CORRESPONDENCE

PDZD8 is not the 'functional ortholog' of Mmm1, it is a paralog [version 1; referees: 2 approved]

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Abstract

Authors of a recent paper demonstrate that, like ERMES (ER-mitochondria encounter structure) in fungal cells, PDZD8 (PDZ domain containing 8) tethers mitochondria to the ER in mammalian cells. However, identifying PDZD8 as a "functional ortholog" of yeast Mmm1 (maintenance of mitochondrial morphology protein 1) is at odds with the phylogenetic data. PDZD8 and Mmm1 are paralogs, not orthologs, which affects the interpretation of the data with respect to the evolution of ER-mitochondria tethering. Our phylogenetic analyses show that PDZD8 co-occurs with ERMES components in lineages closely related to animals solidifying its identity as a paralog of Mmm1. Additionally, we identify two related paralogs, one specific to flagellated fungi, and one present only in unicellular relatives of animals. These results point to a complex evolutionary history of ER-mitochondria tethering involving multiple gene gains and losses in the lineage leading to animals and fungi.

Keywords

Pdz8, ERMES, paralog, ortholog, evolution

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	Invited Referees 1	2
version 1 published 16 Jul 2018	✓ report	✓ report

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Author roles: Wideman JG: Conceptualization, Investigation, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Balacco DL**: Formal Analysis, Investigation, Writing – Review & Editing; **Fieblinger T**: Validation, Writing – Review & Editing; **Richards TA**: Validation, Writing – Review & Editing

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Hirabayashi *et al.*[1] show that PDZD8 (PDZ domain containing 8) is an SMP domain-containing protein involved in ER-mitochondria tethering and regulation of Ca^{2+} dynamics in mammalian neurons. We do not dispute the authors' interesting results with respect to PDZD8 function in mammalian cells. However, claims made by the authors regarding the evolutionary relationship of this gene with fungal homologs represents a misuse of the term ortholog, where the term homolog or, more appropriately, paralog is correct. This misuse has consequences for interpreting data in the paper, including in the domain-swapping experiments conducted as part of the complementation assay. The misclassification affects how their data should be interpreted and results in confused explanations of how ER-mitochondria contact sites evolved in animals and fungi. Approaching the data with correct terminology alleviates these problems and illuminates interesting possibilities about trait evolution in fungi and animals.

Orthology and paralogy are special cases of homology (Figure 1). Orthologous genes arise by speciation events. Mouse α-haemoglobin is orthologous to human α-haemoglobin. Paralogous genes arise by gene duplication events. Duplication of a globin gene in an ancestor of vertebrates gave rise to two haemoglobin families in which α- and β-haemoglobin subsequently evolved. Therefore, α- and β-haemoglobin, regardless of which organisms they appear in, are paralogs. In this simple case, the orthologous proteins perform the same function in different organisms (i.e. they are isofunctional orthologs[2]). However, orthologs can diverge and perform different functions in different lineages (heterofunctional orthologs). An example of heterofunctional orthologs is animal Miro (mitochondrial rho GTPase), which, in yeast, is called Gem1 (GTPase EF-hand protein of Mitochondria) and has been suggested to interact with ERMES (ER-mitochondria encounter structure) in fungi[3,4]. Miro is important for microtubule-dependent mitochondrial motility in animals (reviewed by Reis *et al.*[5]). However, although a Miro ortholog is present in all eukaryotic lineages, it does not function in microtubule-dependent mitochondrial motility in yeast, *Neurospora*, *Arabidopsis*, or *Dictyostelium*[5–7]. Thus, functionality alone does not imply orthology.

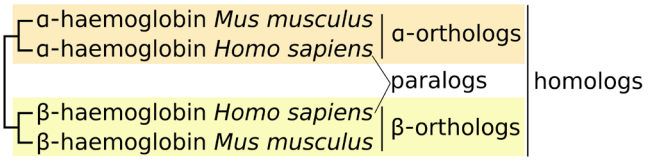

Figure 1. Orthologs versus paralogs: haemoglobin as an example. Orthologs are a consequence of speciation, whereas paralogs are a consequence of gene duplication. Human α- and β-haemoglobin share 43% identity whereas Human α-haemoglobin and Mouse α-haemoglobin share 87% identity. When performing phylogenetic analyses, the orthologous α-haemoglobin subunits from different animals branch together separate from their paralogs, the β-haemoglobin subunits. Taken together, all haemoglobin subunits are homologs.

Any SMP domain is homologous to any other SMP domain because they have shared ancestry, but only those arising via speciation are orthologs. To demonstrate that PDZD8 and Mmm1 (maintenance of mitochondrial morphology protein 1) are "functional orthologs", Hirabayashi *et al.*[1] swap the SMP domain from PDZD8 into the Mmm1 protein in *Saccharomyces cerevisiae* thereby rescuing the defects imparted by loss of Mmm1. But, they also successfully rescue Mmm1 defects by swapping the SMP domain from its paralog, *S. cerevisiae* Mdm12 (mitochondrial distribution and morphology protein 12), into the *S. cerevisiae* Mmm1 protein. The domain swapping experiments should not be interpreted to mean that the proteins (or even the domains) carry out the same function. Instead, these experiments suggest that the paralogous SMP domains from PDZD8, Mmm1, and Mdm12 are biochemically isofunctional in this specific scenario (i.e. the SMP domains can carry out similar functions when they are placed very specifically into the *S. cerevisiae* Mmm1 protein). However, it must be stressed that this does not mean that the proteins themselves are isofunctional. The fact that mammalian PDZD8 and similar proteins from other animals have long C-terminal extensions containing accessory domains (e.g. a PDZ domain and cysteine-rich C1 domains), while Mmm1 and Mdm12 do not, suggests these proteins have different or additional mechanisms of function. Thus, we cannot say that the full proteins are isofunctional homologs and especially not isofunctional orthologs.

The fact that PDZD8 is a homolog of SMP domain-containing proteins like Mmm1 has been demonstrated previously[8–11]; but it has also been demonstrated that PDZD8 is not an ortholog of ERMES components (Figure 2, also see Wideman *et al.*[11]). This can be seen very clearly in the SMP-domain proteins of *Capsaspora owczarzaki*, which include orthologs of all the ER-mitochondrial contact site SMP proteins (PDZD8, Mmm1, Mdm12, Mdm34). This organism is closely related to animals[12], but still retains both a complete ERMES complex and PDZD8 and represents a future model for investigating their differential functions. Interpreting the work of Hirabayashi *et al.*[1] from a comparative perspective demonstrates that mitochondria-ER tethering is a function that is conserved deep within this gene family predating the duplication that gave rise to PDZD8 and Mmm1, and indeed, all known ERMES paralogs. This is important because it allows us to phrase the next questions, 'how have different paralogs expanded, or changed, in function? (is there evidence of heterofunctionality?)' and 'why have some evolutionary lineages maintained multiple paralogs while others have tolerated loss?'. When uninformative terms like 'functional ortholog' are used, especially in exceptional papers like Hirabayashi *et al.*[1], biologists interested in explaining functional differences between organisms are being misled. We stress that only comparative evolutionary methods can identify the starting data for phrasing the above questions and functional data cannot be used when inferring orthology or paralogy. Once questions of orthology and paralogy have been resolved, questions of functional conservation and divergence can be addressed at the bench.

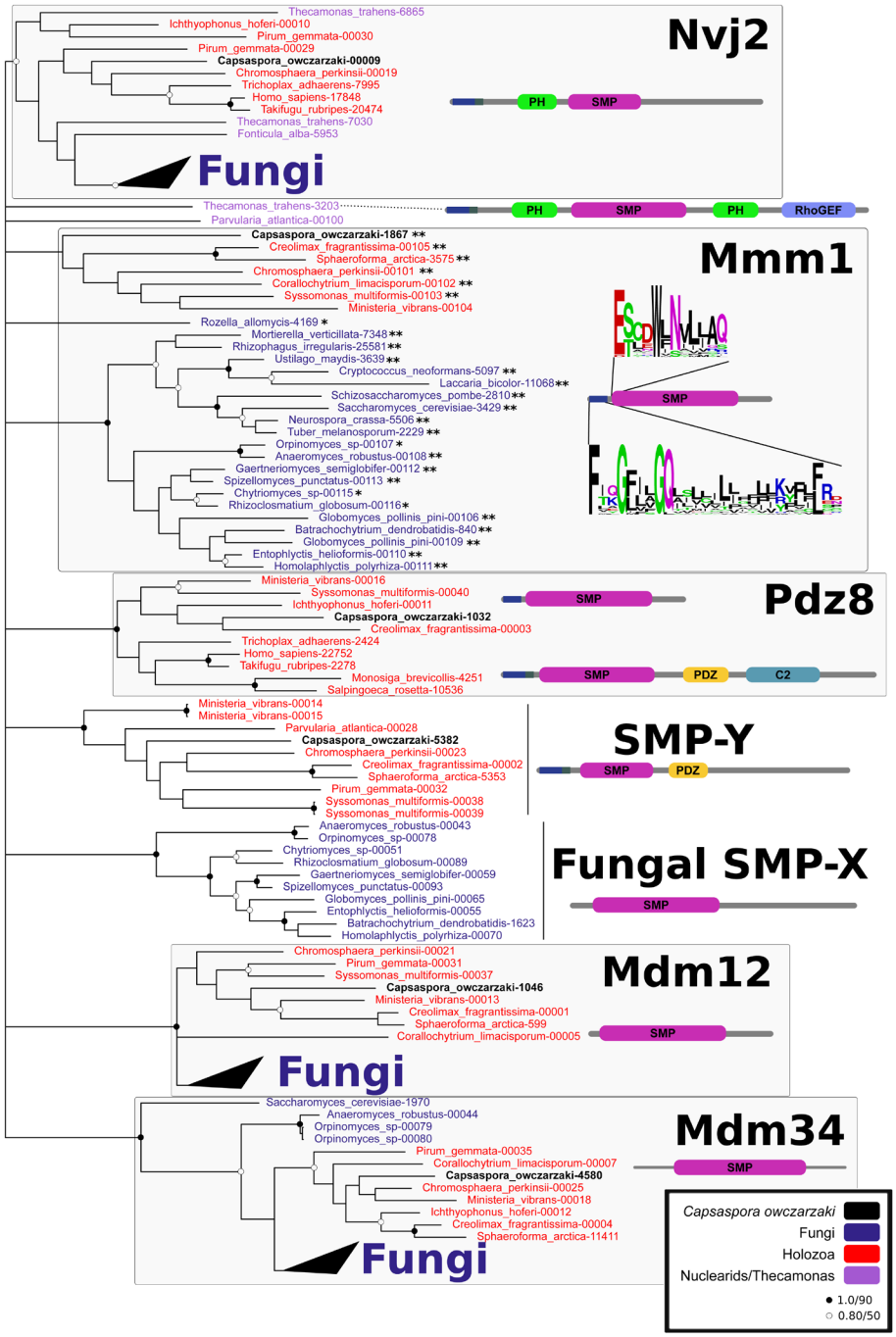

Figure 2. Phylogenetic tree and schematic domain organization of SMP domain-containing proteins. PDZD8 groups to the exclusion of Mmm1, Mdm12, Mdm34, Nvj2 and two unnamed paralogs. SMP domain-containing proteins were gathered from diverse opisthokonts (i.e. animals, fungi, and closely related protists) and their sister species *Thecamonas trahens* (sequences were obtained from public databases (Joint Genome Institute and NCBI) as well as from recently available genomes and transcriptomes[13,14]. The SMP domains were aligned and subjected to phylogenetic reconstruction using RaxML v8.2 (100 pseudoreplicates using the LG model) and MrBayes v3.2 (1 million generations using the WAG model) as in 11. Sequences and alignments are available at https://github.com/mbzdlb/PDZD8. Six strongly supported paralogs, including PDZD8, Nvj2, Mdm12, Mdm34, and two unnamed paralogs comprising sequences from flagellated fungi and opisthokont protists, were recovered. Fungal Mmm1 is recovered in a strongly supported clade whereas some proteins designated as Mmm1 previously[11] do not. However, sequence inspection identified motifs outside the SMP domains present in both fungal and non-fungal Mmm1s, strongly suggesting that proteins designated as Mmm1 are orthologous. Mmm1 proteins lacking these motifs may represent truncated or mispredicted proteins. Similarly, some predicted Pdz8 proteins lack Pdz domains and C-terminal extensions. Human PDZD8 is considered paralogous to Mmm1 because it groups separately and can be found in organisms that already contain Mmm1-like proteins (e.g. *Capsaspora owczarzaki*). Motifs were visualized using WebLogo[15]. One asterisk indicates the presence of Mmm1-specific short motif, two asterisks indicate both the short and the transmembrane motifs are present. Support values are as iconized in inset key (MrBayes/RAxML).

Data availability

All sequences were downloaded from publicly available databases (e.g. Joint Genome Institute or NCBI) or obtained from published transcriptomic and genomic data[13,14]. The sequences used to generate Figure 2 are downloadable at https://github.com/mbzdlb/PDZD8.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

Supplementary material

Supplementary File 1. List of organisms and accession numbers (with database) used to generate Figure 2. The corresponding sequences can be found at https://github.com/mbzdlb/PDZD8 or from the indicated online database.

Click here to access the data.

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Version 1

Referee Report 31 July 2018

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In this correspondence, Wideman *et al.* comment on a recent seminal study by Hirabayashi *et al.*, which describes the biochemical characterization of the SMP domain-containing protein PDZD8, as an ER-mitochondria tether important for the regulation of Ca^{2+} dynamics in mammalian neurons. Although the functional data presented by Hirabayashi *et al.* is sound and of great interest, their interpretation of the homologous relationship between PDZD8 and the yeast SMP domain-containing protein Mmm1 is incorrect. Mmm1 is part of the ERMES complex, which tethers the ER to mitochondria in yeast and is important for the exchange of phospholipids between these organelles. This complex contains two further proteins of the SMP family, Mdm12 and Mdm34. Wideman *et al.*[1] and we[2] have previously shown that some eukaryotes contain all three of these ERMES proteins as well as PDZD8, indicating that they are paralogs. Hirabayashi *et al.*, however, misinterpret PDZD8 to be a functional ortholog of yeast Mmm1, and substantiate this relationship experimentally by substituting the SMP domain of yeast Mmm1 with that of PDZD8 to rescue the phenotypic defects caused by the deletion of Mmm1. Here, Wideman *et al.* present convincing data to show that PDZD8 is a paralog of Mmm1 and not its ortholog. The arguments presented by Wideman *et al.* are sound and will help to clear the confusion regarding the evolutionary relationship between these proteins.

Minor comment:
The human PDZD8 protein contains a C1 domain as well as a coiled-coil segment at its C-terminal end. Do other orthologs also contain these domains? If so, the domain composition of PDZD8 shown in Fig. 2 should be updated to show this. Also, HHpred searches suggest that the PDZ domain in human PDZD8 is inserted into the C2 domain; is this the case for other orthologs too?

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Is the rationale for commenting on the previous publication clearly described?
Yes

Are any opinions stated well-argued, clear and cogent?
Yes

Are arguments sufficiently supported by evidence from the published literature or by new data and results?
Yes

Is the conclusion balanced and justified on the basis of the presented arguments?
Yes

Competing Interests: No competing interests were disclosed.

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.

Referee Report 30 July 2018

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In this correspondence, Wideman and colleagues show that PDZD8 (also called PDZ-8 or PDZK8) is not an ortholog of the ERMES subunit Mmm1p. This conclusion by Wideman et al. is well founded, and the underlying argument is well explained in the text here.

The same point of non-orthology has been said before in Wideman et al., 2015, but was then missed in the first paper that focussed on the function of PDZD8 (Hirabayashi et al., 2017[1]). There, the SMP domain from mammalian PDZD8 substituted for the function of the Mmm1p SMP domain in yeast, which was used to support the view that “The SMP domain of PDZD8 is functionally orthologous to the SMP domain found in yeast Mmm1.” However, a definition of orthologue that I accept is “Homologous sequences are orthologous if they are descended from the same ancestral sequence separated by a speciation event” [https://en.wikipedia.org/wiki/Homology_(biology)]. By that definition, I agree with Wideman et al. that bioinformatic studies indicate PDZD8 and all 3 ERMES subunits with SMP domains, including Mmm1p, are paralogs, not orthologs.

On that basis the function of PDZD8 may have evolved away from that of ERMES at some time, and might also have converged towards it, but we cannot tell by just showing that the activity of one’s SMP domain (most likely to transfer lipid with fairly low specificity) can be substituted by the other’s SMP domain.

There are several points where the current article might be marginally improved by revision:

Improve text and diagram describing domains within PDZD8: apart from the SMP and PDZ domains, PDZD8 is described as containing a C1 domain e.g. NCBI human PDZD8 (1154aa): 841-883. Here that domain is missing from the diagram. In addition, a C2 domain is shown in the diagram in an incorrect way, because the C2 domain sequence in PDZD8 is split. ~65 aa lies between the SMP and PDZ domains (320-372), then the rest (695-800) is before the C1 domain. See Figure 1B in Wong et al. 2017[2].

The text would benefit from a brief analysis of likely branching of the SMP protein family, based on supplementary data from the 2015 paper, possibly plus extra work to place the different Mmm1 groupings and the two new designations (X and Y).

The text should explcitily state that the Mmm1s in C. owczarzaki and other holozoa are variant, rooting onto the main tree separately from fungal Mmm1s, despite the shared presence of the 2 accompanying motifs.

This raises another point. The diagram adds to cladistic analysis of SMP domain sequences alone by looking for accompanying motifs. How can using accompanying motifs be justified, when accompanying domains are excluded from the orthology/paralogy argument? As SMP sequences can be inherited separately from other elements, I do not fully grasp that C. owczarzaki-1867 is included in Mmm1, while C. owczarzaki-5382 is excluded from Pdz8.

Understanding the figure might be improved by more information in the legend:

- Say what "**Fungi**" refers to each of the three times it appears in the diagram. Is it always the same set of fungal sequences or a different group in each case? How many?
- Say that the figure is not the same as appears in ref 11 or its supplementary data, but based on the same data set.
- Say that Tcbs have been excluded
- For "closely related protists" (line 3) say if every protist has been included or a selection has been made (and how)
- Identify falgellated fungi (compared to non-flagellated)
- Add description of one/two asterisks to key, not at bottom of long legend.

Data set on Git Hub:
I note that some sequences are incomplete. This should be made explicit.

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Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Lipid traffic; membrane contact sites; structural bioinformatics using HHsearch

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.