Review of: "Biochemical and Cytological Interactions Between Callose Synthase and Microtubules in the Tobacco Pollen Tube"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

Overview

This manuscript contributes relevant progress in the elucidation of the mechanisms controlling pollen tube elongation by tip growth, with particular reference to callose deposition. The work integrates and completes previously published data by the same group (Cai et al, 2011) and by other Authors. Complementary biochemical approaches have been put in place in order to elucidate the possible involvement of microtubules and tubulin in positioning and/or activating callose synthase in the plasma membrane. The different approaches gave generally according results leading to the proposal of a nice model.

Some criticities

In the Introduction, the description of the role of the different CalS isoforms in is not completely clear to me. Several Arabidopsis CalS genes are mentioned, among which GSL1, which is said to perform overlapping functions with *GSL5* in sporophyte and pollen development. I didn't find this information in the cited reference, I think the original one should rather be (Enns et al., 2005 Two callose synthases, GSL1 and GSL5, play an essential and redundant role in plant and pollen development and in fertility. Plant Mol. Biol. 58: 333–349). Later in the text, GSL5 is mentioned again and reported to be expressed during pollen tube maturation, but in this case the cited reference deals with the rice gene *OsGSL5*, which is not orthologous to AtGSL5. Therefore, I suggest to specify always the plant when mentioning the different CalS encoding genes in order to avoid possible misunderstanding, and to clearly explain ortholog relationships among CalS involved in pollen tube elongation in different plant species.

The most conflicting results seems to be the colocalization of CalS with microtubules by immunogold staining and the presence of tubulin in CalS complexes, in one side, and the lack of co-precipitation of CalS with microtubules by spin-down assays on the other. This is explained by an indirect binding mechanism, possibly mediated by tubulin 'monomers' (page 15 and p.17). This is the first point. Monomers or dimers? Microtubules are in constant equilibrium with αβ tubulin heterodimers, but also true monomers may be present within cells and even form complexes with other proteins, as also reported by the Authors in the Discussion section (p.16).

Moreover, all assays performed to highlight interactions between CalS and tubulin used anti-tubulin

antibodies without further specification. Whether this antibody recognizes a specific monomer (α or β) or both is not known. Therefore, no indication is provided to understand what the Authors means by ‘tubulin’: monomers (α, β or both) or dimers? This point should be clarified throughout the manuscript.

Since interaction of microtubules with other proteins through tubulin have never been described to my knowledge, such hypothesis, although fascinating, is the weakest part of the model and should be more convincingly supported.

Another question remaining open and not mentioned in the Discussion is why CalS was not found in association with the ER membrane fraction, differently to Sucrose synthase, if the two enzymes supposedly share the common secretion pathway, as mentioned in the Introduction. I think a possible explanation for this result should be provided.

Minor points

I would suggest that the model plant used, tobacco, should be mentioned in the Abstract, if not in the title.

In Material and Methods, The sentence ‘Pollen was collected from closed anthers and induced to anthesis under controlled conditions’ is misleading, I believe the word ‘and’ should be deleted.