Author’s response to reviews

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 comments to Authors.

I did not review the original submission of this manuscript, which has since been comprehensively revised to take account of the very extensive comments and criticisms of the two reviewers who did see the original. Considering the in-depth review the manuscript has already received and the resulting changes made by the author, my comments are necessarily limited. The subject of this submission is undoubtedly of considerable interest. As the author points out, none of the numerous extant models of eukaryogenesis compellingly accounts for the all of the known properties of the eukaryotic cell and its genomes (nuclear and mitochondrial). For that reason, new ideas are always welcome as a means of advancing discussion of this challenging problem. The author’s model in which a biofilm-like matrix might have been central to initiating the process of integrating separate bacterial and archaeal partners is an interesting one and, in principle, deserving of publication. The author has argued his case well and, in general, the presentation flows smoothly. I did find parts of the text heavy going, in part because my own knowledge of the biofilm literature is limited. On the other hand, the figures illustrating the main points of the model are quite helpful (but see Minor issues, below). In general, I did not find the overall model particularly persuasive in a number of its aspects. In particular, the biofilm-to-cyttoplasmic membrane transition is, in my view, difficult to imagine.

Author’s comment. I agree it is difficult to imagine. The third-space model is obviously speculative, and is intended as a first pass at developing such a model, but it is based on the normal mode of life of prokaryotes (in communities) and on processes that can be observed in the wild or in laboratory conditions. The model discussed in detail is the “strong” version, but, as outlined in section 1.3, there are other versions, (“weak” and “intermediate”). I chose to focus on the strong version with a mixed population in order to see how far the idea could be pushed. It requires a shift in emphasis from prokaryotic endosymbiosis, or other form of cellular engulfment, which have been rarely observed, and in which only two cell types are the primary players, to an exosymbiotic process which can include more than two cell types. Everything in the model arises by small steps. Originally, I used the term “progressive” for this, but to emphasize this feature I have, in places, switched to the term “incremental” which hopefully better captures the meaning. This avoids unexplained evolutionary leaps across structural and functional chasms. The formation of the boundary membrane is a key step, and this may be hard to
envision. Additional material was, therefore, added (second paragraph of 4.2. “Membrane encapsulation and cytosolic takeover”) to outline three possible ways a boundary membrane could form around a cellular ensemble in a matrix. The first paragraph of this section outlines an observed example of a bacterial colony that is, at least under some conditions, enclosed within an apparent lipidic membrane-like sheath. In addition, it points out that lipid substrates for the formation of the membrane are likely to be available in the matrix.

Once contained within a boundary membrane, the lysis of a subset of cells in the population and/or regulated secretion from the live cells of the population (both process have been observed) would deposit cytoplasmic material into the matrix. Given time, and in a population of cells linked by obligatory exosymbiosis (symbiosis and syntrophy between cells is often observed), these processes initiate the progressive replacement of the matrix by the cytosol. Again, replacement of the matrix by the cytosol would not occur as a step function but as an incremental process. As the ensemble transitions towards a more cell-like lifestyle, the production of the incipient cytosol becomes a more regulated and orderly process. A summary is now added in the final paragraph of section 4.2 (lines 654-661).

Also, in contrast to endosymbiotic models, the three-spaces model does not readily account for the selection of an archaeal cell as the progenitor of the nucleus and a bacterial cell as the forerunner of the mitochondrion.

Author’s comment. The strong-third space model hypothesizes that cells in the matrix-ensemble undergo genetic merging (to become the so called multi-genomes). Extensive gene transfer in both directions between Arcahea and Bacteria has been documented. The multi-genome structures contain both archaeal and bacterial genetic material. This is exactly what is seen in the eukaryotic nucleus, that is, a genome with contributions from both prokaryotic domains. It is from these structure that the nuclei develop. To emphasize the mixed nature of the nuclear precursors in the third space model I have referred to them as bacteriarcheons (lines 451-452). The third space model assumes that systems were selected because they were “fit for purpose” (last sentence, section 4.4, line 751) and not because they were specifically archaeal or bacterial. This is re-stated in lines 827-830, Section 5.2 part 2, “Limitations”. In this view archaeal information processing provided an advantage over the bacterial machinery. Once one part of the archaeal information processing machinery was selected, other systems that interact with it would be brought along in-step in order to maintain the biochemical and functional compatibility of the systems. Once a critical archaeal information processing system was selected, the evolution of supporting nuclear functions would, therefore, be channeled along an archaeal line. The concept of associated mechanisms being carried along by functional linkage is discussed in the final paragraph of 4.3. Partitioning of protein synthesis to the cytoplasm and part 2 of section 5.1. Limitations.

The selection of mitochondrial precursors is discussed in section 4.4. “Steps towards …. Shared metabolism”, and required an aerobic, mesophilic cell capable of generating enough ATP to provide a surplus. Clearly some member of the alpha-proteobacteria must have fit the bill.

Moreover, why the bacterial partner would selectively undergo reductive genome evolution, ceding many genes to the archaeal partner, is also not well developed. Again, endosymbiotic models better account for this distinction (it is well established, e.g., that the bacterial partner in obligate endosymbiosis generally has a much shrunken genome compared to its free-living relatives).

Author’s comment. I have now added a short discussion of this problem in text Line 722-726 “Moreover, as the mitochondrial precursors ….. mosaicism of the mitochondrial proteome”.
Interestingly, exosymbiotic prokaryotic cells can also show genome reduction (192), so the phenomenon is not restricted to endosymbionts.

These and other concerns aside, the model presented here is still worth considering and, if possible, refining.

Minor comments.
1. The author uses the terms “eukaryotization” and “eukaryogenesis” seemingly interchangeably throughout the manuscript. Are these terms equivalent in the author’s view? If yes, perhaps only the more common “eukaryogenesis” could be adopted to avoid possible confusion. If no, the terms should be explicitly defined in order to clarify their distinction.

Author’s comment. This has been altered to eukaryogenesis throughout

2. L68-70 (ref., 27): Additional references linking intracytoplasmic membranes in alphaproteobacteria to mitochondrial cristae could be added: DOI:10.1093/molbev/msw298 and DOI: 10.1016/j.cub.2015.04.006.

Author’s comment. These references have been added

3. L407: Bacteria (cap.) 4. L414-415: should read “an archaeal or a bacterial species” 5. L642: coli 6. L644: vesicle-associated 7. L724: membrane-enclosed 8. L1451: delete “34.” 9. L1812: “243 of bacterial origin” In Fig. 1(A), the number is 234. Also, Fig. 1(A) retains “eubacterial” (rather than “bacterial”) and “eub/arch” (which presumably should be “bact/arch”

Author’s comments. 3-9. Each of these points have been corrected

10. I find Fig. 1(A) confusing in that the figure legend states, “Among the bacterial clades, 41 were clearly alphaproteobacterial …”. This implies that the 41 shown in the figure (and the 198 non-...) are actually contained in the 234/243 bacterial group to the left, rather than being separate. It would help if the figure could be revised in some way to make this point explicit.

Author’s comment. This has now stated in the legend.

11. L1815-1816: “Only 3 Clades ...” The meaning of this sentence is unclear. Does the author mean that the clades in question do not branch with either Bacteria or Archaea? In which case, why are these clades included in the bacterial group (or are they?).

Author’s comment. This paper this was based on is rather complex, and I have used only one level of their analyses. I rewrote the sentence “only 3 clades ...” which now reads as “““Trees were generated for eukaryotic, bacterial and archaeal gene families. These were then analyzed in terms of “configurations”, for example, those that branched cleanly between eukaryote and bacteria, were assigned as bacterial clades, etc. Only 3 clades (labelled bact/arch) have the so-called “three domain configuration”, that branched into Archaea, Bacteria, and Eukarya with no obvious bias between the three domains.”

Reviewer's report 4.
Damien Devos, Affiliation European Molecular Biology Laboratory (EMBL), Heidelberg
Reviewer one-second report

The author has convincingly addressed most of my concerns. I would now recommend the article for publication. All considered, it is an hypothesis as worthy of publication as any other. I do have however, I few minor concerns to consider: Answer to my concern with L45. I was asking about clarification of the age of the archaea in the sentence, “making them much younger than Archaea or Eubacteria”. Changing the sentence to “making them much younger than prokaryotes” is not changing anything at all. This doesn’t clarify anything about the age of archaea. I do however agree that the article is long enough to discourage a more profound discussion about the age of the archaea. Despite the author’s understanding that “eubacteria” is an inadequate historical term and as corrected in the text, fig 1 still refers to “eubacterial”, “eub/arch”,
Author comment. This has been corrected

… Ref 54 is about a prepublication deposited in bioRxiv. I am unsure about this journal’s policy concerning preprints.

Author comment. A more complete article has now appeared and is cited (reference (58)).

The new paragraph “Membrane encapsulation and cytosolic takeover” is still not satisfying to me, as it briefly provides potential lipid sources, not an explanation of how these would have formed around the third space. How would the third space be transferred internal to the lipids? The Myxococcus example is unclear, to say the least.

Author comment: The section is now re-written. I have added additional text that expands upon three possible pathways for membrane formation (paragraph 2 of section 4.2. Membrane encapsulation and cytosolic takeover). The first paragraph of this section discusses extracellular lipid sources in a matrix, and a documented case that these lipids can be assembled into a sheath-like outer boundary. Returning to the Myxococcus example, the observed existence of a membrane-like structure around the Myxococcus colonies shows that under some circumstances some bacterial communities can enclose themselves in a membrane-like structure. It is certainly true, however, that Myxococcus is not representative of typical biofilms as the colonies are motile and predatory. New text “Although Myxococcus, being motile and predatory may not be representative” was added to outline this (paragraph one 4.2. “Membrane encapsulation and cytosolic takeover”).

About our question on the assumption that eubacteria outnumbered archaea in the matrix to justify eubacterialization of the archaeal component, as a posteriorly thinking, author response is not convincing and the point is not addressed in the section “Genomic reorganization towards a eukaryotic pattern”.

Author comment. The model does not require more bacterial and archaeal cell types, it can accommodate only two cell types, although I think there are advantages to a mixed population with more than two bacterial cell types. This was outlined in the previous version of the text lines 246-247, i.e. “In its simplest version, the third-space model requires only two cell types, or a population of two cell-types, embedded in the matrix, one of which must be archaeal and the other bacterial.” Allowing for mixed populations in the matrix, the phylogenetic makeup of the eukaryotic genome (Fig 1) suggests that it arose from more than one bacterial genetic reservoir but does not require multiple archaeal reservoirs. The third-space model accounts for this by allowing for a population of different bacterial cells in the matrix ensemble. New text has been included in the first paragraph of section “Genomic reorganization towards a eukaryotic pattern” to include the following

“The eukaryotic genome arose by merger of archaeal and bacterial genomes. The third-space model can accommodate simple populations of only two cell types (an archaeon and an alpha-proteobacteria) but also more complex mixed populations with more than two cell types. Considering mixed populations, and focusing on the bacterial component, the relative weakness of the alpha-proteobacterial signal (the mitochondrial precursor) compared to the aggregate bacterial signal in the eukaryotic nuclear genome (Fig 1) can be rationalized if the population contained more than one type of bacteria (an alpha-proteobacterium plus others). A mixed population model does not, however, necessarily require the involvement of more than one archaeal cell type. The minimal mixed-population third-space model suggests, therefore, more than one type of bacterial cell interacting with one archaeal cell type (presumably one of the Lokiarchaeota).