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Chloroplast Division and Expansion Is Radically Altered by Nuclear Mutations in *Arabidopsis thaliana*[1]

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ABSTRACT

We have isolated three mutants of *Arabidopsis thaliana* in which there is a sevenfold change in chloroplast number in fully expanded leaf mesophyll cells and increases and decreases in chloroplast number are compensated for by changes in chloroplast size. The changes are stably inherited in sexual crosses for three generations and mutant phenotypes are effected by changes at single recessive nuclear loci, termed *arc* loci. This is the first report of large, stably inherited changes in chloroplast number in higher plants, and represents a major advance toward the genetic dissection of the control of chloroplast division.

The attainment of photosynthetic competence in leaves is entirely dependent on the authentic development of chloroplasts. Chloroplasts arise from undifferentiated plastids in small mitotic cells and replicate during normal mesophyll cell development (1, 5, 7). As a result, the number of chloroplasts increases dramatically during mesophyll cell expansion producing a large population of chloroplasts in each mature mesophyll cell. Chloroplast population size is species specific, can be variety specific, and is always closely related to mesophyll cell size (2, 4, 8). The genetic manipulation of chloroplast number would be one way of influencing the photosynthetic capacity of the leaf. What possibilities exist for the manipulation of chloroplast number? A role for nuclear genes has been suggested (6), yet nothing is known of the genetic factors controlling the division and rate of accumulation of chloroplasts during cell development. Clearly, the isolation of mutants with altered chloroplast accumulation characteristics would be invaluable in the genetic dissection of the control mechanisms responsible for chloroplast replication. Using a novel image analysis screening procedure (9), we have isolated 18 mutants of *Arabidopsis thaliana* with altered chloroplast accumulation and we report here the analysis of three mutants during mesophyll cell expansion.

MATERIALS AND METHODS

Plant Material

The mutants of *Arabidopsis thaliana* (L.) Heynh. var Landsberg *erecta* were isolated from an ethyl methane sulfonate-induced M2 population (Lehle Seeds, Tucson, AZ) by image analysis screening after iodine staining of isolated cells (9). The plants were grown as described previously (9) and first leaves were harvested during the course of leaf expansion; for wild type between 9 and 22 d after sowing and between 14 and 33 d after sowing for mutants. The oldest leaves sampled in each line were fully expanded and the mean mesophyll cell plan area for the whole leaf was also maximal.

Preparation of Mesophyll Cell Suspensions for Chloroplast Counting

The numbers of chloroplasts in individual isolated fixed mesophyll cells in a representative population from the whole leaf were counted using Nomarski differential interference contrast optics (9). Mesophyll cell plan areas were measured using an image analysis system (Seescan Imaging Ltd, Cambridge, UK) linked to a Nikon Optiphot microscope (9). In mesophyll cells in which chloroplast numbers were counted, plan areas of 5 to 15 individual chloroplasts were also measured using the same image analysis procedures as used for cell plan area measurements. For each cell, the total chloroplast plan area is calculated as number of chloroplasts × mean chloroplast plan area.

RESULTS

During wild-type mesophyll cell development, the number of chloroplasts per cell is closely correlated with the size of the cell ($r^2 = 0.86$, Fig. 1a). In three different mutants of *Arabidopsis* (Fig. 1, b–d), this relationship between cell plan area and chloroplast number is significantly different from that shown in wild-type cells. In mutant *arc*1 (Fig. 1b), there is an increased rate of chloroplast accumulation during cell expansion compared with wild type, producing an increase in chloroplast number per cell. In contrast, mutant *arc*2 (Fig. 1c) has a reduced rate of chloroplast accumulation during cell expansion, producing fewer chloroplasts per cell. In the most extreme mutant, *arc*3, chloroplast number per cell remains almost constant during mesophyll cell expansion (Fig. 1d). Figure 2 illustrates the mesophyll cells of three mutant plants and the wild type. The mutant plant phenotype is morphologically normal.

The large differences in the patterns of chloroplast accumulation observed in these mutants are stably inherited through three selfed generations and also after reciprocal backcrossing to wild type (Table I) F2 progeny segregate in a 3:1 ratio of wild type:mutant ($P > 0.05$) in both halves of each backcross, indicating that the different patterns of chlo-

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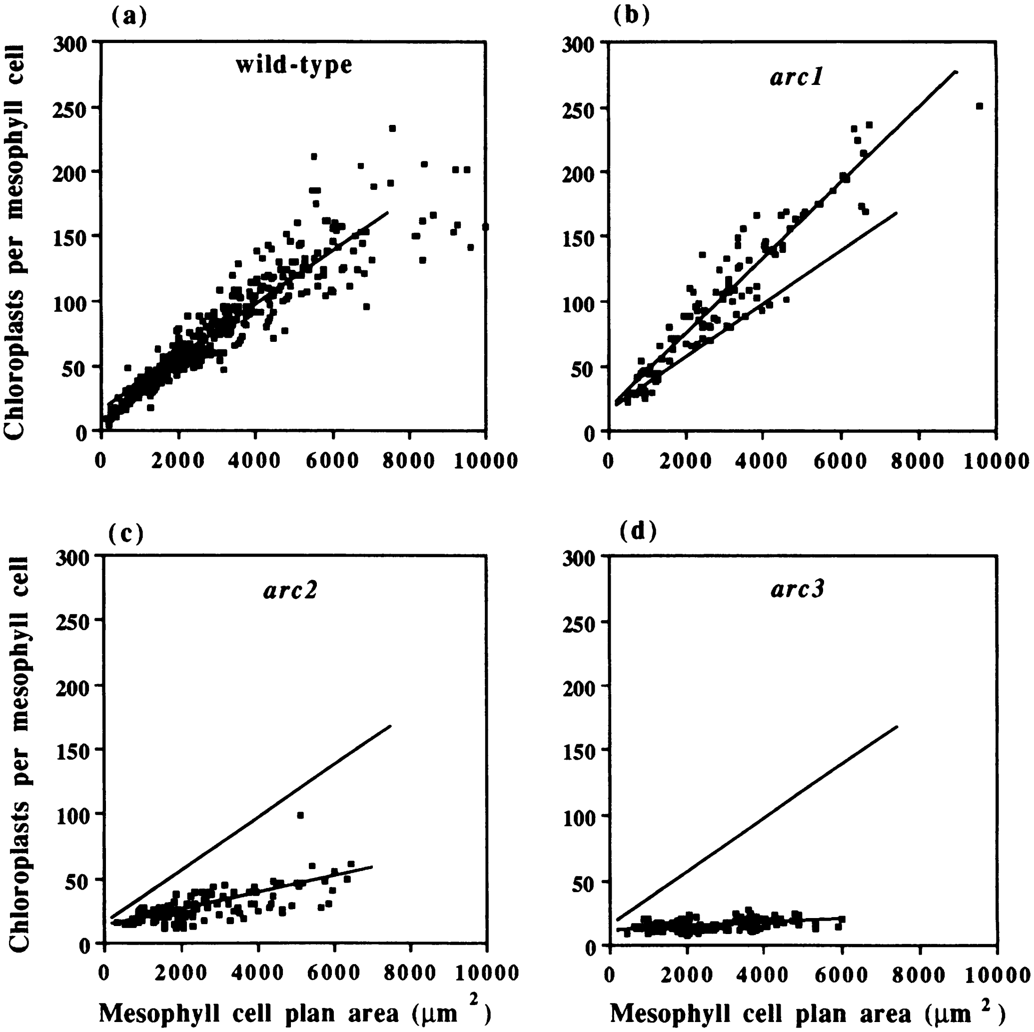

Figure 1. The relationship between chloroplast number per mesophyll cell and mesophyll cell plan area for wild-type and three *arc* mutants of *Arabidopsis thaliana* cv Landsberg *erecta*. Each data point represents the measurement from one cell whose area was measured and the chloroplast complement counted. Values of r^2 for each relationship are for (a) wild type, 0.86; (b) *arc*1, 0.90; (c) *arc*2, 0.46; and (d) *arc*3, 0.17. The wild-type regression line from (a) is shown on each subsequent graph for comparison.

roplast accumulation in *arc*1, *arc*2, and *arc*3 are caused by mutations at single recessive nuclear loci. Complementation analysis of crosses between the three mutants has shown clearly that three different nuclear loci are involved and we have termed these nuclear loci *arc* loci, *i.e.* "*a*ccumulation and *r*eplication of *c*hloroplasts" loci.

In the mutants, modified patterns in chloroplast accumulation are compensated for by differences in chloroplast size (Table II). In *arc*1, the increased number of chloroplasts per unit cell plan area (column 4) is associated with a reduction in chloroplast size compared with wild type, whereas the sequential decreases in mean chloroplast number (column 2) in mutants *arc*2 and *arc*3 are associated with increases in chloroplast size (column 3). Quantitatively, the relationship between total chloroplast plan area per cell (chloroplast number per cell × mean chloroplast plan area per cell) and cell size for individual mesophyll cells during cell expansion is similar for all three *arc* mutants and for wild type (Fig. 3). In marked contrast, the different relationships between chloroplast number and mesophyll cell size show very wide variation (Fig. 1). In all three *arc* mutants, the compensation between chloroplast number and chloroplast size results in 90% of the total chloroplast plan area found in wild-type cells of the same plan area.

In *arc*3, chloroplast number does not increase significantly during cell development (Fig. 1d) and the increase in the chloroplast compartment size during cell expansion is due largely to chloroplast expansion, resulting in a few very large chloroplasts in fully expanded cells (Table II, Fig. 2d). Chloroplast division is clearly not an essential prerequisite for normal mesophyll cell development in this plant. In *arc*1, in contrast, the chloroplasts do not increase in mean size during mesophyll cell expansion and thus the increase in chloroplast compartment size is largely by chloroplast replication. *arc*2 plants are intermediate between these two extremes, *arc*1 and *arc*3, with fewer larger chloroplasts than wild type (Table II).

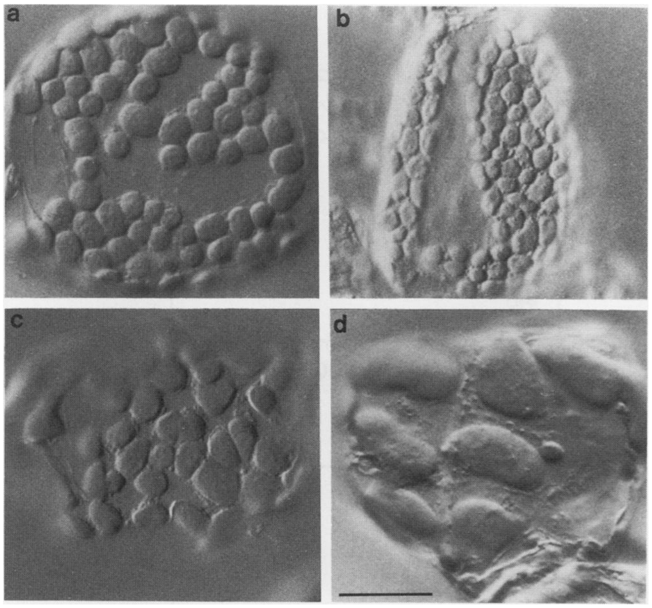

Figure 2. Isolated mesophyll cells from fully expanded first leaves of wild-type and three *arc* mutants of *Arabidopsis* viewed with Nomarski differential interference contrast optics (9). a, Wild type; b, *arc*1; c, *arc*2; and d, *arc*3. The rough surface appearance of some chloroplasts is the result of starch accumulation. Distinct areas of the cell surface in which chloroplasts are absent represent areas where mesophyll cells were joined. Bar = 25 μm.

Table I. *Results of Crosses with arc Mutants*

Mutants were selfed for three generations (M5) and then were reciprocally backcrossed to wild-type Landsberg *erecta* and the progeny examined in the F1 and F2 generations. Mutant phenotypes were scored by microscopic examination of isolated mesophyll cells from first leaves of individual F1 and F2 seedlings harvested after 20 d. The χ^2 calculation is based on an expected segregation of one mutant to three wild type.

Cross	Generation	No. of Plants		χ^2
		Wild type	Mutant	
*arc*1/*arc*1	F1	54	0	
× ARC1/ARC1	F2	130	36	0.89
ARC1/ARC1	F1	45	0	
× *arc*1/*arc*1	F2	132	45	0.002
*arc*2/*arc*2	F1	44	0	
× ARC2/ARC2	F2	168	48	0.75
ARC2/ARC2	F1	81	0	
× *arc*2/*arc*2	F2	181	49	1.58
*arc*3/*arc*3	F1	22	0	
× ARC3/ARC3	F2	141	47	0
ARC3/ARC3	F1	32	0	
× *arc*3/*arc*3	F2	131	46	0.07

Table II. *Mean Mesophyll Cell Size, Mean Chloroplast Number, and Chloroplast Size for Populations of Fully Expanded Mesophyll Cells from First Leaves of Wild-Type and arc Mutants of Arabidopsis thaliana*

Mesophyll cell plan areas and chloroplast plan areas were measured by image analysis of fixed isolated cells (9). Mean chloroplast number for each line was determined from a regression of chloroplast number per cell on mesophyll cell plan area using the value for mean mesophyll plan area (column 1). Mesophyll cell plan area is a mean of 250 cells per line and mean chloroplast plan area is a mean of at least 200 chloroplasts from 30 different mesophyll cells. SEs are shown in parentheses.

	Mean Mesophyll Cell Plan Area	Mean Chloroplast Number	Mean Chloroplast Plan Area	Chloroplasts
	μm²	*No. per mesophyll cell*	*μm²*	*No. per 1000 μm² mesophyll cell plan area*
Wild type	4778 (135)	121	50.3 (0.5)	25
*arc*1	3388 (86)	108	25.8 (0.3)	32
*arc*2	4339 (106)	40	111.3 (3.9)	9
*arc*3	3582 (56)	18	202.1 (9.3)	5

The cell size/chloroplast number relationships for the three mutants and wild type have similar *y* intercepts (Fig. 1), indicating that the mean chloroplast number in the smallest cells for all three mutants and wild type are similar, *i.e.* 14 (SE = 1.0). This suggests that the effects of the genetic lesions in the mutants are specific to chloroplast development.

Analysis of *arc* mutants of *Arabidopsis* has shown that the accumulation and expansion of chloroplasts in mesophyll cells is under the influence of several nuclear genes and has highlighted the considerable degree of plasticity that can occur during development of the chloroplast compartment as a result of compensation between the rate of initiation of new chloroplast divisions and the rate of chloroplast expansion. The fact that *Arabidopsis* mutants with very different numbers of chloroplasts per cell are stable and grow normally indicates that chloroplast division *per se* is not critical for mesophyll cell development as long as changes in chloroplast number are compensated for by changes in chloroplast size. As a result of this compensation, plants in which chloroplast division appears to be largely absent, such as *arc*3, are viable. Mutants lacking this compensatory mechanism are likely to be greatly retarded or lethal. The balance between chloroplast number and size seen in wild-type cells presumably represents an optimized configuration of the chloroplast compartment within the cell. The compensation between chloroplast number and size in the *Arabidopsis* mutants suggests that chloroplast division and expansion are largely independent processes that interact (4, 9). Consequently, the genetic manipulation of chloroplast number may only be achieved when accompanied by changes in chloroplast size. The underlying nature of the genetic changes in these mutants that affect chloroplast division and accumulation is unknown, but by using *Arabidopsis* wild-type and mutant plants the isolation of the nuclear genes responsible should be greatly facilitated.

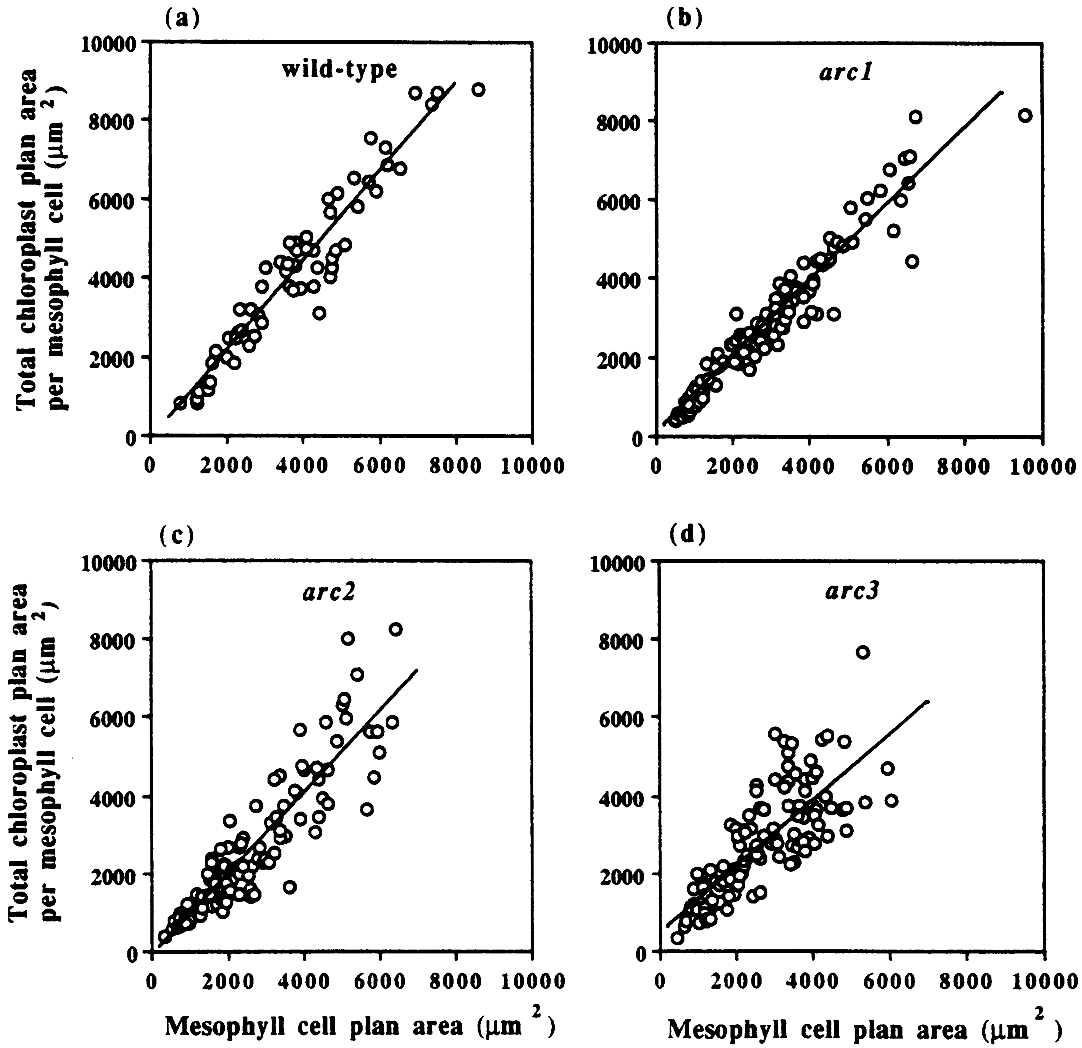

Figure 3. The relationship between total chloroplast plan area per mesophyll cell and mesophyll cell plan area for wild-type and three *arc* mutants of *Arabidopsis*. Values of r^2 are (a) wild-type, 0.92; (b) *arc*1, 0.93; (c) *arc*2, 0.81; and (d) *arc*3, 0.61.

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LITERATURE CITED

1. **Boffey SA, Ellis JR, Sellden G, Leech RM** (1979) Chloroplast division and DNA synthesis in light-grown wheat leaves. Plant Physiol **64:** 502–505
2. **Ellis JR, Leech RM** (1985) Cell size and chloroplast size in relation to chloroplast replication in light-grown wheat leaves. Planta **165:** 120–125
3. Deleted in proof
4. **Leech RM, Pyke KA** (1988) Chloroplast division in higher plants with particular reference to wheat. *In* SA Boffey, D Lloyd, eds, Division and Segregation of Organelles. Cambridge University Press, Cambridge, UK, pp 39–62
5. **Leech RM, Thomson WW, Platt-Aloia KA** (1981) Observations on the mechanism of chloroplast division in higher plants. New Phytol **87:** 1–9
6. **Possingham JV, Hashimoto H, Oross J** (1988) Factors that influence plastid division in higher plants. *In* SA Boffey and D Lloyd, eds, Division and Segregation of Organelles. Cambridge University Press, Cambridge, UK, pp 1–20
7. **Possingham JV, Sauer W** (1969) Studies on the growth of spinach leaves (*Spinacia oleracea*). Planta **86:** 186–194
8. **Pyke KA, Leech RM** (1987) The control of chloroplast number in wheat mesophyll cells. Planta **170:** 416–420
9. **Pyke KA, Leech RM** (1991) A rapid image analysis screening procedure for identifying chloroplast number mutants in mesophyll cells of *Arabidopsis thaliana*. Plant Physiol **96:** 1193–1195