Review of: "CURT1A and CURT1C mediate distinct stages of plastid conversion in Arabidopsis"

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The authors investigated the transformation of crystalline structure of prolamellar bodies (PLBs) during light-induced etioplast-to-chloroplast differentiation in *Arabidopsis thaliana* by scanning transmission electron tomography (STET). To avoid fixation artifacts, they prepared cotyledon specimens of dark-grown *Arabidopsis* seedlings by high-pressure freezing. Their STET observations indicate that pre-granal thylakoids develop from PLB tubules at the PLB-stroma interface, and grana stacks arise from the pre-granal thylakoids. Because membranes at the PLB surface curve sharply toward the stroma and form pre-granal thylakoids and grana stacks after illumination, they hypothesized that CURT1 family proteins, which are known to facilitate the formation of the thylakoid membrane curvature, are involved in stack assembly from PLB tubules. In fact, the localization of CURT1A-GFP and immunogold-labeled CURT1A indicates frequent association of the CURT1A with the PLB surface. The importance of CURT1A for the thylakoid assembly from PLB tubules is supported by the observation of swollen thylakoids and the absence of grana stacks in the *curt1A* mutant during etioplast-to-chloroplast differentiation. In addition, analysis of CURT1C-GFP and the *curt1c* mutant suggests a role of CURT1C in cubic crystal growth of PLBs in etioplasts and thus the functional differentiation between CURT1A and CURT1C in *Arabidopsis*.
This study provides important insights into the unique structure of PLBs in etioplasts and the mechanism of PLB-to-thylakoid transformation during etioplast-to-chloroplast differentiation. Their data further indicate the different roles of CURT1A and CURT1C in these processes.
We have some comments on this paper as described below.

1. First of all, we cannot find supplementary data that are mentioned in the article so this review has been done without them. In addition, we are not familiar with the computational 3D modeling study and thus we do not go deep into the methodology and the interpretation of the 3D modeling of the PLB structures. Also, we do not comment on grammatical aspects of English.

2. This study focuses on the PLB structures in etioplasts and their transformation to the thylakoid membrane in chloroplasts. Meanwhile, the word “plastid” in the title includes other various types of chloroplast relatives such as proplastids, amyloplasts, and chromoplasts. Therefore, the title “CURT1A and CURT1C mediate distinct stages of plastid conversion in Arabidopsis” may not represent their study well

because they report only the etioplast-to-chloroplast differentiation. The observation of the pre-granal thylakoid formation at the PLB surface, in which CURT1A would play a crucial role, would be one of the main and specific findings of this study.

3. It will be helpful for readers particularly those who are not familiar with the chloroplast studies if the background of CURT1 protein is described in more detail in the introduction section.

4. L 106. The authors identified PLBs as small fluorescent spots of 1.5-20 µm. However, because the lengths of the scale bars in Fig. 1D and 1E are not indicated in the legend, the sizes of the fluorescent particles are unknown to us. Considering the possibility of protochlorophyllide accumulation in the envelope and prothylakoid membranes of etioplasts, we wonder if the fluorescent particles exactly correspond to PLBs.

5. L. 114-128. As described in the article, stacked membranes are observed around the periphery of PLBs in Fig. 1G (1 HAL) and 1H (2 HAL). The authors mentioned them as “grana stacks”. However, to our eyes, the stacked membranes observed at 1 HAL and 2 HAL look like transient structures and may be different from typical grana observed in developing and mature chloroplasts, as seen in Fig. 1J (8 HAL) and 1K (12 HAL). Although the authors propose the assembly of grana-forming super-complexes containing LHCII in PLBs at 2 HAL (L. 127), with showing accumulation of Lhcb transcripts between 1 HAL and 2 HAL (Fig. S3, but we cannot refer to those supplemental data), we suspect the possibility that the stacked membranes are transiently formed early during etioplast-to-chloroplast transition independently of LHCII, because LHCII proteins are very few at these time points (e.g. Fujii et al., 2019, https://doi.org/10.1093/pcp/pcz041). This point may be worth further investigation in detail.

6. In Fig. 7A, the authors suggest that CURT1C proteins are located at the core of the PLB crystallin structure, whereas CURT1A proteins are at the periphery of the PLB, in agreement with their GFP and immunogold observations. With this model, we assume that CURT1C is required for the assembly of core PLB structures particularly at the beginning of PLB formation, while CURT1A proteins accumulate mainly after the formation of core PLB structures. In this context, we are interested in the temporal patterns of CURT1A and CURT1C expression during etioplast development. There is the possibility that CURT1C is expressed earlier than CURT1A during the growth of etiolated seedlings reflecting the different roles of these two proteins in the formation and conversion of PLBs.

7. Description of white arrows in Fig. 1F is missing in the legend.