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RESEARCH NOTE

The ubiquitous and ancient ER membrane protein complex (EMC): tether or not? [version 1; peer review: 1 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.

Keywords

Evolutionary cell biology, ER membrane protein complex (EMC), Membrane contact sites (MCS), ERMES, ER-mitochondria contact sites

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

2. **Courtney Stairs**, Dalhousie University, Halifax, Canada

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

Any reports and responses or comments on the article can be found at the end of the article.

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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 eukaryotes 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, which are related). 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). Only Emc9 was found to be absent from most taxa and is very likely the result of a vertebrate-specific duplication of Emc8.

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 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.

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 domestica; 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, Sphaeroloma arctica; Sros, Salpingoeca rosetta; Fungi: Spom, Schizosaccharomyces pombe; Scer, Saccharomyces cerevisiae; Nora, Neurospora crassa; Cneo, Cryptococcus neoformans; Umay, Ustilago maydis; Bden, Batrachochytrium dendrobatidis; Ncer, Nosema ceranae; Ecu, Encephalitozoon cuniculi; Pir, Piromyces sp.; Spun, Spizellomyces punctatus; Rirr, Rhizophagus irregularis; Crev, Coemansia reversa; Ccor, Conidiobolus coronatus; Cang, Catenaria anguillulae; Rall, Rozella allomycetes; Apusozoa: Ttra, Thecamonas trahens; Fungi: Fonticula alba, Fonticula alba; Amoebozoa: Acas, Acanthamoeba castellanii; Ddis, Dictyostelium discoideum; Ehis, Entamoeba histolytica; Ppal, Polysphondylium pallidum; Excavata: Ngru, Naegleria gruberi; Gint, Giardia intestinalis; Tvag, Trichomonas vaginalis; Bsal, Bodo saltans; Lmaj, Leishmania major; Tbru, Trypanosoma brucei; Stramenopiles: Bhom, Blastocystis hominis; Alim, Aurantiochytrium limacinum; Aana, Aureococcus anophagefferens; Tpsa, Thalassiosira pseudonana; Ptri, Phaeodactylum tricornutum; Psoj, Phytophthora sojae; Esil, Ectocarpus siliculosus; Ngad, Nannochloropsis gaditana; Alveolates: Ppet, Parametium tetraurelia; Tthe, Tetrahymena thermophila; Otri, Oxytricha trifallax; Tpar, Theileria parva; Smin, Symblodinium minutum; Tgon, Toxoplasma gondii; Cpar, Cryptosporidium parvum; Plal, Plasmodium falciparum; Rhizaria: Bnat, Bigelowiella natans; Rfil, Reticulomyxa filosa; Archaeplastida: Crei, Chlamydomonas reinhardtii; Cmer, Cyanidioschyzon merolae; Cyp, Cyanophora paradoxa; Aha, Arabidopsis thaliana; Ppat, Physcomitrella patens; Otau, Ostreococcus tauri; Gsul, Galdieria sulphuraria; Mpus, Micromonas pusilla; Ccri, Chondrus crispus; CCTH, Ehux, Emiliana huxleyi; Gthe, Guillardia theta.

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.

It explains the connection to ERAD as a secondary effect of mis-assembled 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 (Mačasev *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 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

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

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Supplementary materials

Supplementary table S1

Protein sequences retrieved in this study.

[Click here to access the data.](#)

References

- Adl SM, Simpson AGB, Lane CE, *et al.*: **The revised classification of eukaryotes.** *J Eukaryot Microbiol.* 2012; **59**(5): 429–93.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- AhYoung AP, Jiang J, Zhang J, *et al.*: **Conserved SMP domains of the ERMES complex bind phospholipids and mediate tether assembly.** *Proc Natl Acad Sci U S A.* 2015; **112**(25): E3179–88.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Altschul SF, Madden TL, Schäffer AA, *et al.*: **Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.** *Nucleic Acids Res.* 1997; **25**(17): 3389–402.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Christianson JC, Olzmann JA, Shaler TA, *et al.*: **Defining human ERAD networks through an integrative mapping strategy.** *Nat Cell Biol.* 2012; **14**(1): 93–105.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Field HI, Coulson RMR, Field MC: **An automated graphics tool for comparative genomics: the Coulson plot generator.** *BMC Bioinformatics.* 2013; **14**: 141.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Finn RD, Clements J, Eddy SR: **HMMER web server: interactive sequence similarity searching.** *Nucleic Acids Res.* 2011; **39**(Web Server issue): W29–37.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Jonikas M, Collins S, Denic V, *et al.*: **Comprehensive characterization of genes required for protein folding in the endoplasmic reticulum.** *Science.* 2009; **323**(5922): 1693–1697.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Kornmann B, Currie E, Collins SR, *et al.*: **An ER-mitochondria tethering complex revealed by a synthetic biology screen.** *Science.* 2009; **325**(5939): 477–81.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Lahiri S, Chao JT, Tavassoli S, *et al.*: **A Conserved Endoplasmic Reticulum Membrane Protein Complex (EMC) Facilitates Phospholipid Transfer from the ER to Mitochondria.** *PLoS Biol.* 2014; **12**(10): e1001969.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Li Y, Zhao Y, Hu J, *et al.*: **A novel ER-localized transmembrane protein, EMC6, interacts with RAB5A and regulates cell autophagy.** *Autophagy.* 2013; **9**(2): 150–63.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Luo W, Gong X, Chang A: **An ER membrane protein, Sop4, facilitates ER export of the yeast plasma membrane [H⁺]ATPase, Pma1.** *Traffic.* 2002; **3**(10): 730–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mačasev D, Whelan J, Newbigin E, *et al.*: **Tom22', an 8-kDa trans-site receptor in plants and protozoans, is a conserved feature of the TOM complex that appeared early in the evolution of eukaryotes.** *Mol Biol Evol.* 2004; **21**(8): 1557–64.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Meisinger C, Pfannschmidt S, Rissler M, *et al.*: **The morphology proteins Mdm12/ Mmm1 function in the major beta-barrel assembly pathway of mitochondria.** *EMBO J.* 2007; **26**(9): 2229–39.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Meisinger C, Wiedemann N, Rissler M, *et al.*: **Mitochondrial protein sorting: differentiation of beta-barrel assembly by Tom7-mediated segregation of Mdm10.** *J Biol Chem.* 2006; **281**(32): 22819–26.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Richard M, Boulton T, Robert VJP, *et al.*: **Biosynthesis of ionotropic acetylcholine receptors requires the evolutionarily conserved ER membrane complex.** *Proc Natl Acad Sci U S A.* 2013; **110**(11): E1055–63.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Satoh T, Ohba A, Liu Z, *et al.*: **dPob/EMC is essential for biosynthesis of rhodopsin and other multi-pass membrane proteins in *Drosophila* photoreceptors.** *Elife.* 2015; **4**: e06306.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wideman JG, Gawryluk RMR, Gray MW, *et al.*: **The ancient and widespread nature of the ER-mitochondria encounter structure.** *Mol Biol Evol.* 2013; **30**(9): 2044–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wideman JG, Go NE, Klein A, *et al.*: **Roles of the Mdm10, Tom7, Mdm12, and Mmm1 proteins in the assembly of mitochondrial outer membrane proteins in *Neurospora crassa*.** *Mol Biol Cell.* 2010; **21**(10): 1725–36.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Wideman JG, Lackey SWK, Srayko MA, *et al.*: **Analysis of mutations in *Neurospora crassa* ERMES components reveals specific functions related to β -barrel protein assembly and maintenance of mitochondrial morphology.** *PLoS One.* 2013; **8**(8): e71837.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

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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-immunoprecipitation 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.

Competing Interests: No competing interests were disclosed.

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

Author Response 26 Sep 2015

Jeremy G Wideman, Arizona State University, USA

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.

Reviewer Report 04 September 2015

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Sujoy Lahiri

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

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.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and 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.

Author Response 26 Sep 2015

Jeremy G Wideman, Arizona State University, USA

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.

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