appear in the figure legend to Figure S1 - Associated references have also been added). These results suggest that vertebrate Emc8 and Emc9 should be renamed Emc8a and Emc8b, respectively. Figure 1 has been modified accordingly.

See referee reports

Introduction

Recent studies suggest that the EMC (Endoplasmic Reticulum Membrane Complex) is a multifunctional, multi-subunit protein complex. In *Homo sapiens*, the EMC comprises ten subunits, Emc1-10, whereas in *Saccharomyces cerevisiae* the complex comprises only Emc1-6 (Jonikas *et al.*, 2009). The EMC has been implicated in several cellular processes. It has been implicated in ERAD (ER-associated degradation) (Christianson *et al.*, 2012; Jonikas *et al.*, 2009; Richard *et al.*, 2013) but the molecular mechanism for how EMC triggers ERAD has remained elusive. Emc6 contains a Rab5 interacting domain and has been shown to interact with Rab5A in humans during autophagosome formation (Li *et al.*, 2013). It has also been shown that the EMC is an ER-mitochondria tether in *S. cerevisiae* that interacts with the outer membrane protein Tom5 of the TOM (Translocase of the Mitochondrial Outer Membrane) complex (Lahiri *et al.*, 2014). Most recently, the EMC has been implicated in the proper assembly of multi-pass transmembrane (TM) proteins (Satoh *et al.*, 2015). These recent findings suggest that EMC involvement in ERAD may be due to secondary effects, as cells devoid of EMC components may result in either disruption of ER-mitochondria tethering, or the misfolding of multipass membrane proteins. Thus, the primary function of the EMC is still open for debate.

The ER-mitochondria encounter structure (ERMES), also involved in ER-mitochondria tethering, is a multifunctional protein complex implicated in both lipid transfer and mitochondrial outer membrane protein assembly (AhYoung *et al.*, 2015; Kornmann *et al.*, 2009; Meisinger *et al.*, 2006, Meisinger *et al.*, 2007; Wideman *et al.*, 2013; Wideman *et al.*, 2010). However, ERMES as an ER-mitochondria tether is limited to a subset of eukaryote taxa (Wideman *et al.*, 2013), suggesting that a universal ER-mitochondria tethering complex might exist. Lahiri *et al.* (2014) state in their title that the EMC is a conserved protein complex. However, by stating that a protein is conserved, cell biologists and biochemists often simply mean that the protein is present in *S. cerevisiae* (fungi) and animals. Since the clade comprising animals and fungi only accounts for one fifth of the diversity of eukaryotes (Adl *et al.*, 2012), more work is necessary in order to support the claim made by Lahiri *et al.* Thus, I was prompted to investigate the taxonomic distribution of the EMC in order to (1) determine if it *really* is a conserved protein complex and (2) if it could possibly represent the pan-eukaryotic ER-mitochondria tether.

Methods

Sequences of experimentally validated EMC components (see Table S1 for accession numbers) from *H. sapiens* and *S. cerevisiae* were used as queries in BLAST (Altschul *et al.*, 1997) and pHMMer (Finn *et al.*, 2011) searches into the predicted proteomes of 70 organisms spanning the diversity of eukaryotes. Retrieved sequences were considered orthologous if they retrieved the original *H. sapiens* or *S. cerevisiae* EMC sequences as top hits when used as reciprocal BLAST or pHMMer queries into *H. sapiens* or *S. cerevisiae* predicted proteomes and did not retrieve any other closely related sequences (except in the case of Emc8 and Emc9, see below). In cases in which EMC components could not be identified in this manner, transcriptomes and genomes were searched using bioinformatically validated sequences from the previous step that were retrieved from closely related species. Genomes were downloaded from public repositories and genome project websites. See Table S1 for retrieved sequences.

Results and discussion

The EMC is an ancient and highly conserved protein complex

Using homology-searching algorithms EMC candidate proteins were identified in the vast majority of sequenced genomes representing the complete diversity of eukaryotes (Figure 1). Emc8 and Emc9 are homologues but only a single homologue could be detected in most genomes. A subset of opisthokont Emc8/9 sequences were subjected to a phylogenetic analysis demonstrating that vertebrate Emc8 and Emc9 are the result of a vertebrate-specific duplication of Emc8 (Figure S1; see legend for methods). Based on this knowledge, I suggest that the vertebrate Emc8 and Emc9 be renamed Emc8a and Emc8b, respectively.

A complete EMC (Emc1-8, 10) was found in at least one representative from each major lineage including animals, fungi, excavates, amoebozoa, green algae, plants, stramenopiles, alveolates, rhizaria, and haptophytes (Figure 1). The relative sequence conservation of EMC components across diverse taxa suggests that the EMC has an ancient and critical role in cellular function.

Yeast Sop4 and YDR056C are Emc7 and Emc10, respectively

Although previous reports suggest *S. cerevisiae* EMC comprises only six subunits, I identified Sop4 and YDR056C as orthologues of Emc7 and Emc10, respectively. Supporting this, Jonikas *et al.* (2009), the original discoverers of the EMC, show by co-immunoprecipitation analyses that Sop4 and YDR056C are interacting partners of FLAG-tagged Emc3. This experiment not only confirms my bioinformatic classification but also puts into perspective a previous study on Sop4's role in membrane protein quality control (Luo *et al.*, 2002). Furthermore, tracing the evolutionary history of the EMC in fungi demonstrates that Emc8 was lost only in Ascomycetes and a few basally diverging fungi whereas most fungi retain Emc8 (as well as Emc7 and 10).

The EMC has been independently lost in several lineages

Although the EMC was identified in representative taxa from every major eukaryote supergroup, I was unable to identify even a single EMC member in the genomes of the microsporidians *Nosema ceranae* and *Encephalitozoon cuniculi*, the metamonad *Giardia*

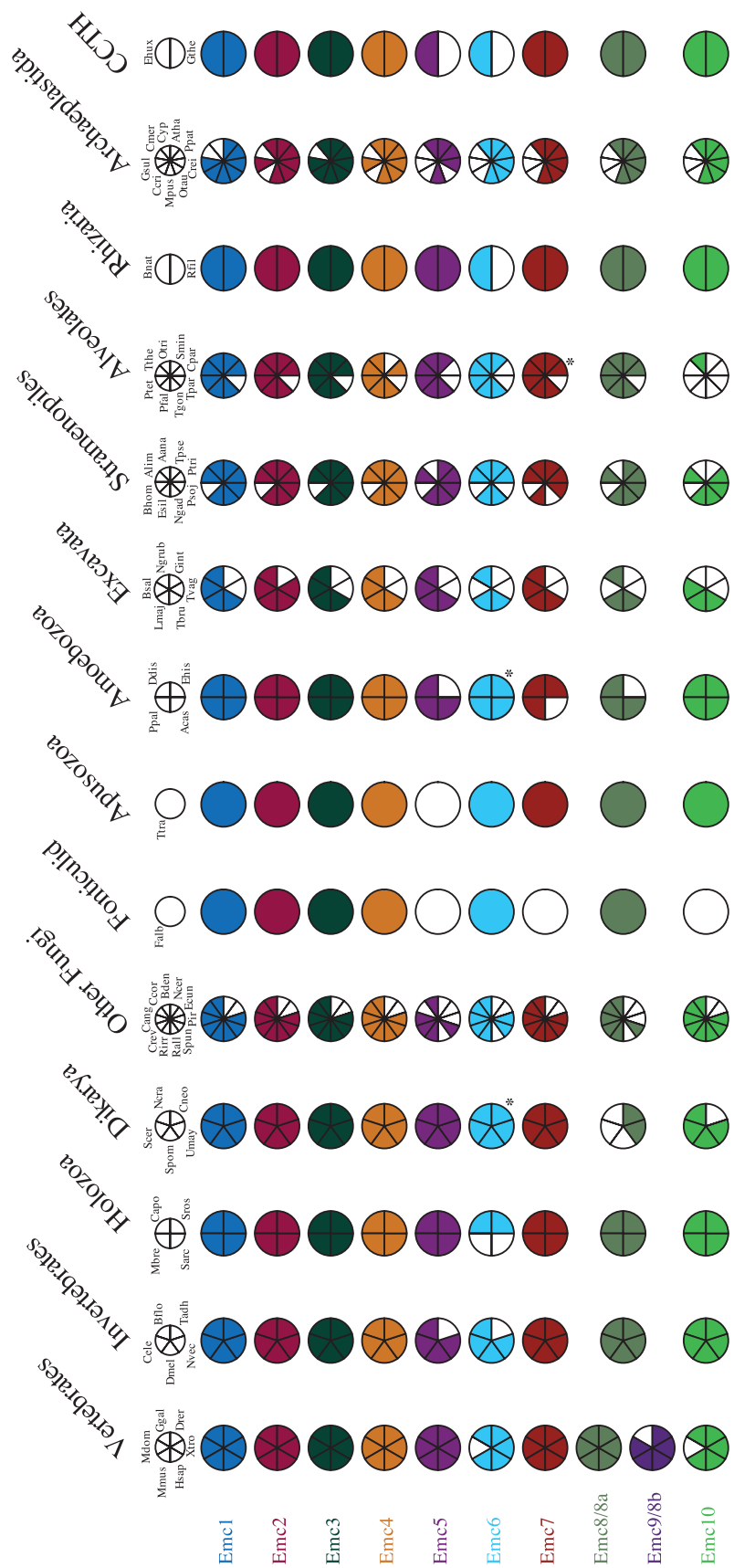


Figure 1. Coulson plot showing distribution of EMC components across eukaryotes. Coloured pies indicate presence of a particular subunit. Plot was generated using the Coulson plot generator (Field *et al.*, 2013). Asterisks indicate presence of orthologue in a different member of the genus but absent in the indicated species (see Table S1). Abbreviations: Vertebrates: Hsap, *Homo sapiens*; Mdom, *Monodelphis domesticus*; Drer, *Danio rerio*; Xtro, *Xenopus tropicalis*; Ggal, *Gallus gallus*; Mmus, *Mus musculus*; Invertebrates: Cele, *Caenorhabditis elegans*; Dmel, *Drosophila melanogaster*, Bflo, *Branchiostoma floridae*; Nvec, *Nematostella vectensis*; Tadh, *Trichoplax adhaerens*; Unicellular Holozoa: Mbre, *Monosiga brevicollis*; Cowc, *Capsaspora owczarzaki*; Sarc, *Sphaeroforma arctica*; Sros, *Salpingoeca rosetta*; Fungi: Spom, *Schizosaccharomyces pombe*; Scer, *Saccharomyces cerevisiae*; Ncra, *Neurospora crassa*; Cneo, *Cryptococcus neoformans*; Umay, *Ustilago maydis*; Bden, *Batrachochytrium dendrobatidis*; Ncer, *Nosema ceranae*; Ecun, *Encephalitozoon cuniculi*; Pir, *Piromyces sp.*; Spun, *Spizellomyces punctatus*; Rirr, *Rhizophagus irregularis*; Crev, *Coemansia reversa*; Ccor, *Conidiobolus coronatus*; Cang, *Catenaria anguillulae*; Rall, *Rozella allomyces*; Apusozoa: Ttra, *Thecamonas trahens*; Fonticulids: *Fonticula alba*; Amoebozoa: Acas, *Acanthamoeba castellanii*; Ddis, *Dictyostelium discoideum*; Ehis, *Entamoeba histolytica*; Ppal, *Polysphondylium pallidum*; Excavata: Ngru, *Naegleria gruberi*; Gint, *Giardia intesinalis*; Tvag, *Trichomonas vaginalis*; Bsal, *Bodo saltans*; Lmaj, *Leishmania major*; Tbru, *Trypanosoma brucei*; Stramenopiles: Bhom, *Blastocystis hominis*; Alim, *Aurantiochytrium limacinum*; Aana, *Aureococcus anophagefferens*; Tpse, *Thalassiosira pseudonana*; Ptri, *Phaeodactylum tricornutum*; Psoj, *Phytophthora sojae*; Esil, *Ectocarpus siliculosus*; Ngad, *Nannochloropsis gaditana*; Alveolates: Ptet, *Paramecium tetraurelia*; Tthe, *Tetrahymena thermophila*; Otri, *Oxytricha trifallax*; Tpar, *Theileria parva*; Smin, *Symbiodinium minutum*; Tgon, *Toxoplasma gondii*; Cpar, *Cryptosporidium parvum*; Ptal, *Plasmodium falciparum*; Rhizaria: Bnat, *Bigelowiella natans*; Rfil, *Reticulomyxa filosa*; Archaeplastida: Crei, *Chlamydomonas reinhardtii*; Cmer, *Cyanidioschyzon merolae*; Cyp, *Cyanophora paradoxa*; Atha, *Arabidopsis thaliana*; Ppat, *Physcomitrella patens*; Otau, *Ostreococcus tauri*; Gsul, *Galdieria sulphuraria*; Mpus, *Micromonas pusilla*; Ccri, *Chondrus crispus*; CCTH: Ehux, *Emiliania huxleyi*; Gthe, *Guillardia theta*.

intestinalis, the stramenopile *Blastocystis hominis*, the alveolate *Theileria parva*, and the red alga *Cyanidioschyzon merolae* (Figure 1 and Figure 2). *Trichomonas vaginalis*, another metamonad retains only a rather divergent Emc2, that passed the test for orthology, but only weakly, suggesting that this protein is under relaxed selection, perhaps repurposed, or in the process of being lost. All other genomes from the remaining 65 species investigated contained clear representatives of EMC homologues (Figure 1).

These disparate organisms that lack the EMC prompted the question: What cellular or biochemical features tie these diverse organisms together? The microsporidians, metamonads and *B. hominis* all contain reduced anaerobic mitochondria-related organelles (MROs) and also lack the EMC. However, the amoebozoan *Entamoeba histolytica* retains Emc1-4, 7 and 10, the apicomplexan *Cryptosporidium parvum* retains Emc1-4, and 8, and the fungus *Piromyces sp*. retains Emc1-4, 6, 7, and 10, but all three organisms also contain extremely reduced MROs. *T. parva* and *C. merolae* contain relatively normal mitochondria but completely lack the EMC. Thus, it seems that further insight into the cell biology of these organisms is required to understand why only these few species from unrelated lineages have lost the EMC. At this point, of the proposed functions of the EMC, its involvement in multipass membrane protein assembly is the best candidate for generalization to other eukaryotes. It explains the connection to ERAD as a secondary effect of misassembled multipass proteins and explains why an organism with extremely reduced mitochondria (*E. histolytica*) might retain the EMC. Finally, although EMC involvement as an ER-mitochondria tether is attractive, the distribution of the only known MOM-localized interactor of EMC (Tom5) has not been identified in organisms other than animals and fungi (Maćasev *et al.*, 2004). Thus, until an ancient interaction partner is identified, the role of EMC as an ancient tether remains speculative.

Conclusions

Since the vast majority of species from each major branch of eukaryotes retain the EMC it can be inferred that it was present in the last eukaryote common ancestor (LECA). Since the sequences of most of the identified EMC homologues are very similar, it can be inferred that its function has likely been retained in most

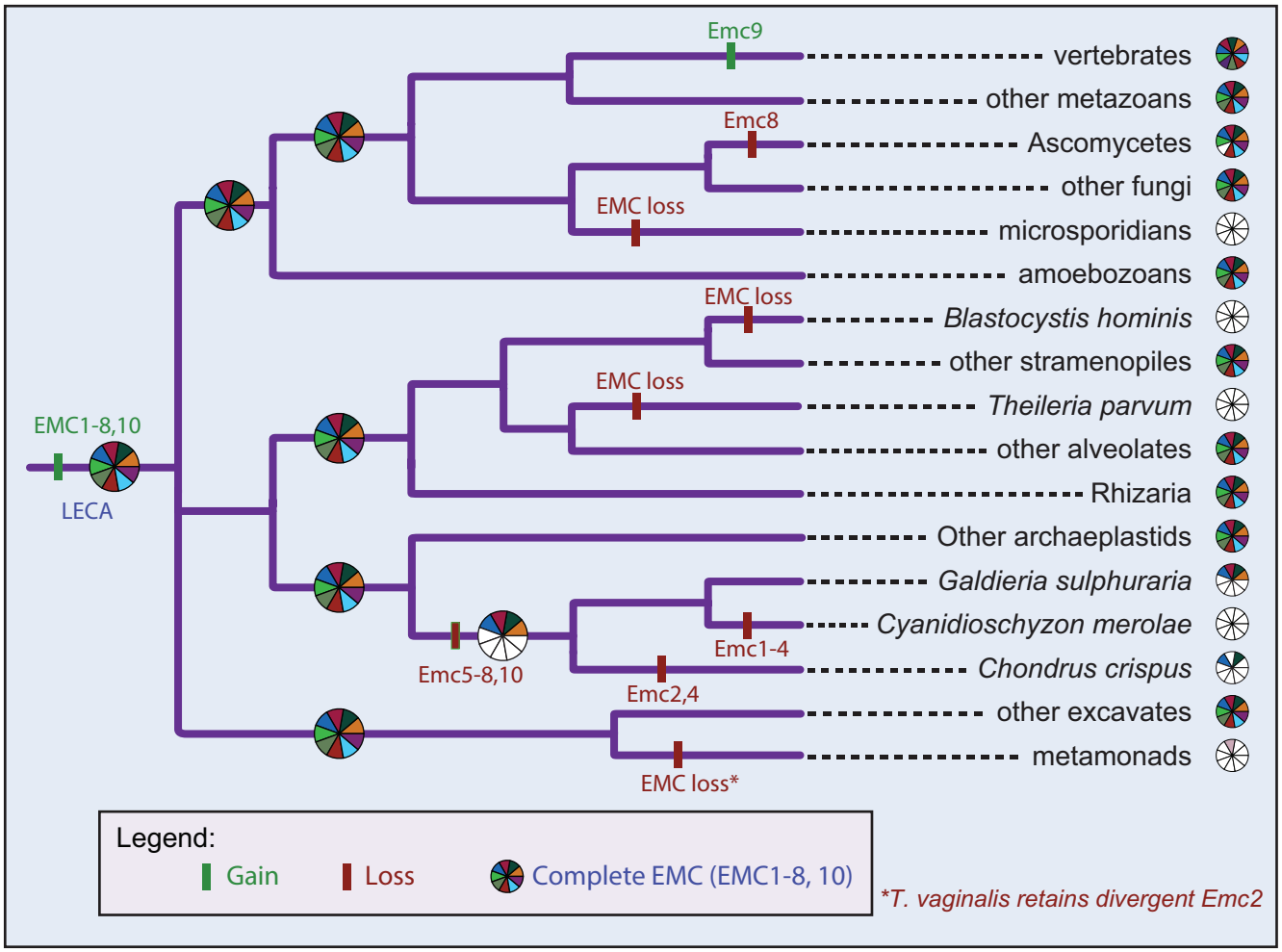


Figure 2. Evolutionary history of the EMC. EMC 1-8 and 10 evolved prior to the divergence of the major eukaryote lineages. Green and red dashes represent gains and losses of EMC components, respectively. Coloured pies are schematic representations of which EMC components were present at different points over the course of evolution.

eukaryote lineages. Thus, the EMC is a generalizable eukaryotic feature as is its function—whatever it might be.

Data availability

All sequence data are freely available in online databases (NCBI, JGI, or independent genome sequencing project websites).

Competing interests

No competing interests were disclosed.

Grant information

JGW is the recipient of the European Molecular Biology Organization (EMBO) Long-term Fellowship (ALTF 761-2014).

I confirm that the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements

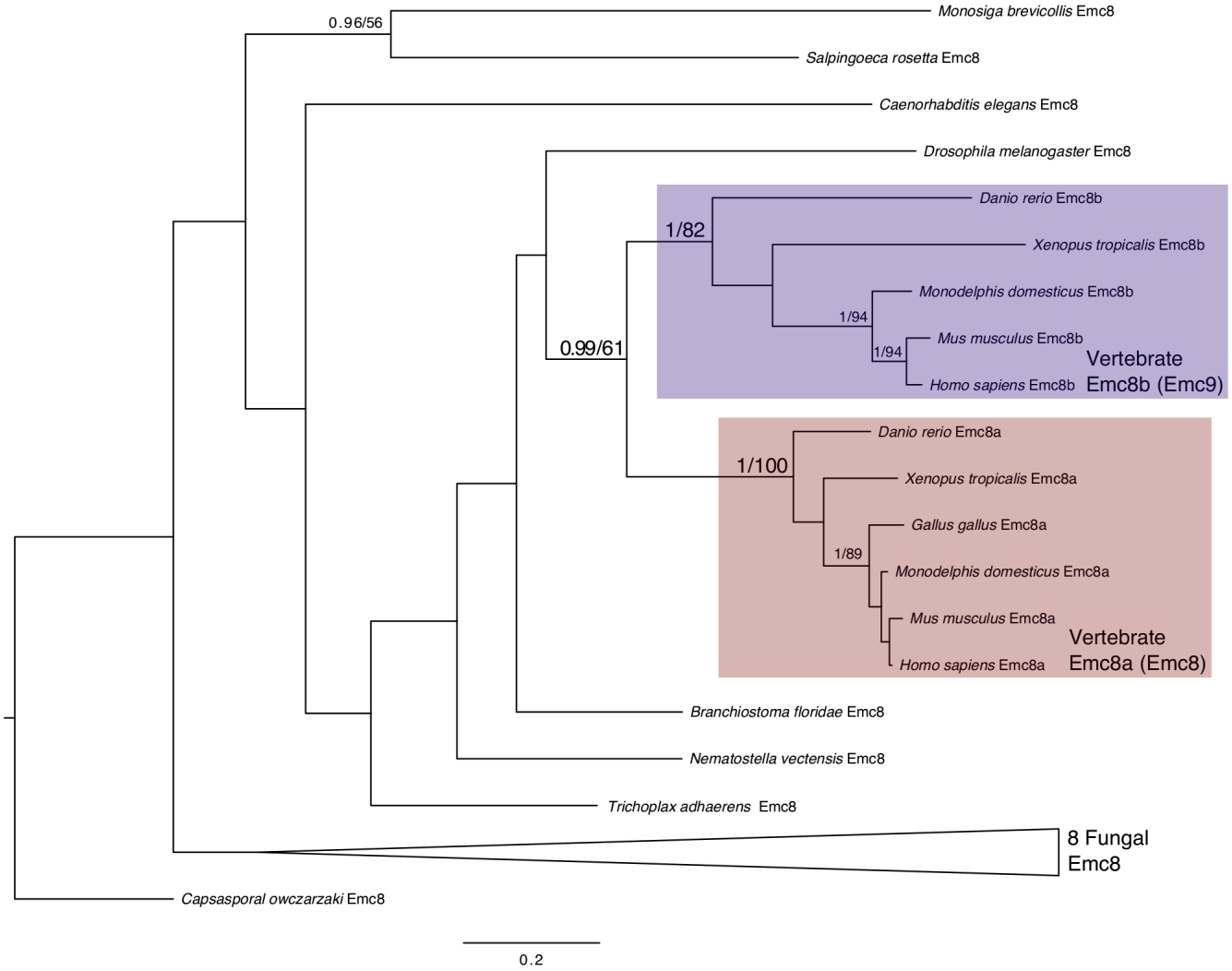
I thank Joel Dacks for server access, computational time, and inspiration.

Supplementary materials

Supplementary Table S1

Protein sequences retrieved in this study.

Click here to access the data.



Supplementary Figure S1. Phylogenetic analysis of opisthokont Emc8/9 proteins. Proteins were aligned using MUSCLE (Edgar, 2004) and manually adjusted as needed using Mesquite (http://mesquiteproject.org). Phylogenetic tree reconstructions were carried out using MrBayes v3.2.2 (Ronquist *et al.*, 2003) for Bayesian analysis. Maximum likelihood bootstrap values were obtained using RaxML (Stamatakis, 2006) with 100 pseudoreplicates using the LG model (Le & Gascuel, 2008). Support values: MrBayes/RAxML. Only support values >0.90/50 are shown.

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Current Referee Status: ✓ ✓ ?

Version 2

Referee Report 09 October 2015

doi:10.5256/f1000research.7713.r10104

Christopher Loewen
Department of Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia, Vancouver, BC, Canada

The article entitled “The ubiquitous and ancient ER membrane protein complex (EMC): tether or not?” authored by Jeremy Wideman presents a solid bioinformatics argument that the EMC protein complex is highly conserved amongst eukaryotes. The EMC was originally identified in budding yeast as a 6 subunit complex (Emc1-6) with a role in protein folding in the endoplasmic reticulum (Jonikas *et al.*, 2009). It was later expanded to 10 subunits (Emc1-10) in mammals based on proteomic work studying ER-associated degradation (Christianson *et al.,* 2011). This current bioinformatics study makes an nice contribution by showing that the whole complex (all ten subunits, with a few exceptions) is widely present in eukaryotes (including invertebrates, fungi and plants). Although overlooked in the original submission, the issue raised by one reviewer about Emc8 and Emc9 being paralogs has now been resolved by the author.

My main issue with the article is the conclusion drawn by the author that the tethering function of the EMC (discovered by Lahiri *et al.*, 2014) is likely not its conserved function. This is arrived at by comparing EMC distribution among species with the presence of mitochondria/MROs. The article title also implies that the findings in this report call into question the role for the EMC in ER-mitochondrial tethering and PS transport (as its conserved function). I feel these are overstatements given the current analysis presented in the paper. Lahiri *et al.*, show interactions between the EMC and Tom5, although these are not demonstrated to be direct, and also state in their discussion that the EMC likely interacts with multiple subunits of the TOM complex (and cite unpublished data to the effect). Hence, judging a role for the EMC in tethering solely based on the presence Tom5 in species seems hardly sufficient to make such an argument. Lahiri *et al.*, demonstrate a role for tethering by the EMC in PS transport to mitochondria, the location of the phosphatidylserine decarboxylase (PSD) that converts the PS into PE. Hence, this transport step is required for PE synthesis by the PSD. Perhaps the author should investigate the coincidence of the EMC and mitochondrial-localized PSD enzymes in the species for which he uses to build arguments against a role for the EMC in tethering. A quick search revealed that two species mentioned in the paper, *C. merolae* and *T. parva*, which completely lack the EMC, contain PSD enzymes that lack mitochondrial-targeting signals (28% and 50% probability, respectively; compared to 95% for *S. cerevisiae* PSD); hence, the EMC would not be needed in these organisms for PE synthesis. The third EMC-lacking species mentioned, *B. hominis*, has a mitochondrial-targeted PSD (99.9% probability) and a second non-mitochondrial PSD (0.01% probability), indicating that there is not an absolute requirement for ER-mitochondrial PS transport and hence, for the EMC in PE synthesis in this species.

If the author feels that an analysis of the co-occurrence of the EMC and mitochondrial-localized PSD enzymes (and/or TOM complex - all subunits) is beyond the scope of this paper, I feel the paper should

be revised, including the title, to de-emphasize the argument that tethering is not a conserved function of the EMC. An additional minor point, the author should name Emc7 and Emc10 on the SGD website for the benefit of the yeast bioinformatic community.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Referee Report 06 October 2015

doi:10.5256/f1000research.7713.r10674

Courtney Stairs
Centre for Comparative Genomics and Evolutionary Bioinformatics, Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada

Thank you for addressing my concerns and including a phylogenetic analysis. I think that renaming EMC8 is an excellent way to avoid confusion. I look forward to reading future studies on the topic.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Referee Report 06 October 2015

doi:10.5256/f1000research.7713.r10675

Sujoy Lahiri
Department of Pharmacology, University of Virginia, Charlottesville, VA, USA

It was gratifying to see that the author has further expanded his study based on my observations. The new finding of Wideman that Emc8 and Emc9 are vertebrate-specific paralogues explains the gain of EMC components in vertebrates. We'll, however, have to wait for future research to know whether the duplication of Emc8 in these higher eukaryotes has any functional relevance. I feel that the author has rightfully addressed my major concern and thus approve the indexation of this revised manuscript.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Version 1

Referee Report 24 September 2015

doi:10.5256/f1000research.7479.r10381

Courtney Stairs
Centre for Comparative Genomics and Evolutionary Bioinformatics, Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada

The article "The ubiquitous and ancient ER membrane protein complex (EMC): tether or not?" presents the distribution of EMC components across eukaryotic diversity. Using a strictly bioinformatic approach, Wideman identified homologues of the majority of the EMC in every major eukaryotic supergroup, suggesting that this complex was likely present in the last eukaryotic common ancestor. One of the most interesting findings of this study was the identification of two previously unreported EMC components (Emc7 and Emc10) in yeast. In fact, the *in silico* findings presented here are supported by previously published co-immunopreciptation study (Jonikas *et al*, 2009) that identified these two components. Surprisingly, the EMC also seems to be present in some organisms that possess highly reduced mitochondria (i.e. mitochondrion-related organelles; MROs). Although beyond the immediate scope of this study, it would be interesting to correlate the presence of various TOM components in these 'amitochondriates' with the various EMC components. Perhaps a brief comment on this in the discussion would be informative - especially since the interaction of TOM and EMC is known in yeast.

In another review for this article, Sujoy Lahiri commented on the assignment of the Drosophila and Anopheles Emc8 as Emc9 on the Homologene database (NCBI). It appears as though these organisms have only one homologue of Emc8 (OR Emc9) by BLAST. It would be helpful if the author could comment on this observation - is this a mistake by Homologene? A phylogenetic analysis of these two related proteins could be helpful to determine the evolutionary origins of these proteins in animals. They also brought up concerns about the homology between these two proteins - however I think the author addresses this in the methods section where he states '...Emc8 and Emc9, which are related)...'.

A system so fundamental to the cellular biology of eukaryotes is likely the result of vertical inheritance, however phylogenetic analysis of each component could help solidify this hypothesis and exclude any concerns over lateral gene transfer. Also, a single sentence describing if any of these components have distant homologues in prokaryotes (especially the recently described Lokiarchaeota) could also be informative for a non-expert audience (such as this reviewer).

The data presented by Wideman (2015) is well within the scope of F1000Research and will be an invaluable resource for those studying the interactions between the ER and the mitochondria. I have no major concerns on the article and fully support its continued publication in F1000Research.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Competing Interests: No competing interests were disclosed.

Author Response 26 Sep 2015

Jeremy Wideman, University of Exeter, UK

Thank you for your insightful review. I have addressed the major concern from Sujoy Lahiri's review by including additional data (opisthokont Emc8/9 phylogeny).

As you suspected, some of your other comments are out of the scope of the paper, but I would like to comment on them here for anyone that is interested.

First, regarding your comment “it would be interesting to correlate the presence of various TOM components in these 'amitochondriates' with the various EMC components”: yes this would be interesting, however, I believe best included in a larger study on the evolution of protein import pathways. Tom5, the only known MOM interactor of EMC is found only in Opisthokonts (Macasev et al. 2004), although this has not been investigated in detail for quite some time. The protein is so short (~50aa) that it is easily missed in bioinformatic analyses; even if the protein is more widespread, it may be that it will only be identified biochemically. Additionally, most amitochondriates have extremely divergent Tom complexes (e.g. *Entamoeba*, microsporidians, *Giardia*), and it is unlikely that even if a small protein like Tom5 is present in these organisms that it will be detectable by phylogenetic analysis.

Second, prokaryotes do not seem to have any close homologues (based on a preliminary BLAST into NCBI prokaryote database) but some weak homology can be detected. Further investigation is beyond the scope of this project.

Third, the likelihood of HGT of EMC components is quite low in this case given the high frequency of retention of EMC across all eukaryotes. Also, for many of the proteins it is unlikely that phylogenies would resolve HGTs as many of the proteins are very short and support values would be low.

Competing Interests: No competing interests were disclosed.

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The article titled “The ubiquitous and ancient ER membrane protein complex (EMC): tether or not?” authored by Jeremy G. Wideman is a comprehensive study to determine if the EMC proteins are truly conserved. Using homology searching algorithms the author has shown that except for a few branches the EMC proteins are present in the vast majority of the eukaryotes and reasoned in favor of the presence of EMC proteins in the last eukaryote common ancestor (LECA). In addition the author has also identified two genes in *S. cerevisiae* to be orthologues of Emc7 and Emc10 which supports the finding of Jonikas *et al*. where these two proteins were co-immunoprecipitated along with the other EMC proteins.

In light of the increasing scientific attention on the EMC proteins over past few years and their multifaceted roles in cell physiology I find this article to be quite relevant in delivering a thorough understanding of this protein complex from the evolutionary perspective. Thus this study by Wideman will be helpful in further understanding of the biology of the EMC proteins. On its scientific merit I consider this article to be substantially important for getting published with F1000Research.

However there is one major concern, which I'd like to be addressed before endorsing the acceptance of

this article. The author has described Emc9 to be present only among the vertebrates. However the HomoloGene database of NCBI shows Emc9 homologs to be present in *Drosophila melanogaster* and *Anopheles gambiae* (http://www.ncbi.nlm.nih.gov/homologene/41095). I assume that the homology searching algorithm used by the author has designated the Drosophila Emc9 homolog protein NP_611731.1 as Emc8 which calls for a discussion by the author. Furthermore, this led me to explore whether Emc8 and Emc9 share any sequence homology. Using pairwise alignment of NCBI Blast (http://goo.gl/B8T0P3) between human Emc8 (NP_006058.1) and Emc9 (NP_057133.2) I found 44% sequence identity between these two proteins with 93% query coverage and an E value of 2e-57. No other Emc proteins, besides Emc8 and Emc9, share such high degree of sequence identity. This makes me curious of whether Emc8 and Emc9 could be paralogs in the vertebrates. In such case the gain of Emc9 among the vertebrates could be explained by a possible duplication of Emc8. In light of this I would request the author to elucidate possible reasons for the high degree of sequence homology between Emc8 and Emc9 and discuss the anomaly between his data as presented in this article and the HomoloGene database.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Competing Interests: No competing interests were disclosed.

Author Response 26 Sep 2015

Jeremy Wideman, University of Exeter, UK

Thank you for your very positive review. I have addressed your major concern by including additional phylogenetic data. Emc8 and Emc9 are now clearly shown as paralogues due to a duplication in the ancestral lineage leading to vertebrates. As such, to prevent future confusion I suggest that vertebrate Emc8 and Emc9 be renamed to Emc8a and Emc8b respectively. I hope you now find the article sufficient for approval.

Competing Interests: No competing interests were disclosed.