Reviewer’s report

Title: Division of labour in a matrix, rather than phagocytosis or endosymbiosis, as a route for the origin of eukaryotic cells

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Reviewer: Damien Paul Devos

Reviewer's report:

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**Reviewer summary**
Please provide a brief overview of your review, stating plainly your opinion of the manuscript’s overall validity, significance and originality.

In the manuscript “Division of labour in a matrix, rather than phagocytosis or endosymbiosis, as a route for the origin of eukaryotic cells”, the author proposes a novel model for the origin of the eukaryotic cell. The author discusses the introduction of a novel third-space that could be have played an import part in the development of the ancestral eukaryotic cell. In summary, the author proposes that biofilms have played a structural role in the development of the eukaryotes. The issue is of course of interest, of actuality and still an important unresolved question. The manuscript is well written, the ideas developed are of interest and well argued. I believe that the manuscript is of interest to a wide community of readers.

**Reviewer recommendations to authors**
Please make your report as constructive as possible, if necessary, recommending specific improvements so that the authors have the opportunity to overcome any serious deficiencies that you find. Please divide your comments into major and minor recommendations.

Main issues: One of the disturbing point is that the manuscript keep calling archae and bacteria as “Archaea” and “Eubacteria”. This is an historical mistake, as the term eubacteria was introduced to differentiate them from archaeabacteria (by Woese). But now, these terms have been defined as “Archaea” and “Bacteria”. Still mixing both is a conceptual mistake. This has to be fixed thorough the manuscript.

The model introduce the concept of “third-space”, a novel class of frameworks for the origins of complex cellular structures. In particular, it has three requirements L264. The third one is to be “more than a passive arena” for evolution. Please develop as this is quite limited. How so? How could it promote evolution? However, my main issue is with the point ii, L265, where the third-space is required to be ‘stable’ enough to provide the opportunity for eukaryotes to emerge. How long exactly would that be? How long do the author estimate that the presence and stability of the third-space would be required? And related to this, how long are biofilms known to be stable? Would it be realistic to think that particular biofilms could be stable for so long? If so, in which environment? Please develop this fundamental issue. Examples from biofilms or microbial mats would nicely illustrate the point. Please also detail how this proposal differ from refs 95 and 96. Also, if the third-space is required to be so stable, why can we not find any intermediary forms in current biofilms? This deserves mention. Most of the model is based on gene exchange inside the “third-space”, assimilating it to a genomic reservoir to support gene
exchange L250. However, the chapter (L245-254) is quite theoretical and ill supported by examples. Could such examples be provided by example based on known interchanges in communities and biofilms? Specifically discuss bacteria/archaea interchanges. A major issue with the proposal is related to the lipid composition of eukaryotic membranes versus archaeal ones. Could the author elaborate around line 120? In the allowable assumptions, I have a few comments of interest. L303, L-form bacteria are discussed and it is stated that L-form cells have been proposed to have played a significant role in early cellular evolution. However, this is a proposal about early cellular division, not about division of the eukaryotic cell. Please clarify or remove. L306, fusion of mitochondria in the eukaryotic cell is assimilated to fusion events that could have occurred between the participating cells within the third-space matrix. There is however a major difference in the fact that participating cells in the third-space matrix would be of different or differentiating types. To the best of my knowledge, fusion between different cell types have not been observed. Please comment. Related to this point, L374 states that “the nascent nuclear membrane arises from the cell membranes of the fused L-form-like multi-genomes”. This is an important jump and worth much more discussion. Why would membrane surround the DNA, and how do the author envision such transition? Relatedly, how could membrane then form around the third-space? Another issue is with the assumption that eu-bacteria outnumbered archaea in the matrix to justify the eu-bacterialization of the archaeal component of the matrix. Why would the author assume this, if not to justify a posteriori a known bias in eukaryotic genomes? If this is so, this is a deduction, not an assumption. It would be important to clarify how an increase in cellular complexity is required in order to “overcome problems that occur when molecules brought together from different lineages fail to interlock efficiently”. Please detail and comment. However, the most important point to me, is related to the transformation of the cytoplasm of one prokaryote into the nuclear AND of the extracellular space into the cytoplasm. Both are dramatic modifications that are not explained at all in the current manuscript and that deserves much more details as they are so central to the proposal. L508 and others and also figure 3 b. In particular, if the archaea is proposed to become the nucleus, as in other proposals, and stated L694, how typically archaeal lipids are modified to become typically eukaryotic and bacterial lipids in the nucleus? L586 deserves more details. An important issue is that some biofilms are compatible with the existence of a bounding lipid membrane L655. I find this argument extremely weak and not supported at all by the reference provided. Please revise.

Minor issues
Please detail any minor comments for the authors attention (spelling, typographical errors, grammatical errors, stylistic suggestions etc.) so that, once addressed, the authors may remove them from the review.

Minor issues: L20, the author states that “the question was addressed by enumerating the classes of potential pathways”. I however see only the two classical pathways, autogenous and fusion, followed by the novel model. There is no ‘enumerating’. I would just remove this line. L45 states that the age of the first eukarytes is between 2.1 and 1.84 Ga, “making them much younger than Archaea or Eubacteria”. If we would agree that bacteria are ancestral, there is much less consensus on the age of the archaea. Could the author develop on the question of the age of the Archaea? Please define at first mention, eg L72, what is meant by a proto-eukaryote. L421, modify ‘consistent’ as this is not consistent, but this is the same thing. L479 is in favor of chromosome linearization arguing that two circular chromosomes cannot segregate properly after recombination
if the number of crossovers is odd. I believe that this is pure speculation and not supported by the
two theoretical analyses used as refs. L481 states that mitosis is either closed or open. This is
untrue as there are plenty of intermediary, as reviewed in Sazer et al., 2014 curr biol. L499 states
that increase in cell size follows obviously. It is unclear to me, why this should be so obvious.
Please detail. L599, reference 160 is NOT the correct one to this statement the correct one is
Devos et al., Plos biolgoy 2004. Advantage listed a #11, L682 is not supportive as matrix
membrane vesicles still needs the ability to fuse in the external environment Figure 4, blue stream
left, “ribosomes in prokaryotic cells” is stated twice.

Reviewer confidential comments to Editor
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or part of the comments in your review for the authors. These comments will not be included in
the report passed to the authors.