Argon-ion-induced mutations in *Arabidopsis EGY1* gene affect chloroplast development in leaf guard cells†

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Leaf tissues of higher plants such as *Arabidopsis thaliana* contain chloroplasts in the mesophyll and epidermis. Until now, it has been indicated that there exists a tissue- or cell-type-dependent control of chloroplast division in leaves. This raises a fundamental question about the control of chloroplast development in the leaf epidermis, or, more specifically, whether chloroplast biogenesis-related factors in leaf mesophyll cells play an equivalent role in the leaf epidermis.

To address this issue, we examined the phenotype of leaf epidermal chloroplasts using two argon ion irradiation-derived, pale green mutants of *A. thaliana*, Ar50-33-pg1 and Ar-28-pg1 (*egy1-4*).[1–3] Both represent loss-of-function mutants of the *Ethylene-dependent Gravitropism-deficient and Yellow-green 1* (*EGY1*) gene, which encodes a thylakoid membrane-localized protease for chloroplast development in the mesophyll.[4] Moreover, the defects in the thylakoid structure and pigmentation in the mutant mesophyll chloroplasts are known to be pronounced during the later stages of leaf (seedling) development.[3,4]

Fluorescence microscopy observations indicated severe chlorophyll deficiency in the chloroplasts of leaf guard cells and guard mother cells of *egy1* (Fig. 1). This finding was in contrast to that observed in mesophyll cells, which retained a considerable amount of chlorophyll. Labeling of plastids with stroma-targeted fluorescent proteins revealed that *egy1* guard cells contained a normal number of plastids; however, their size was moderately reduced compared to those of the wild-type guard cells (Fig. 1). Transmission electron microscopy further revealed that, in the *egy1* chloroplasts, thylakoid development was impaired not only in mature guard cells but also in the guard mother cell (Fig. 2). Thus, the disorganization of the thylakoid structure and the reduction in the chlorophyll contents showed a positive correlation.

Collectively, these observations demonstrate that *EGY1* is critical for chloroplast differentiation in guard cells: *egy1* guard cell chloroplasts apparently exhibit permanent defects, starting from their development. This prompts a revision to the current understanding of chloroplast development, which has been based mainly from on studies using mesophyll cells.

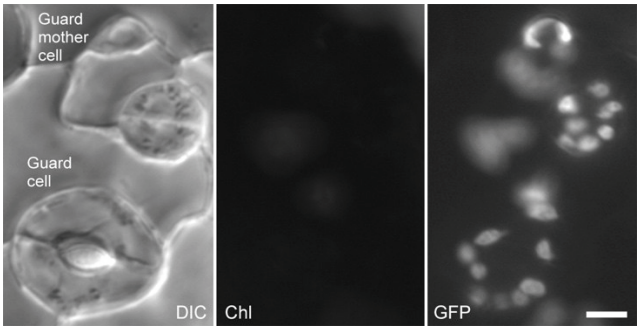

Fig. 1. Fluorescence microscopy images of chloroplasts during guard cell development in Ar50-33-pg1 expressing a stroma-targeted GFP. Bright field (DIC), chlorophyll autofluorescence (chl), and GFP images of leaf epidermis from 18-day-old seedlings are shown. Bar = 10 μm.

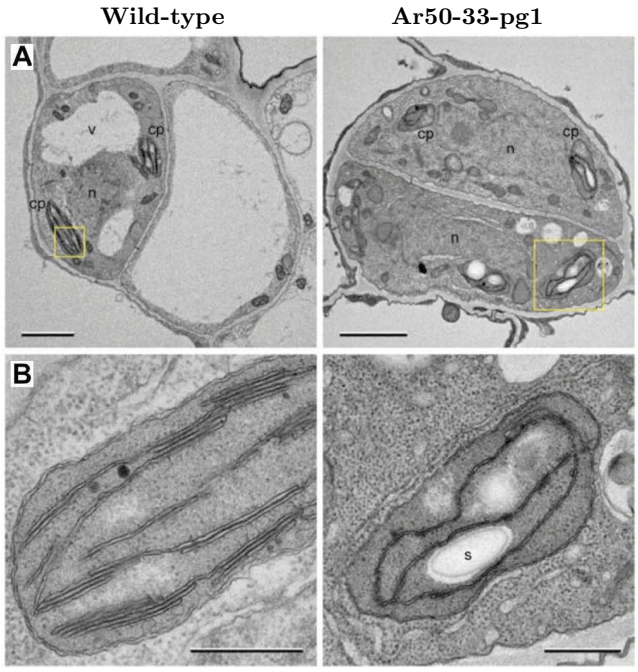

Fig. 2. Transmission electron microscopy analysis of chloroplasts in dividing guard mother cells of expanding leaves. Primary leaves of 10-day-old wild-type and Ar50-33-pg1 plants were analyzed. Guard mother cells (A) and the chloroplasts therein (B). cp, chloroplast; n, nucleus; s, starch; v, vacuole. Bar = 2 μm (A) and 0.5 μm (B).

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