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High Pressure Freezing/Freeze Substitution of Plant Cells for 3D Electron Tomographic Studies

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The quality of a 3D electron tomogram depends primarily on the quality of the sample that is imaged in the electron microscope. Thus, specimen preparation is of the utmost importance for the success of an electron tomographic study. High Pressure Freezing/Freeze Substitution has been long recognized as the premier technique for the preservation of ultrastructure in biological samples [1]. In most cases a basic HPF/freeze substitution protocol can be used to obtain superior ultrastuctural preservation and structural contrast staining (freeze substitution), and allows one to use more advance techniques such as 3D electron tomography [2-4]. In all of these studies, not only was the cellular ultrastructure well preserved, but also the objects of interest were well contrasted, which makes 3D modeling easier [5]. However, for plant tissues, which have a thick cell wall, large water filled vacuoles, and air spaces (all of which can derail the cryo-preservation of the sample), basic HPF/freeze substitution protocol often yield un-desirable results. In particular, the staining of the membrane systems is often negatively stained [6], and makes 3D segmentation of a tomogram difficult. To overcome these problems, various aspects of the high-pressure freezing protocol can be altered, including the cryo-protectant used, the freeze substitution cocktail, and the resin infiltration process. These changes, which will be discussed, allowed us to study the process of cell plate formation during cytokinesis [7-9], and more recently study the ultrastructure of the thylakoid membranes of the chloroplast.

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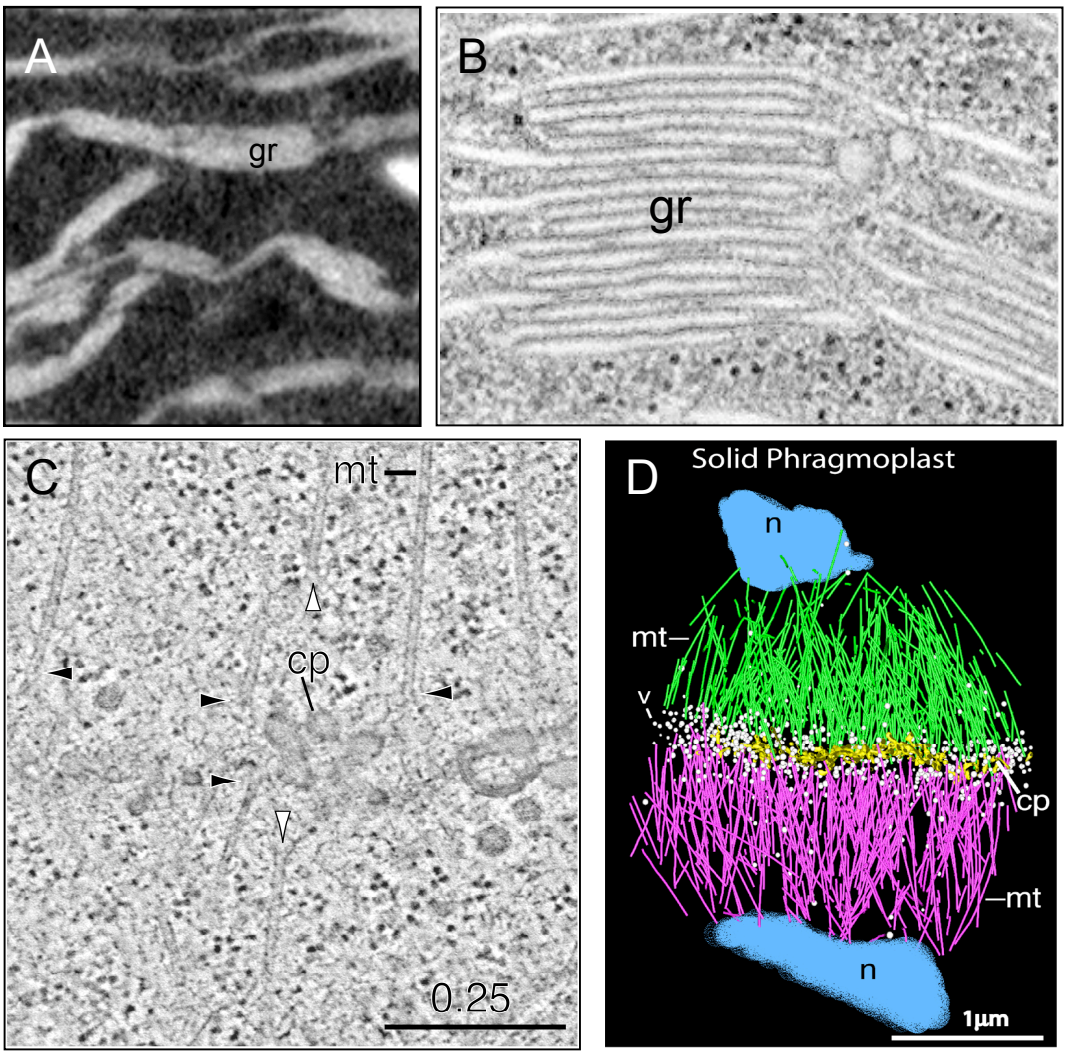

A) Electron Micrograph of a HPF/freeze substituted chloroplast prepared as in [6]. Notice how the grana stack (gr) thylakoid membranes are negatively stained.

B) A tomographic image of the grana stack (gr) using a modified HPF/freeze substitution protocol.

C) A tomographic image of an Arabidopsis root meristem cell in the process of cytokinesis. The arrowheads are indicating different microtubule (mt) plus end geometries, black (blunt, meta-stable), and white (horned, disassembling). Cell-plate (cp).

D) A 3D tomographic model depicting the Solid Phragmoplast stage of cell plate formation. Microtubules (mt), cell plate (cp), nucleus (n).

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