Review of: "In situ architecture of the lipid transport protein VPS13C at ER-lysosomes membrane contacts"

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The manuscript by Cai and coworkers combine Cryo-Focused-Ion-Beam (Cryo-FIB) milling and Cryo-Electron Tomography (Cryo-ET) to provide the first evidence indicating that VPS13C forms a rod of approximately 30 nm that bridges cellular membranes. The structure of several domains of different members of the VPS13 family has been solved by Cryo-EM or crystallography, starting from the purified polypeptides. However, no structural data within the cellular context is available. Therefore, the work presented by Cai et al is of key importance to the field. The data in the article would need though further quantification and extra-controls to robustly demonstrate that the structures under observation correspond to VPS13C. Further, the figure legends could be more informative and a more accurate discussion on the structural data on the VPS13 family members other than the VPS13 isoforms would also be acknowledged. An interesting observation made by the authors is that after subtomogram averaging and 3D classification, the data indicates that the 30 nm rod does not directly contact the ER, suggesting that either a linker is missing in the experimental system used or the VPS13C contact with the ER membrane is somehow regulated. In this context, the ER-attached configuration would be the less abundant, and therefore, it will not appear after averaging. Further experimental work to evaluate those possibilities would be very interesting.

Some specific comments follow:

1. The cryo-ET analysis is performed in Hela cells overexpressing VPS13C and VAPB to produce massive expansion of the ER to endo-lysosome contacts. Controls are done with cells over-expressing only one of the proteins or none. A proper quantification of the ER endo-lysosome contacts in each case would help the reader to understand if the structures under study in the cells overexpressing VPS13C and VAPB are really massively occurring as compared with the controls.

2. The authors study by FIB-CryoET, followed by subtomogram 3D classification and averaging, some rod-like structures that appear in cells overexpressing VPS13C and VAPB (identified by CLEM). However, the resolution of the system does not allow to unequivocally identify those rods as VPS13C. The authors then nicely demonstrate that eliminating a fragment of the protein results in shorter rods, suggesting that indeed, those rods correspond to VPS13C. It would be important to back up this information with additional experiments to either elongate the rod or add additional domains to see that new densities appear. The authors comment that they do not visualize the Halo tag because it might be in a flexible loop. It might also be that the Halo tag does not have a symmetric distribution.

Attempts to fusion the Halo tag elsewhere using alfa-fold to predict a rotational symmetry would very much reinforce the data.

3. It would really help the reader to have more informative figure legends: the type of electron microscopy performed should be clearly specified (transmission electron microscopy, cryo-EM, chemically-fixed samples…), the Halo tag ligand used in the fluorescence microscopy experiments should also be indicated and the statistics on the experiments needs to be described (i.e. number of subtomograms averaged…).

4. In the context of the hypothesis that the VPS13C rods do not permanently contact the ER, it would be very interesting to increase the number of rods under study to see if a second less-abundant ER-attached conformation shows. Also, it could be interesting to force a more rigid interaction between VAPB and VPS13C to see if the ER-interaction can be forced.