Reviewer's report

Title: Biological constraints limit the use of rapamycin-inducible FKBP-Inp54p for depleting PIP2 in dorsal root ganglia neurons

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The manuscript by Coutinho-Budd et al attempts to use a rapamycin-inducible 5-phosphatase system in vivo in DRG neurons. To this end they produced two transgenic lines, crossing of which generated a mouse with both components expressed in CGRP positive DRG. The authors find that rapamycin did not induce a translocation of the 5-ptase in vivo and in cultured DRG neurons. Rapamycin did not induce any change in thermosensitive behavior in the transgenic mice either. The authors find that DRG has higher levels of endogenous FKBP12 than HEK cells, where successful translocation was observed. They conclude that FKBP12 levels preclude using this system in vivo in sensory neurons.

The reviewer highly appreciates the author’s willingness to publish negative data and inform the scientific community on the limitations of this system. Nevertheless, there are some problems with both the presentation and the interpretation of the data, which need to be resolved before publication.

1. The key finding of the paper is that rapamycin does not induce translocation of the FKBP construct. All the data shown are long-term rapamycin treatment 24 h or more, either intrathecal application or treating cultured neurons. In such a time scale various compensatory mechanisms may alter localization. Also these experiments are not self-controlled, i.e. different populations of cells are compared. Membrane to cytoplasm ratio is quite arbitrary and cell selection is also subjective. While the images and statistics overall are quite convincing that there is no robust translocation, some partial movement can be over looked. Furthermore on page 6 it is stated that translocation was determined histochemically in Fig. 3. What antibody was used? Please clarify in figure legend. If it is anti FKBP, would not it also measure endogenous FKBP? Also if rapamycin was introduced intrathecally, is not is expected to wash out during DRG / cell preparation? Please comment.

2. The rapamycin-induced translocation happens on a fast time scale, seconds to a couple of minutes. In the legend of Figure 1 they also mention that in HEK cells translocation happened within 3 s. For all the reasons mentioned in point 1, the key experiment in the paper is the short-term treatment of cultured DRG neurons with rapamycin, which is mentioned as data not shown, on page 8. This data should be shown. I would strongly prefer that it is done in a self-controlled manner, i.e. showing the same cells before and after rapamycin treatment.
3. Figure 1 is not convincing. Most images are so faint that it is hard to see anything on them. In panels B & F it is hard to see the translocation. Panel G shows that there was a statistically significant movement; a better image, or adjusting brightness and contrast would make the message a lot clearer. Panel C & F is problematic. While some translocation of the PLCD-PH from the PM to the cytoplasm can be suspected, much of the probe is still in the PM after rapamycin. While the statistics shows significant translocation, the representative image suggests partial translocation. This raises doubts on how robust this system is, especially that the authors modified the constructs used by others. Looking at similar experiments in the literature this PIP2 probe usually completely disappears from the PM after rapamycin treatment. It is unclear why the authors used one component from one lab the other from another, and it is also unclear why they modified the PM targeting construct to “destabilize” it. They cite an article, but the paper should be self contained, and the rationale should be explained.

4. The authors claim that the reason why the system does not work is the high expression levels of FKBP in DRG neurons. Figure 6 shows a western blot where the FKBP band is a lot thicker in DRG compared to HEK cells. The actin band, however, is also somewhat more dense, and when normalized data are shown, FKBP is only ~1.5 higher in DRG compared to HEK cells. It is somewhat difficult to imagine that this small difference can account for the lack of translocation. Would the difference be so little also if they normalize to another housekeeping protein? Please comment.

5. Have the authors checked the endogenous expression levels of mTOR? High expression levels of mTOR can also explain the lack of translocation. In that case, an alternative experimental solution may be possible. In Varnai et al, a rapamycin analogue (rapalogue) was used that does not bind to endogenous FRB, but can be heterodimerize a mutant FRB with FKBP using a rapamycin analogue (rapalog). The authors may consider this alternative in future work.

Minor comments:

1. To my knowledge the full length FKBP12 (108 amino acids) is used in the Ptase constructs, and not its “FKBP-domain”. This is used throughout the paper including the abstract. Please correct.

2. Page 15: “Twenty micrograms were loaded”. Please specify, I assume it is twenty ug of protein

3. Excitation and emission settings for CFP and venus fluorescence should be described

4. As mentioned earlier, in legend of Figure 3 DRG a detailed description of what was done is needed. Is it slices or isolated neurons? What antibodies were used, if it is immunohistochemistry?
Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.