Involvement of AtMinE1 in Plastid Morphogenesis in Various Tissues of Arabidopsis thaliana

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While it has been established that binary fission of leaf chloroplasts requires the prokaryote-derived, division site determinant protein MinE, it remains to be clarified whether chloroplast division in non-leaf tissues and the division of non-colored plastids also involve the MinE protein. In an attempt to address this issue, plastids of cotyledons, floral organs, and roots were examined in the Arabidopsis thaliana mutant of the MinE (AtMinE1) gene, which was modified to express the plastid-targeted cyan fluorescent protein constitutively, and were quantitatively compared with those in the wild type. In the cotyledons, floral organs, and root columella, the plastid size in the atminE1 mutant was significantly larger than in the wild type, while the plastid number per cell in atminE1 appeared to be inversely smaller than that in the wild type. In addition, formation of the stroma-containing plastid protrusions (stromules) in the cotyledon epidermis, petal tip, and root cells was more active in atminE1 than in the wild type.

Key words: amyloplast; chloroplast division; leucoplast; MinE; stromule

The presence of homogeneously sized chloroplasts is one of the conspicuous morphological traits of leaf mesophyll cells in land plants. This homogeneity of chloroplasts is considered to be attained by symmetric binary division of the organelles.1 Chloroplast division requires two types of plant homologues of prokaryotic cell division protein FtsZ, FtsZ1 and FtsZ2,2) which assemble into a ring structure just beneath the inner envelope membrane at the mid-chloroplast point.3–6) The confinement of the FtsZ ring assembly within the mid-chloroplast is known to involve other prokaryote-derived, stromal proteins, MinD7,8) and MinE.9–11) Overexpression of MinD in leaf mesophyll cells resulted in inhibition of chloroplast division and failure of the FtsZ ring assembly,4,7,8,12) while reduced expression or a loss-of-function mutation of MinD resulted in asymmetric chloroplast division with the formation of non-central and/or multiple FtsZ ring(s).7,12–15) Precisely conversely, overexpression of MinE led to the latter results,16,11,14,15) and reduced expression or T-DNA inactivation of MinE, the former.14,16) These data imply an inhibitory role of MinD and an antagonistically facilitative role of MinE in chloroplast division and FtsZ ring assembly, in accord with the "Min system" model of Escherichia coli.17–19) In E. coli, MinD in the ATP-bound form is associated with the plasma membrane and covers a polar zone extending toward the midcell, just like a test tube. MinE is localized mainly at the edge of the MinD tube as a ring, and detaches MinD from the membrane by activating the ATP hydrolysis of MinD. This results in shrinkage of the MinD tube and the following movement of the MinE ring toward the pole. Then a new MinD tube appears at the other polar side, and MinE-induced shrinkage of the tube occurs again. This oscillation of MinD and MinE in the bacterial cell ensures the lowest concentration of the division inhibitor MinD at the midcell and thus permits FtsZ ring assembly and cytokinesis exclusively therein. Nevertheless, recent studies using Arabidopsis thaliana indicate that the above scheme should not be simply applicable to chloroplast division, because AtMinD1, the sole MinD homologue in A. thaliana,7,8) has been found to be located all over the chloroplast envelope membrane and preferentially at the mid-chloroplast constriction site20) and to be capable of rescuing the minicell phenotype of the E. coli minD minE double deletion mutant without oscillation.21) This suggests the uniqueness of the chloroplast Min system, as distinct from that of E. coli.

In land plants, the chloroplast is one member of diverse, interconvertible forms of plant-specific, double membrane-limited organelles, collectively called as plastids.22) In the last decade, the machinery of chloroplast division in leaf tissues has been characterized in detail (for a recent review, see Yang et al.23). Despite this, studies on the molecular processes of chloroplast division in non-leaf tissues and the division of non-green plastids, such as amyloplasts, leucoplasts, chromoplasts, and proplastids, have been less frequently conducted.24–27) Holzinger et al.27) extensively investi-
gated the effects of mutations in three ARC (ACUMULATION AND REPLICATION OF CHLOROPLASTS) genes \(^{28-32}\) of \(A.\ thaliana\), \(ARC2\) \(30,33,34\), \(ARC5\) \(30,35,36\), and \(ARC6\) \(12,24,31\), which are required for the normal progression of chloroplast division, on plastid morphology in both green and non-green tissues. This study showed that the effects of \(arc3\) on plastid morphology appeared mostly similar regardless of the tissues examined, while the effects of \(arc5\) appeared to be limited to chloroplasts within green tissues, and the effects of \(arc6\) were somewhat variant, depending on the tissue. This alteration of the division machinery accompanying plastid differentiation appears to be another aspect that highlights the difference between plastid division and prokaryotic cell division.

In this study, we examined plastid phenotypes in the cotyledons, floral organs, and root tissues of the \(A.\ thaliana\) mutant of the \(MinE\) (\(AtMinE1\)) gene.\(^{14}\) By the use of transgenic plants that constitutively express the plastid-targeted cyan fluorescent protein (CFP), we visualized non-colored plastids in roots and weakly pigmented plastids in the cotyledon epidermis, as well as chlorophyll-free, stroma-containing membranous tubules, known as stromules.\(^{37}\)

**Materials and Methods**

**Plant materials and growth conditions.** \(Arabidopsis\ thaliana\) (L.) Heynh. was used in all experiments. Transgenic \(A.\ thaliana\) lines (Columbia background), which express the \(N\)-terminal transit peptide (90 amino acids) of \(AtFtsZ1\)\(^{12,38}\) fused to the \(N\)-terminal end of CFP (AtFtsZ1-1TP–CFP), were generated by \textit{Agrobacterium}-mediated stable transformation.\(^{39}\) Binary vector pSMB704 (kindly provided by Dr. Hiroaki Ichikawa) was inserted with the AtFtsZ1-1TP–CFP gene, the upstream cauliflower mosaic virus 35S promoter, and the terminator, and the resulting expression vector was used in the above transformation. Among the transformants, a line with stable high expression of the transgene was selected. A recessive, T-DNA insertional mutant of \(AtMinE1\)\(^{14}\) was used in all experiments. The accumulation level of the correctly spliced \(AtMinE1\) transcripts was estimated to be approximately 2,000-fold reduced as compared with that in the WT, indicating that \(AtminE1\) is a severe knockdown mutation.\(^{14}\) The overall plant morphology, growth, flowering, and fertility of the \(AtminE1\) plants appeared normal under laboratory conditions. On the other hand, the \(atminE1\) plants were found to possess giant chloroplasts, with extremely reduced numbers per cell, in the developing and mature leaf mesophyll cells and parenchyma cells of the leaf petioles. Such chloroplast phenotypes imply severe inhibition of chloroplast division, which has been associated with defective organization of the FtsZ1 (AtFtsZ1)–filaments in the mutant.\(^{14,15}\) In this study, we utilized the \(atminE1\) line to assess the global effects of the mutation in the \(AtMinE1\) gene on plastid morphology in various green and non-green tissues.

**Plastid morphology in cotyledons, rosette leaves, and floral organs**

We examined the mesophyll or epidermal plastids in the rosette leaves and cotyledons of \(atminE1\) and \(WT\), which expressed the \(AtFtsZ1-1TP–CFP\) transgene (Figs. 1, 2). Based on observation by chlorophyll autofluorescence, the chloroplast morphology in the \(AtFtsZ1-1TP–CFP\)-expressing WT line was basically same as in the non-transgenic \(A.\ thaliana\) WT plants (data not shown). Furthermore, the plastid morphology in the \(AtFtsZ1-1TP–CFP\)-expressing WT line was quite similar to that in the WT line expressing \(RbcS\-R–green\) fluorescent protein (data not shown), which has been used widely as a plastid-labelling marker.\(^{32}\) This confirmed that the expression of \(AtFtsZ1-1TP–CFP\) \(per\ se\) did not affect plastid morphology. The mesophyll chloroplasts in the \(atminE1\) cotyledons were giant and few in number per cell as compared to those in WT (Fig. 1, Table 1), similarly to the leaf mesophyll chloroplasts (Fig. 1, Table 1).\(^{14}\) The mean plan areas of the \(atminE1\) mesophyll chloroplasts in the rosette leaves and the cotyledons were approximately 27- and 12-fold larger respectively than those of WT (Table 1). In addition, the cotyledon mesophyll chloroplasts in \(atminE1\) were heteromorphic, while those in WT were uniform in size and spherical morphology (Fig. 1). The epidermal chloroplasts in the cotyledons, which were immature and hence only weakly pigmented with chlorophyll, were visualized by the use of plastid-targeted \(AtFtsZ1-1TP–CFP\), and were also found to be significantly larger in \(atminE1\) than in WT (Fig. 1,
Table 1). The mean plan area of the atminE1 plastids in the cotyledon epidermis was approximately 5-fold larger than that of WT (Table 1). The morphology of the cotyledon epidermis plastids in atminE1 was typically spherical or spindle-shaped, but more irregular and heterogeneous than in WT (Fig. 1). Besides this, active formation of stromules was observed in atminE1 (see below). In the cotyledon epidermis of WT, there was a correlation between the plastid number per epidermal cell and the epidermal cell plan area (Fig. 2; \( r^2 = 0.842 \)), in the same manner as reported for the leaf mesophyll.\(^{30,43}\) Whereas a similar correlation was also observed in atminE1 (\( r^2 = 0.481 \)), the plastid number per epidermal cell was consistently smaller in atminE1 than in WT (Fig. 2). In a cotyledon epidermal cell of the same size, the plastid number in atminE1 was calculated to be 4-fold fewer than in WT (Fig. 2). The same tendency of as to chloroplast number—cell size correlation was also confirmed in the mesophylls of the rosette leaves, while the chloroplast number per leaf mesophyll cell in atminE1 appeared to be substantially constant, from 1 to 4, irrespective of cell size, and thus the difference between WT and atminE1 in the leaf mesophyll was more extreme than in the cotyledon epidermis (Fig. 2). Moreover, we found that the occurrence of stromules\(^{37} \) in the cotyledon epidermis was significantly (8-fold) more frequent in atminE1 than in WT (Fig. 1, Table 2), and that the stromule length in atminE1 was twice as high as in WT (Table 2). The stromules in the epidermis of the atminE1 cotyledons often exhibited branching and interconnection of two or more plastids (Fig. 1); this has been described as a typical behavior of stromules.\(^{37,44,45} \)

Next we extended our observations to the floral organs (tissues), including the sepalas (adaxial parenchyma), petals (epidermis at the tip and epidermis and parenchyma in the basal regions), stamens (anther epidermis and filament epidermis), and pistils (style and outer valve epidermis) of atminE1 and WT (Fig. 3). Non-colored plastids (leucoplasts) in the petal epidermis in the tip region were observed by CFP fluorescence, and the chloroplasts and plastids in the other floral tissues were observed by chlorophyll autofluorescence. The anther epidermal plastids were not green-colored, but emitted red autofluorescence under chlorophyll-excitation conditions. For all the tissues examined, the plan area of the plastids was significantly larger in atminE1 than in WT (Fig. 3, Table 1). The mean plan areas of the atminE1 plastids in the floral tissues were 2.4- to 17-fold larger than those of WT, and the extent of plastid enlargement varied according to the tissue (Table 1). The most extreme enlargement of plastids (17-fold) was observed in the basal petal region, and the slightest enlargement (2.4-fold) the tip region (Fig. 4, Table 1).

### Table 1. Mean Plastid Plan Area in Various Tissues of WT and atminE1\(^{a}\)

<table>
<thead>
<tr>
<th>Tissue(^{b})</th>
<th>Mean plastid plan area ± SD ((\mu m^2))^(c)</th>
<th>WT</th>
<th>atminE1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf mesophyll(^{**})</td>
<td>44.9 ± 11.4 [10.132]</td>
<td>1198.1 ± 393.5 [10.56]</td>
<td></td>
</tr>
<tr>
<td>Cotyledon mesophyll(^*)</td>
<td>53.0 ± 0.7 [3.102]</td>
<td>668.3 ± 170.2 [3.32]</td>
<td></td>
</tr>
<tr>
<td>Cotyledon epidermis(^**)</td>
<td>10.9 ± 3.0 [14.443]</td>
<td>49.9 ± 14.7 [8.78]</td>
<td></td>
</tr>
<tr>
<td>Sepal parenchyma (adaxial)(^*)</td>
<td>19.9 ± 0.6 [3.55]</td>
<td>133.4 ± 37.8 [3.21]</td>
<td></td>
</tr>
<tr>
<td>Petal epidermis (tip)(^**)</td>
<td>1.2 ± 0.2 [6.158]</td>
<td>2.9 ± 1.0 [6.110]</td>
<td></td>
</tr>
<tr>
<td>Petal epidermis and parenchyma (base)(^*)</td>
<td>5.6 ± 1.7 [3.97]</td>
<td>94.3 ± 22.2 [3.20]</td>
<td></td>
</tr>
<tr>
<td>Anther epidermis(^**)</td>
<td>1.9 ± 0.4 [6.131]</td>
<td>5.4 ± 2.6 [6.64]</td>
<td></td>
</tr>
<tr>
<td>Filament epidermis(^*)</td>
<td>3.7 ± 1.3 [3.75]</td>
<td>27.7 ± 10.6 [3.18]</td>
<td></td>
</tr>
<tr>
<td>Style epidermis(^*)</td>
<td>3.0 ± 1.4 [6.97]</td>
<td>10.7 ± 3.3 [6.43]</td>
<td></td>
</tr>
<tr>
<td>Valve epidermis (abaxial)(^*)</td>
<td>7.5 ± 1.7 [3.94]</td>
<td>45.9 ± 27.5 [3.47]</td>
<td></td>
</tr>
<tr>
<td>Root columella(^**)</td>
<td>5.3 ± 0.7 [6.53]</td>
<td>8.9 ± 1.4 [6.57]</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)For each tissue, measurement was performed using plants of the same age as those shown in Figs. 1, 3, 4 (see legends). When measuring the plastid plan area in mesophyll tissues, the plant samples were gently pushed by application of pressure to the coverslip. The numbers of plants and total plastids examined are shown in square brackets in that order.

\(^{b}\)Tissues for which pairs of WT versus atminE1 values are significantly different at \( p < 0.05 \) or \( 0.01 \), as determined by Mann-Whitney U test, are marked with single (*) and double (**) asterisks respectively.

\(^{c}\)SD, standard deviation.
plastids and stromules in the petal tip of atminE1 observed in emanating stromules along the cell periphery were observed. As for the leucoplasts in the petal epidermis in the tip parenchyma in the basal regions and filament epidermis.]

In the floral tissues of the atminE1 mutant, chloroplast morphologies were found to be diverse: crescent-shaped, prolate ellipsoid, amorphous, spherical, dumbbell-shaped, arcuate, or multilobed (Fig. 3). In general, the chloroplasts appeared to be appressed to the cell periphery. In the elongated cells, as seen in the petal epidermis and parenchyma in the basal regions and the filament epidermis, the chloroplasts tended to have a prolate-ellipsoid morphology. Additionally, in the filamen
ted plastids the for atminE1 open circle). As for the cotyledon epidermis, 28 cells were analyzed for WT and 16 cells for atminE1. The regression lines for WT (solid line) and atminE1 (broken line) are also shown.

In the floral tissues of the atminE1 mutant, chloroplast morphologies were found to be diverse: crescent-shaped, prolate ellipsoid, amorphous, spherical, dumbbell-shaped, arcuate, or multilobed (Fig. 3). In general, the chloroplasts appeared to be appressed to the cell periphery. In the elongated cells, as seen in the petal epidermis and parenchyma in the basal regions and the filament epidermis, the chloroplasts tended to have a prolate-ellipsoid morphology. Additionally, in the filament epidermis of atminE1, mini-sized chloroplasts were frequently observed (Fig. 3, arrowheads). In contrast to the case of atminE1, the chloroplast (plastid) morphologies in WT were generally more simple-shaped: spherical or oval (sepal parenchyma, anther epidermis, style epidermis, and outer valve epidermis) or dumbbell-shaped or pairs of adjacent spheres (petal epidermis and parenchyma in the basal regions and filament epidermis). As for the leucoplasts in the petal epidermis in the tip region, few spindle-shaped plastids with both ends emanating stromules along the cell periphery were observed in atminE1, while spherical to rod-shaped leucoplasts were commonly observed in WT (Fig. 3). The morphology and the subcellular location of the plastids and stromules in the petal tip of atminE1 were quite similar to those observed in arc6.7) We investigated quantitatively the occurrence frequency and the mean length of the stromules in the petal tips, confirming significant differences for both values between WT and atminE1 (Table 2). Stromules appeared 2.6 times more frequently in atminE1 than in WT, and their length was 2.6 times greater in atminE1 than in WT.

### Table 2. Occurrence Frequency and Mean Length of Stromules in the Cotyledon Epidermis and the Petal Tip of WT and atminE1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Line</th>
<th>Frequency (%)</th>
<th>Mean length ± SD (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledon epidermis</td>
<td>WT</td>
<td>46 [413]</td>
<td>8.4 ± 1.1 [25]</td>
</tr>
<tr>
<td></td>
<td>atminE1</td>
<td>48 [114]</td>
<td>17.8 ± 1.2 [55]</td>
</tr>
<tr>
<td>Petal tip</td>
<td>WT</td>
<td>4 [372]</td>
<td>2.0 ± 0.2 [43]</td>
</tr>
<tr>
<td></td>
<td>atminE1</td>
<td>4 [177]</td>
<td>5.2 ± 0.3 [54]</td>
</tr>
</tbody>
</table>

*Counting and measurement for the cotyledon epidermis were performed using six 1-week-old individuals. Counting and measurement for the petal tip were performed using nine individuals up to 10 weeks old. Clearly shaped plastids for which we could unmistakably judge the presence or absence of attached stromules were chosen as samples. The numbers of total plastids examined are shown in square brackets.

*Frequency was defined as the percentage of stromule-emanating plastids.

*Stromule length was defined as the length of the longest track of the main stromule and did not include the length of laterally branched parts.

*Pairs of WT versus atminE1 values that are significantly different at *p* < 0.001, as determined by *χ*² test.

*Pairs of WT versus atminE1 values that are significantly different at *p* < 0.01, as determined by Mann-Whitney U test.

Plastid morphology in root tissues

Roots contain several types of non-colored plastids, including leucoplasts and amyloplasts. At present, the division machinery of those plastids in the roots remains poorly understood. In this study, we observed and quantitatively analyzed the plastid morphology in the root tissues of atminE1 and WT. The columella cells at the root tip are known to contain well-developed amyloplasts, which are thought to be involved in gravity sensing in the cells.46) The amyloplasts in the columella cells of atminE1 were larger than those in WT (Figs. 4, 5, Table 1), as is the case of the aerial tissues examined, although the enlargement ratio (atminE1/WT) was 1.7, and thus not so high as those observed in the aerial tissues (*i.e.*, 2.4 at the lowest). There were no significant differences in total starch content in the columella cells between atminE1 and WT, as estimated by starch staining with iodine potassium iodide (data not shown). In relation to this, we also conducted a gravitropism assay of the roots and hypocotyls of dark-grown, etiolated seedlings by the method of Caspar and Pickard.37) Again, we did not detect major differences in gravitropism between atminE1 and WT (data not shown).

In the outer parenchyma cells of the mature regions of the main roots and the root hairs, the difference in plan area of stromule-free plastids between similarly developed and spatially equivalent regions of atminE1 and WT was not significant (Fig. 5, Table 3). Nevertheless, the plan area of the stromule-emanating plastids in the root hairs and the entire length of the stromule-emanating plastids in both the main roots and the root hairs were greater in atminE1 than in WT (Fig. 5, Table 3), while the frequencies of stromule formation in the main roots and the root hairs of atminE1 were not
significantly different from those of WT (Table 3). The mean plan area of the stromule-emanating plastids in the root hairs was 2.4-fold larger in \textit{atminE1} than in WT, and the mean total length of the stromule-emanating plastids in the root tissues was approximately 1.7-fold longer in \textit{atminE1} than in WT (Table 3).

### Table 3. Mean Plastid Plan Area, Mean Plastid Length, and Occurrence Frequency of Stromules in the Root Tissues of WT and \textit{atminE1}*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Line</th>
<th>Mean plan area (±SD [μm²]) of stromule-free/-emanating plastids</th>
<th>Mean length (±SD [μm]) of stromule-emanating plastids</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mature main root</td>
<td>WT</td>
<td>4.1 ± 2.1 [9;32]/11.4 ± 5.3 [3;35]</td>
<td>*10.8 ± 5.1 [9;28]</td>
<td>56 [8;147]</td>
</tr>
<tr>
<td></td>
<td>\textit{atminE1}</td>
<td>3.1 ± 1.4 [7;19]/12.7 ± 5.6 [9;40]</td>
<td>*19.2 ± 9.0 [9;40]</td>
<td>70 [13;137]</td>
</tr>
<tr>
<td>Root hair</td>
<td>WT</td>
<td>2.2 ± 1.3 [11;38]/4.5 ± 1.2 [10;24]</td>
<td>*3.5 ± 1.0 [10;25]</td>
<td>40 [13;73]</td>
</tr>
<tr>
<td></td>
<td>\textit{atminE1}</td>
<td>2.7 ± 1.0 [6;19]/8.4 ± 4.8 [8;21]</td>
<td>*5.8 ± 1.8 [8;21]</td>
<td>57 [9;44]</td>
</tr>
</tbody>
</table>

*Counting and measurement were performed using 1-week-old plants. The numbers of plants and total plastids examined are shown in square brackets in that order.

*Plastid length was defined as the total length of the main plastid body and its stromules.

*Frequency was defined as the percentage of stromule-emanating plastids.

*Pairs of WT versus \textit{atminE1} values that are significantly different at \( p < 0.001 \) (d) or 0.05 (e, f), as determined by Mann-Whitney \( U \) test.

Fig. 3. Plastid Morphology in the Floral Organs of \textit{atminE1}.
Chloroplasts in sepal parenchyma (adaxial side), basal regions of petal epidermis and parenchyma, anther epidermis, filament epidermis, style epidermis, and valve epidermis (abaxial side) from open flowers of WT and \textit{atminE1} plants up to 10 weeks old were visualized by chlorophyll autofluorescence, and leucoplasts in tip regions of petal epidermis were visualized by CFP fluorescence. The arrows in the \textit{atminE1}—Petal (Tip) panel indicate stromules. The arrowheads in the \textit{atminE1}—Filament panel indicate mini-sized chloroplasts. Scale bar, 10 μm.

Discussion

In our former study,\(^9\) we monitored tissue-specific expression of the \textit{AtMinE1} gene \textit{in situ} by means of histochemical β-glucuronidase (GUS) staining of transgenic \textit{A. thaliana} lines harboring an \textit{AtMinE1} promoter::GUS reporter fusion. This revealed specific activation of the promoter in green tissues, including the shoot apices, leaves, cotyledons, sepals, anthers, pollen grains, stigma, and siliques, while GUS staining was hardly detectable in the roots. Our results are consistent with the microarray data from Arabidopsis eFP Browser, the comprehensive gene expression atlas of \textit{A. thaliana}.\(^{48}\) From these results, it appears likely that the \textit{AtMinE1} gene is globally required for chloroplast division in the cotyledons and floral organs, in addition to the rosette leaves, as previously found.\(^{14}\) In this study, we found that the chloroplasts in the \textit{atminE1} cotyledons and floral organs were giant and few in number per cell as compared to those in WT. Enlargement of the chloroplasts is a typical symptom of inhibition of chloroplast division, and is considered to reflect a yet unexplained compensatory mechanism of a
chromoplasts in the ripe red fruits. During the differentiation in ripening fruits of the tomato (Solanum lycopersicum) different phenomenon has been found to occur upon chromoplast enlargement at the petal tip (Figs. 3, 4, Table 1). A similar extreme enlargement at the petal base, and the smallest decrease in the chloroplast number per cell. Exceptionally, we found mini-sized chloroplasts in the filament epidermis of atminE1 (Fig. 3). Mini-sized plastids in the filaments have also been observed in the arc3 and arc6 mutants, although those plastids were identified as “non-green” (see Table 4 for a brief summary of plastid morphology in certain tissues of atminE1 and arc mutants). The occurrence of mini-sized plastids in the filament epidermis of the plastid division mutants might be explained by a malfunction of the division site selection system for plastids, which normally specifies the mid-plastid site upon plastid division in WT. Moreover, we also noticed that in the floral tissues of atminE1, plastids exhibited the most extreme enlargement at the petal base, and the smallest enlargement at the petal tip (Figs. 3, 4, Table 1). A similar phenomenon has been found to occur upon chloroplast differentiation in ripening fruits of the tomato (Solanum lycopersicum) suffulta mutant, in which aberrantly enlarged chloroplasts of green developing fruits degenerated and gave rise to a wild-type-like population of chromoplasts in the ripe red fruits. During the redifferentiation of chloroplasts into leucoplasts upon petal development, giant plastids in atminE1 are perhaps downsized, accompanied by a reduction in the size of the petal cells. This might make the leucoplast enlargement in the atminE1 petal tip modest.

In the root columella cells, there was a significant difference in the size of amyloplasts between WT and atminE1 (Figs. 4, 5, Table 1). This coincides with an earlier observation of amyloplasts in the columella cells of arc12, another functionally defective allele of AtMinE1, by means of starch staining with iodine potassium iodide. The phenotype of amyloplast enlargement in the columella cells of atminE1 (Figs. 4, 5, Table 1) and arc12 also resembles that in arc6, which were examined by both iodine staining and transmission electron microscopy. As is the case of atminE1, in both arc12 and arc6, there were no major differences in total starch content or total mass of plastids per cell in columella cells as compared to WT. These results imply that proplastid or amyloplast division during the differentiation of columella cells from stem cells in the root cap might be susceptible to mutations in the genes encoding the chloroplast division factors, though starch synthesis in those plastids was not inhibited. Besides, it has been found that the arc12 and the arc6 mutations slightly reduced the gravitropism of light- and dark-grown inflorescence stems and light-grown hypocotyls, but not of dark-grown hypocotyls or light- or dark-grown roots, by means of kinetic analysis of gravicurvature. Along with this, we did not detect significant differences in the gravitropism of the hypocotyls or roots of dark-grown, etiolated seedlings between atminE1 and WT (data not shown).

In root tissues, except for the columella cells, the differences in the size distribution of plastids between WT and atminE1 were obscure as compared to those observed in the aerial tissues (Fig. 5, Table 3). It is
possible that these weak effects of the atminE1 mutation reflect the low expression level of this gene in the roots of WT, as determined by GUS staining53) and microarray analysis.54) However, there were significant differences between WT and atminE1 in the total length of the stromule-emanating plastids of the mature main roots and root hairs, and in the plan area of the plastids of the root hairs, while the occurrence frequency of stromules was not significantly different (Table 3). Vigorous elongation of the stromules in atminE1 was also observed in the cotyledon epidermis and the petal tip (Figs. 1, 3 and Table 2). The occurrence frequency of stromules in atminE1 was concomitantly greater than that in WT in the cotyledon epidermis and the petal tip (Table 2). In arc6, increases in both the occurrence frequency and the length of stromules were observed for several organs, including the roots and the hypocotyl epidermis.27) Our results for atminE1 were different from those for arc6, in that atminE1 displayed increased stromule length without elevating the stromule frequency in the roots, although this might merely reflect differences in experimental or growth conditions, and might not be attributable to the functional difference between ARC6 and AtMinE1. At present, it remains unknown whether the active formation (unusual elongation and/or frequent occurrence) of stromules in atminE1 should be attributable directly to a dysfunction of AtMinE1. One explanation is that AtMinE1 is directly involved in the control of stromule formation.

Anther is that the atminE1 mutation affects the plastid density within the cell, which secondarily affects the degree of stromule formation. Waters et al.54) found that the stromule length and frequency were negatively correlated with the plastid density. It is possible that the atminE1 mutation lowered the plastid density in certain tissues, through inhibition of plastid division. Nevertheless, it has been found that the plastid density was not affected in the hypocotyl epidermis of arc6 and arc3, in spite of the increase in stromule length in both mutants.27) From this, it follows that the inverse relationship between plastid density and stromule formation does not necessarily hold true of mutant cases.

In conclusion, our observations imply that the atminE1 mutation affects plastid morphogenesis and/or division globally in the aerial and root tissues as far as examined, and positively affects stromule formation and/or elongation in a tissue-dependent manner, although it is not clear whether this effect is directly attributable to the molecular function of AtMinE1 (i.e., a secondary result due to alterations in plastid size or number per cell). Further study of this issue might shed light on the possible link between stromule formation and size control of plastids.

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