Evaluation of the PhD Thesis by Elvia Amparo Rosero Alpala:

Role of Formins in the Organization and Dynamics of Intracellular Structures in *Arabidopsis thaliana*

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PhD Thesis of Elvia Amparo Rosero Alpala is composed of 16 pages of the general text, including the *Abstract*, 11 pages of the *Introduction*, *Aims of the Thesis*, *Thesis Ourtline*, and 9 pages of the *Discussion*, followed by the *References*. The main part of the Thesis consists of one paper published in the internationally relevant journal '*Journal of Experimental Botany*' in 2013, one manuscript is under submission, and one methodical book chapter. The central topic of her PhD Thesis is the role of AtFH1 and AtFH2 Arabidopsis formins in cytoskeletal organization and arrangements, as well as in control of cell morphogenesis and development. In two of these papers, Elvia Amparo Rosero Alpala is the first author and they represent the central part of her Thesis.

Formins are evolutionary ancient nucleators of actin polymerization which allow very effective coupling between signaling at the plasma membrane – cell wall adhesion domains with the internal actin cytoskeleton. They are well studied in animal and yeast cells, but relatively less

understood in plants. Elvia Amparo Rosero Alpala focused on two of the Arabidopsis formins: AtFH1 and AtFH2. These belong to the class I plant formins which are targeted to plasma membrane and insert into cell walls via their large extracellular domains, bridging cell wall with plasma membrane and cytoplasm. Their anchoring within cell walls is promoting F-actin assembly and bundling. This indicates that the class I plant formins, which are unique from the whole formin family, act akin integrins of animal cells (which are absent from plants). In the first paper published by Elvia Amparo Rosero Alpala et al, *fhi* mutants have been analyzed and characterized. Cells of these mutants showed increased sensitivity to the actin polymerization inhibiotor latrunculin B. Root cells of mutant lines showed inhibited cell elongation and several morphological abnormalities in root epidermis cells. Moreover, F-actin was more bundled but less dynamic, whereas microtubules were more dynamic in cells of mutant roots. Importantly, most of these features could be mimicked with exposure of wild-type roots to formin inhibitor SMIFH2. In her second study, manuscript of which is still under review, she studied roles of the AtFH1 and AtFH2 Arabidopsis formins on leaf pavement cell shapes, vascular patterning and development of gametophyte. It turned out that phenotypes of single *fh1* and *fh2* mutants show similar features. However, it is not possible to obtain double mutants, indicating that at least one of these proteins must be present in cells of developing gametophytes. As published for roots, also leaf cells showed increased F-actin bundling and enhanced dynamicity of microtubules. Moreover, also clear defects in the endocytosis and membrane trafficking has been scored in leaf cells. It turns out that FH1 and FH2 formins play a central role in cytoskeleton – membrane integration in plant cells. The high scientific quality of the manuscript under review allows me to be very optimistic about its acceptance in one of the internationally relevant journals. The Thesis by Elvia Amparo Rosero Alpala fulfills all the criteria required from the PhD Thesis and I am very happy to suggest its acceptance after her public defense.

I have few questions and comments:

1/ What particular process driven by actin polymerization could be critical for driving clathrin based endocytosis?

2/ Please, characterize in detail the large extracellular domains of FH formins. Might these domains act as receptors for clathrin based endocytosis?

3/ Did you check plasmolysis and re-plasmolysis in mutant roots? If FH formins have roles in cell wall – plasma membrane – cytoskeleton continuum, then one could expect that cells of mutant roots will be less effective in these rapid structural responses to changes in osmotic conditions.

4/ How could FH formins regulate the structural cross-talks between F-actin filaments and microtubules?

5/ Several aspects of mutant phenotypes, such as pavement cell shapes and vascular patterning, suggest also impaired polar auxin transport. In future, it would be important to analyze auxin distribution and polar auxin transport.