REPLY TO KU AND SUN:

Ancestors of modern giant and large eukaryotic dsDNA viruses infected proto-eukaryotes

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In Guglielmini et al. (1), we analyze the evolutionary relationships between Nucleo-Cytoplasmic Large DNA Viruses (NCLDVs) and the cellular domains based on the two largest universal markers, that is, the two largest RNA polymerase subunits (RNAP). We conclude that NCLDVs diversified before the emergence of the last eukaryotic common ancestor (LECA). Our results now draw criticism from Ku and Sun (2).

They notably describe as a flaw the three-domain (3d) topology displayed by our RNAP trees, exposing an unjustified prejudice in favor of the two-domain (2d) topology. Indeed, the debate between the 2d and 3d scenarios is still open, as exemplified by the still unsolved phylogenomic challenges and the recent robust multigene phylogenies supporting the 3d topology (3, 4). Nonetheless, as repeated throughout our manuscript, our hypothesis can be interpreted in both scenarios, since proto-eukaryotes had to exist in both. Importantly, most deep evolution studies, including our own, combine single and multigene analyses to obtain complementary information required for a rational interpretation.

Ku and Sun (2) also list as flaws the "contradictory positions" of the Asfarviridae between figures 1 and 3 and figure 2 in our article (1) and the paraphyly of the Phycodnaviridae–Asfarviridae–Megavirales (PAM) superclade in figure 2 of our article. However, the position of Asfarviridae is exactly the same relative to the other NCLDV families, and the paraphyly of the PAM is due to the branching of eukaryotic RNAP-I and RNAP-II within it combined with a different rooting. This is explained in our original article, and notably illustrated in its SI Appendix, figure S12. Finally, although the three eukaryotic RNAP clades exhibit topological differences, they also display some congruent groupings. Particularly, the three eukaryotic RNAPs are monophyletic with high supports and represent the same diversity: Their branches correspond to LECA.

The "issues with phylogenetic inference" listed by Ku and Sun (2) are not flaws. They are already described, investigated, and explained in our article (1). The additional "weaknesses" they mention reveal a clear misunderstanding. Indeed, Ku and Sun (2) assimilate our scenario to an "NCLDVs-before-eukaryotes hypothesis," as if we suggest that NCLDVs could parasitize contemporary prokaryotes. They fail to realize that LECA was not predated by a single proto-eukaryotic lineage resembling prokaryotes but was the end point of a long evolutionary period with increasingly complex intermediates (5, 6). Our data suggest that ancestors of modern NCLDVs (containing at least the core genes that we analyze) have indeed coevolved with communities of such proto-eukaryotes (displayed as a single lineage in trees due to the lack of extant intermediates). Our hypothetical scenario could thus be labeled NCLDV-before-modern-eukaryotes, as has indeed been mentioned in the title of our article. The alternative hypothesis to this timeline hinted by Ku and Sun would posit that NCLDVs obtained their RNAP after the eukaryotic diversification from LECA, a scenario rejected by all our analyses.

Finally, we have never claimed that eukaryotes were older than bacteria or archaea, and functional redundancy is a characteristic of eukaryotic cells with both multiple RNA and DNA polymerases. In conclusion, while we remain open to questioning our results and interpretations, the points raised by Ku and Sun are irrelevant and unjustified.

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The authors declare no competing interest.

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