Ultrastructural & Analytical Methods in Life Sciences

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Light-induced transformation of prolamellar body (PLB) during early stages of bean chloroplast biogenesis visualized by electron tomography.

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Chloroplast biogenesis is a multistage process leading to fully differentiated and functionally mature plastid. At ultrastructure level this process consists of the transformation of prothylakoids (flat porous membranes, PT) and prolamellar bodies (paracrystalic tubular membrane structures, PLB) into grana and stroma thylakoids This model of differentiation of etioplast to mature chloroplast is based on natural scotomorphogenesis (etiolated growth), which represents the initial seedling growth occurring beneath the soil's surface.

Three-dimensional structure of thylakoid membrane arrangement has been a subject of many studies during last fifty years [1 and literature therein]. In last few years electron tomoghraphy and 3D modelling techniques in particular have contributed to creation detailed model of grana stacks arrangements [1,2].

In our study we focused on visualization of spatial model of thylakoid differentiation during the chloroplast biogenesis. We followed this process step by step from paracrystaline structure of PLB to first stacked membranes observed in ultrastructure of bean chloroplasts. Therefore we selected five main stages of plastid internal membranes arrangements during early stages of photomorphogenesis. Changes in membranes arrangement during subsequent hours of illumination. are schematically shown in Figure 1. Initially the paracrystaline structure of PLB observed in 1.0 plants (8 days of etiolation) transforms into irregular one already after one hour of light exposition (1.1 plants). During subsequent hours degradation of PLB proceeds and more prothylakoids are detected in plastid stroma (1.2 plants) until no PLB structures are found. In this stage only thylakoids and prothylakoids parallely arranged to each other exists(1.4 – plants after four hours of light). In last examinated stage, after eight hours of light exposition (1.8) first stacked membranes are observed in developing chloroplasts.

Electron tomography technique was performed with JEM 1400 (Jeol) microscopy from +60 to -60 at 1 intervals in one axis. Tomograms collected from samples from five selected stages (1.0, 1.1, 1.2, 1.4, 1.8) were reconstructed using tomoJ (ImageJ) software and then isosurfaces of membrane arrangements were prepared using Imaris software. Additionally models of selected details of structures were made with 3dmod software. In Figure 2 the isosurface of paracrystaline PLB (1.0) is presented.

1. J.R. Austin and A. Staehelin Plant Physiol. 155 (2011) p1601.
2. B. Daum and W. Kuhlbrandt J. Exp. Bot. 22(4) (2011) p1299
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4. TEM images were performed in the Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology on JEM 1400 (JEOL Co. Japan) electron microscope. This equipment was installed within the project sponsored by the EU Structural Funds: Centre of Advanced Technology BIM – Equipment purchase for the Laboratory of Biological and Medical Imaging.

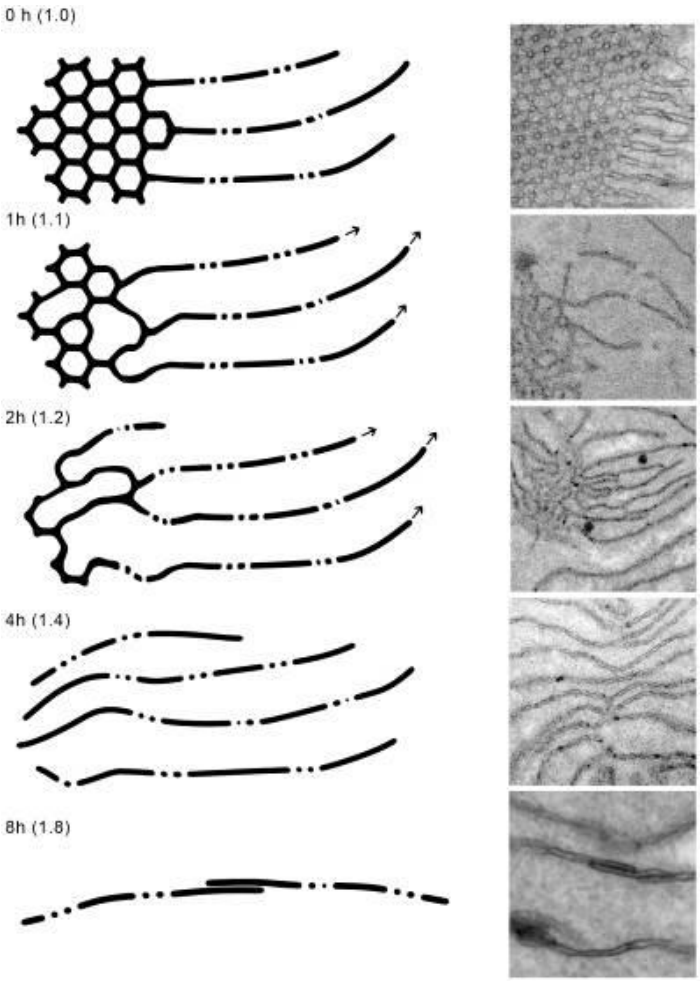

Figure 1. Theoretical model (with corresponding electron micrographs) showing arrangement of inner plastid membranes during prolamellar body transformation in subsequent hours of illumination.

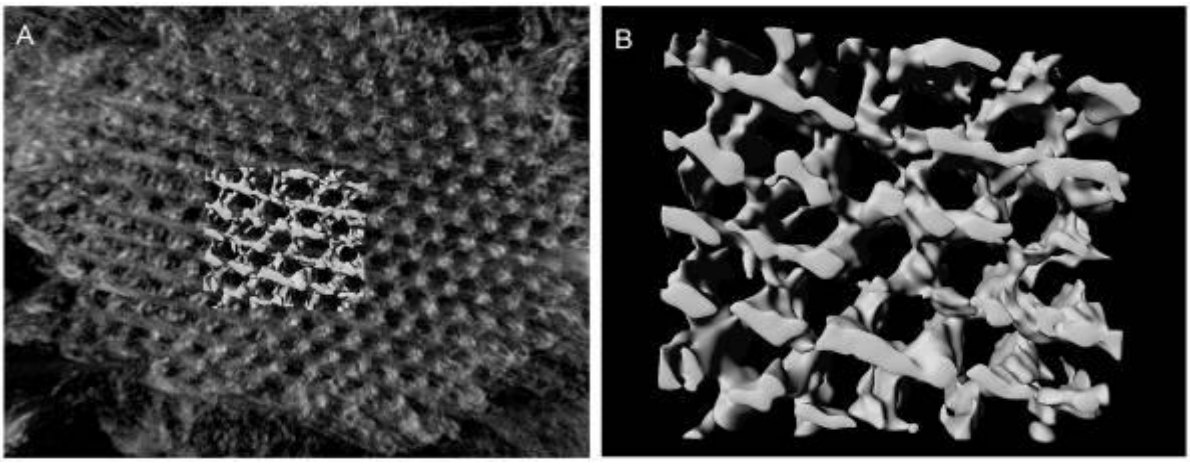

Figure 2. Isosurface visualisation of 3D reconstruction of paracrystaline prolamellar body structure (A volume with isosurface, B – enlargement) prepared with electron tomography technique.