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A Genetic Analysis of Chloroplast Division and Expansion in *Arabidopsis thaliana*[1]

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A nuclear recessive mutant of *Arabidopsis thaliana*, *arc*5, has been isolated in which there is no significant increase in chloroplast number during leaf mesophyll cell expansion and in which there are only 13 chloroplasts per mesophyll cell compared with 121 in wild-type cells. Mature *arc*5 chloroplasts in fully expanded mesophyll cells are 6-fold larger than in wild-type cells. A large proportion of *arc*5 chloroplasts also show some degree of central constriction, suggesting that the mutation has prevented the completion of the chloroplast division process. To examine the interaction of *arc* loci, a double mutant was constructed between *arc*1, a mutant possessing many small chloroplasts, and *arc*5. A second double mutant was also constructed between *arc*3, a previously discovered mutant also possessing few large chloroplasts per cell, and *arc*1. Analysis of these double mutants shows that chloroplast number per mesophyll cell is greater when *arc*5 and *arc*3 mutations are expressed in the *arc*1 background than when expressed alone. The cell-specific nature of *arc* mutants was also analyzed. The phenotypic traits characteristic of *arc*3 and *arc*5 are a reduction in chloroplast number and an increase in chloroplast size in mesophyll cells: these changes are also observed in reduced form in the epidermal and guard cell chloroplasts of *arc*3 and *arc*5 plants. Analysis of parenchyma sheath cell chloroplasts suggests that in leaves of *arc*1 plants the normal developmental distinction between mesophyll and parenchyma sheath chloroplasts is perturbed. The relevance of these findings to the analysis of the control of chloroplast division in mesophyll cells is discussed.

Chloroplast division during leaf mesophyll cell expansion results in a population of individual chloroplasts within each cell, and the size of the population is dependent on several factors, including mesophyll cell size (Pyke and Leech, 1987), cell ploidy level (Butterfass, 1983; Pyke and Leech, 1987), and plant genotype (Leech and Pyke, 1988). The division of partially differentiated chloroplasts occurs in all higher plants, both monocotyledons (Boffey et al., 1979; Hashimoto and Possingham, 1989) and dicotyledons (Saurer and Possingham, 1970; Possingham and Smith, 1972; Possingham, 1980), and is a process central to the correct development of the chloroplast compartment and the attainment of photosynthetic competence of the leaf (Boffey, 1992). It seems most likely that chloroplast division is controlled by nuclear genes (Possingham et al., 1988; Boffey, 1992). Although some progress has been made in understanding the interaction between the nuclear genome and the chloroplast and its genome (Taylor et al., 1987; Susek and Chory, 1992), little is known of the molecular basis for the control of chloroplast division itself (Boffey, 1992). The only molecular traits shown to be associated with chloroplast division are an increased rate of ctDNA replication (Scott and Possingham, 1980; Boffey and Leech, 1982) and an associated increase in levels of topoisomerase II enzyme (Marrison and Leech, 1992) prior to division. A morphological feature of the chloroplast division process that has been well characterized is the dumbell shape of the chloroplast division profile caused by a central constriction that occurs just prior to the separation of the two daughter chloroplasts (Leech et al., 1981) and is only a relatively short-lived part of the division process (Leech and Pyke, 1988).

The isolation in the higher plant *Arabidopsis thaliana* (L.) Heynh. of *arc* (accumulation and replication of chloroplasts) mutants in which the dynamics of chloroplast division in expanding mesophyll cells are radically altered (Pyke and Leech, 1991, 1992) provides a valuable new genetic resource to aid the understanding of the genetic basis for chloroplast division. The analysis of mutants *arc*1, *arc*2, and *arc*3 showed that a reduction in chloroplast division is closely compensated for by an increase in the size of individual chloroplasts, resulting in a similar chloroplast compartment size per unit mesophyll cell size but with no obvious loss in plant vigor (Pyke and Leech, 1992). We report here the isolation of a novel *arc* mutant, *arc*5, in which the separation of the daughter chloroplasts during division is prevented. We also analyzed the phenotypes resulting from the genetic interaction between *arc*1, another locus also shown to be important in the control of chloroplast division (Pyke and Leech, 1992), and both *arc*3 and *arc*5.

MATERIALS AND METHODS

Plant Material

Ethyl methanesulfonate-induced mutants of *Arabidopsis thaliana* var Landsberg *erecta* with altered chloroplast division characteristics were isolated using an image analysis-screening procedure after iodine staining of isolated cells (Pyke and Leech, 1991). Plant growth conditions and harvesting of first leaves were as described previously (Pyke and Leech, 1992). The oldest leaves sampled for each mutant and wild type were fully expanded, and the mean mesophyll cell plan area for the whole leaf was also maximal.

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Table I. *Genetic analysis of arc5*

arc5 plants were selfed for two generations (M_4) and reciprocally backcrossed to wild-type Landsberg *erecta*, and the progeny were examined in the F_1 and F_2 generations. Mutant phenotypes were scored by microscopic examination of isolated mesophyll cells from first leaves of individual F_1 and F_2 seedlings harvested after 24 d. Allelic crosses were performed between combinations of *arc* mutants, and the F_1 progeny from the allelic crosses were scored in the same manner. χ^2 values indicate no significant difference ($P > 0.05$) from an expected segregation of one mutant to three wild-type seedlings. na, Not applicable.

Genotype	Generation	Wild Type	Mutant	χ^2
		no. of plants		
Backcrosses				
arc5/arc5 ×	F_1	37	0	
ARC5/ARC5	F_2	210	60	0.86
ARC5/ARC5 ×	F_1	32	0	
arc5/arc5	F_2	166	46	1.06
Allelic crosses				
arc1 × *arc5*	F_1	13	0	na
arc2 × *arc5*	F_1	23	0	na
arc3-1 × *arc5*	F_1	19	0	na
arc3-2 × *arc5*	F_1	36	0	na

Analysis of Chloroplast Numbers in Leaf Cells

Chloroplasts in individual isolated, fixed mesophyll cells were counted using Nomarski differential interference contrast optics (Pyke and Leech, 1991), and mesophyll cell plan areas and individual chloroplast plan areas were measured directly from the microscope image using an image analysis system (Seescan Imaging Ltd., Cambridge, UK). Chloroplasts in parenchyma sheath cells, which were identified by their large size and cylindrical shape, were measured in a similar manner. Chloroplasts in guard cells and epidermal cells were measured in pieces of epidermis stripped from fresh, fully expanded first leaves of *Arabidopsis*. To facilitate observation of these pale chloroplasts, epidermal strips were mounted in 1% (w/v) silver nitrate, which causes brown staining of the chloroplasts.

RESULTS

Characterization of the *arc5* Mutant

Genetic Analysis

A novel mutant of *Arabidopsis* was isolated from an ethyl methanesulfonate-induced M_2 population of plants, in which the mean chloroplast number per mesophyll cell is only 13 compared to a mean of 121 in wild-type cells (Table I). Reciprocal backcrossing of this mutant to wild type and analysis of F_1 and F_2 progeny show that this mutation is nuclear and recessive (Table I). Complementation analysis between this new mutant and preexisting *arc* mutants (Pyke and Leech, 1992) show that this mutation represents a new locus (Table I). We have termed this new locus *arc5*. Plants of *arc5* show no gross morphological differences from wild type and show no loss of vigor.

Chloroplast and Cell Characteristics of arc5

The relationship between chloroplast number per mesophyll cell and mesophyll cell size in *arc5* leaves (Fig. 1) shows

Table II. *Mean mesophyll cell size, mean chloroplast number, and chloroplast size for populations of mesophyll cells from fully expanded first leaves of wild type, arc mutants, and double arc mutants of A. thaliana*

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 cell plan area. Mesophyll cell plan area is a mean of 150 cells, and mean chloroplast plan area is a mean of at least 150 chloroplasts from 30 different mesophyll cells. The mean cell plan area of cells in which chloroplasts were measured was not significantly different from the mean cell plan area of 150 cells and was a representative sample. Lines used had been selfed for three generations and backcrossed to wild type once. SE values are shown in parentheses.

Genotype	Mesophyll Cells			
	Mean cell plan area	Chloroplasts	Mean chloroplast plan area	Chloroplasts
	μm^2	*mean no.*	μm^2	*no. per 10^3 μm^2 mesophyll cell plan area*
Wild type	4778 (135)	121	50 (0.5)	25
arc1	2910 (68)	94	31 (0.5)	32
arc3	3890 (80)	16	294 (13)	4
arc5	3531 (83)	13	312 (11)	4
Double mutants				
arc1/arc3	4221 (192)	26	118 (5)	6
arc1/arc5	4765 (145)	49	80 (5)	10

that chloroplast number per cell remains low at an average of 13 per cell throughout the period of mesophyll cell expansion. The slope of the regression in Figure 1 is not significantly different from zero ($P > 0.05$), i.e. *arc*5 chloroplast number is independent of changes in mesophyll cell size. In contrast, in wild-type cells chloroplast number and cell size are closely correlated (Fig. 1). The reduction in chloroplast number per mesophyll cell is associated with an increase in chloroplast size. *arc*5 chloroplasts from fully expanded mesophyll cells are, on average, 6-fold larger in plan area than wild-type chloroplasts (Table II). Consequently, the relationship between total chloroplast plan area per mesophyll cell and mesophyll cell size for *arc*5 cells (Fig. 2a) is similar to the relationship between total chloroplast plan area per mesophyll cell and mesophyll cell size for wild-type cells (Fig. 2b).

The size and number of chloroplasts in the mutant *arc*5 resemble very closely the mutant *arc*3 (Pyke and Leech, 1992), which also possesses few but large chloroplasts (Table II). Although *arc*3 and *arc*5 chloroplasts have a similar mean size (Table II), *arc*5 chloroplasts are less variable in plan shape, and, in particular, many of the *arc*5 chloroplasts show some degree of permanent central constriction (Fig. 3), similar to that shown by wild-type chloroplasts undergoing division. About 50% of chloroplasts isolated from protoplasts made from fully expanded leaves of *arc*5 seedlings clearly show a degree of central constriction similar to that seen in fixed isolated cells (Fig. 3). The reduction in chloroplast number in the *arc*5 phenotype appears to be caused by a major disruption of the chloroplast division process and completion of the constriction phase, and, in addition, the separation of the daughter chloroplasts does not occur. Failure of the chloroplasts to divide normally, followed by continued chloroplast expansion, gives, on average, 13 very large chloroplasts in the mature cell. The dumbbell shape of many *arc*5 chloroplasts is not a function of abnormally large chloroplast size alone, because chloroplasts of *arc*3 are as large as those of *arc*5, yet *arc*3 chloroplasts have no centrally constricted region.

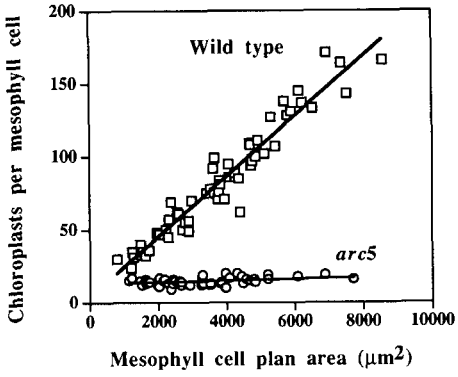

Figure 1. The relationship between chloroplast number per mesophyll cell and mesophyll cell plan area for wild type (□) and *arc*5 mutant (○) of *A. thaliana* var Landsberg *erecta*. Each data point represents the measurement from one cell whose area was measured and the chloroplast complement counted. Values of r^2 are 0.93 for wild type and 0.1 for *arc*5.

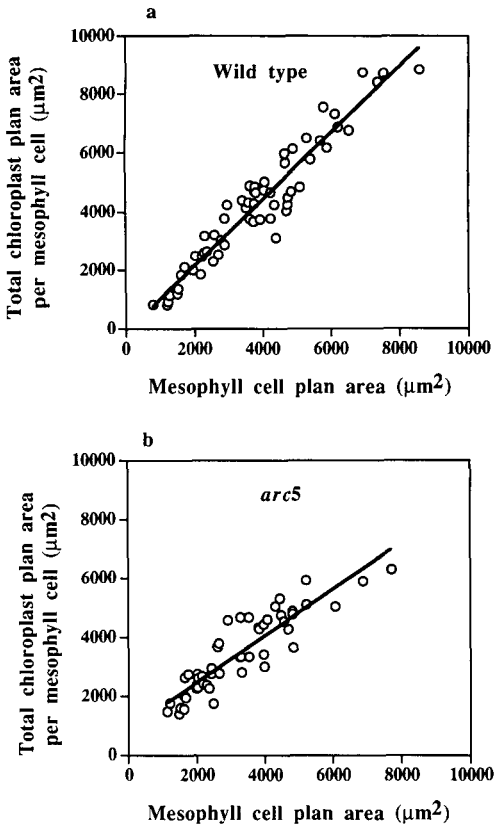

Figure 2. The relationship between total chloroplast plan area per mesophyll cell (determined as the number of chloroplasts in an individual mesophyll cell × the mean chloroplast plan area for that cell) and mesophyll cell plan area for wild type (a) and *arc*5 (b). Values of r^2 are 0.92 (a) and 0.84 (b).

Construction and Analysis of Double Mutants

We questioned whether chloroplasts in *arc*3 and *arc*5 leaves are capable of division or whether the genetic lesions resulting from these mutations have caused complete loss of the ability of the chloroplasts to divide. We also wished to determine whether it was possible to modify genetically the makeup of the chloroplast compartment by combining different mutant *arc* loci. The most extreme *arc* mutant phenotypes (Pyke and Leech, 1992) were chosen for combination as follows: *arc*1, which has a 25% increase of smaller chloroplasts per cell and has a pale seedling phenotype, and *arc*3 and *arc*5, both of which have fewer large chloroplasts per cell than the wild type. In the F_2 segregating populations of crosses between *arc*1 × *arc*3 and *arc*1 × *arc*5, plants with a possible double-mutant phenotype were selected, and seeds were collected. The criteria used for a double-mutant phenotype was a pale seedling containing mesophyll cells with some chloroplasts larger than wild type (Fig. 3, e and f). Putative double-mutant seedlings were confirmed by their lack of segregation in the subsequent F_3 generation, and analysis of F_1 progeny from test crosses to both parental lines confirmed that the selected double mutants were indeed homozygous recessive at both mutant loci. Chloroplasts were

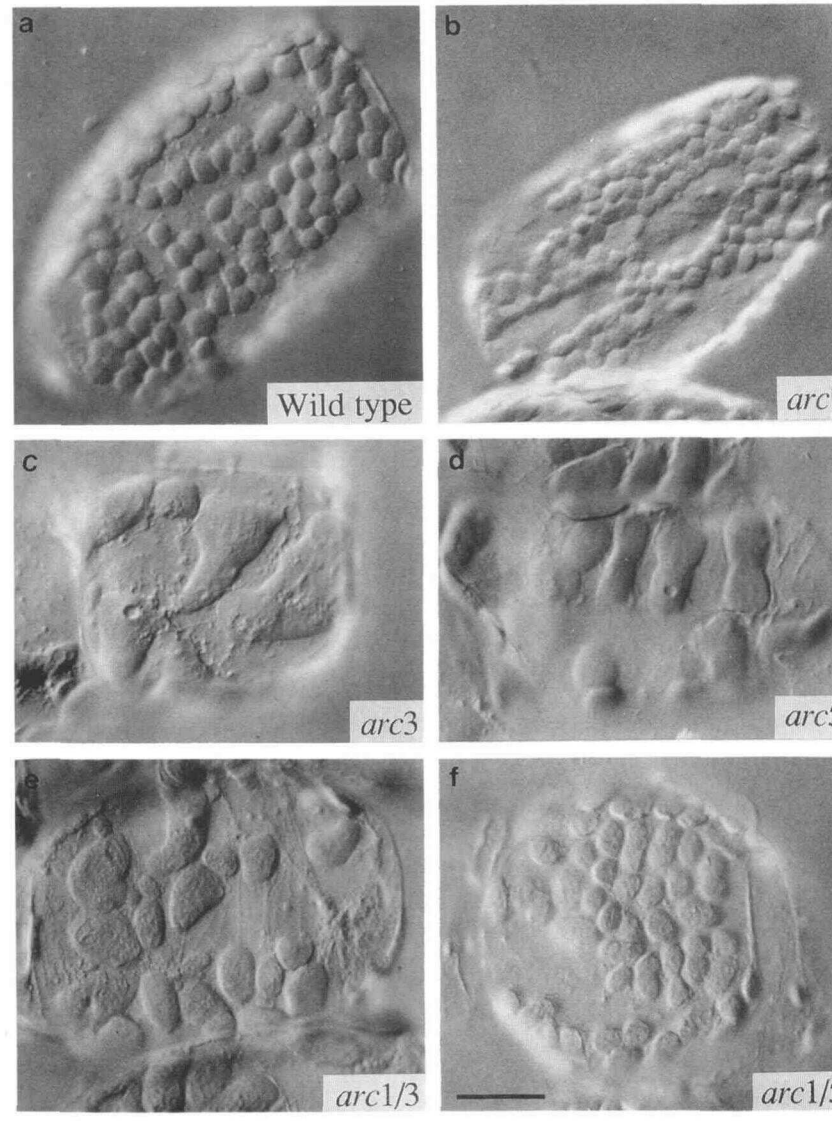

Figure 3. Isolated leaf mesophyll cells from fully expanded leaves of wild type and mutants of *A. thaliana* var Landsberg *erecta* viewed with Nomarski differential interference contrast optics. a, Wild type; b, *arc*1; c, *arc*3; d, *arc*5; e, double mutant *arc*1/*arc*3; f, double mutant *arc*1/*arc*5. Bar = 25 μm.

counted in mesophyll cells from fully expanded first leaves of both *arc*1/*arc*3 and *arc*1/*arc*5 double mutants, and chloroplast and mesophyll cell plan areas were measured using image analysis (Table II).

In both double mutants the relationship between number of chloroplasts per mesophyll cell and mesophyll cell size is intermediate between that of two parental mutants (Table II, Fig. 4). The mean chloroplast number per cell is greater in both the *arc*1/*arc*3 double (26 chloroplasts) and the *arc*1/*arc*5 double (49 chloroplasts) than in the *arc*3 (16 chloroplasts) and *arc*5 (13 chloroplasts) mutants (Table II). Clearly, the *arc*3 and *arc*5 mutations do not cause a permanent loss of the chloroplast division function, and when expressed in a different genetic background, in this case *arc*1, some chloroplast division can occur. Chloroplast size in these double mutants is also intermediate between the two parental mutants (Table II), and the changes in the distribution of chloroplast plan area between single and double mutants demonstrate this clearly (Fig. 5). The fully expanded mesophyll cells of the *arc*1/*arc*5 mutant (Fig. 3f) also contain many chloroplasts in arrested division, a phenotypic trait of the *arc*5 mutation. In contrast, chloroplasts in arrested division are not observed in the *arc*1/*arc*3 double mutant (Fig. 3e).

Tissue Specificity of *arc* Mutants

Because *arc* mutations cause such radical changes to chloroplast number and size in leaf mesophyll cells, we wished to determine whether the mutations also caused changes in chloroplast development in other types of chloroplast-containing leaf cell. We examined three leaf cell types: guard cells, epidermal cells, and parenchyma sheath cells.

Chloroplast number in wild-type *Arabidopsis* guard cells and epidermal cells is considerably lower than in mesophyll cells, and the chloroplasts are also smaller, particularly in guard cells (Table III). In the *arc*1 mutant, chloroplast number and size in both guard cells and epidermal cells showed no significant differences from wild type. In mutant *arc*3 both guard cell and epidermal cell chloroplasts were significantly larger than wild type ($P < 0.001$) (Table III), although chlo-

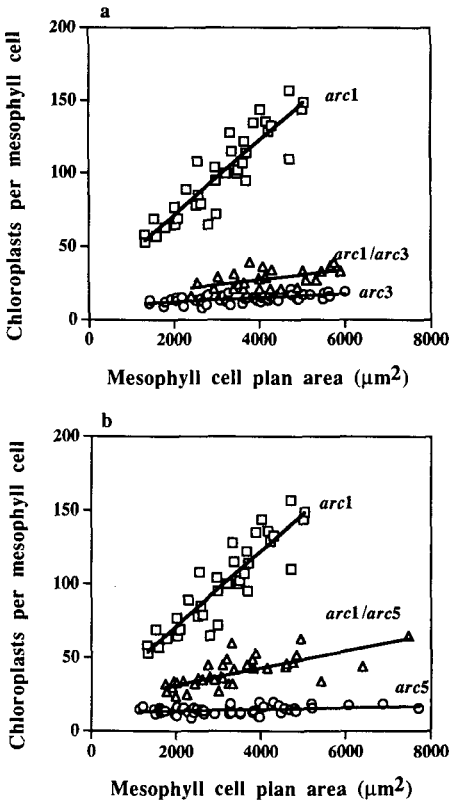

Figure 4. The relationship between chloroplast number per mesophyll cell and mesophyll cell plan area for double mutant *arc1/arc3* (△) in comparison with *arc1* (□) and *arc3* (○) (a) and double mutant *arc1/arc5* (△) in comparison with *arc1* (□) and *arc5* (○) (b).

roplast number was the same as in wild type. In contrast, mutant *arc5* showed a significant decrease in chloroplast number in both guard cells and epidermal cells ($P < 0.001$) and an increase in chloroplast size in epidermal cells ($P < 0.001$) (Table III). Clearly, the phenotypic traits associated with mesophyll cell chloroplasts of *arc3* and *arc5* are also seen in the epidermal cells of the mutants and to a lesser extent in guard cells.

The specialized mesophyll cells associated with the vascular tissue in *Arabidopsis*, termed parenchyma sheath cells, also contain chloroplasts. In wild-type leaves parenchyma sheath chloroplasts are, on average, 28% smaller than chloroplasts in mesophyll cells (Table IV). A similar decrease in size of parenchyma sheath cell chloroplasts compared with mesophyll cell chloroplasts is also observed in mutants *arc3* and *arc5* (Table IV). In contrast, in mutant *arc1* parenchyma sheath cells, the chloroplasts are slightly larger than the mesophyll cell chloroplasts (Table IV). This difference in the size relationship between the two chloroplast types in different *arc* mutants is reflected in the characteristics of the chloroplasts in the double mutants. In both *arc1/arc3* and *arc1/arc5* double mutants there is a considerable reduction in mesophyll chloroplast size compared with *arc3* and *arc5*, whereas the parenchyma sheath chloroplasts are more similar in size to those in *arc3* and *arc5* (Table IV).

DISCUSSION

Mutation at the *arc5* locus causes a severe disruption to the chloroplast division process, preventing the normal increase in chloroplast number per mesophyll cell during cell expansion. The lack of successful chloroplast divisions and the presence of many very large *arc5* chloroplasts in arrested division suggests that the *arc5* mutation specifically disrupts the division process at the point of separation of the two daughter chloroplasts. Because chloroplast division has been shown to occur only in chloroplasts smaller than a certain size (Ellis and Leech, 1985), a retarded division process and continued chloroplast expansion are likely to result in a

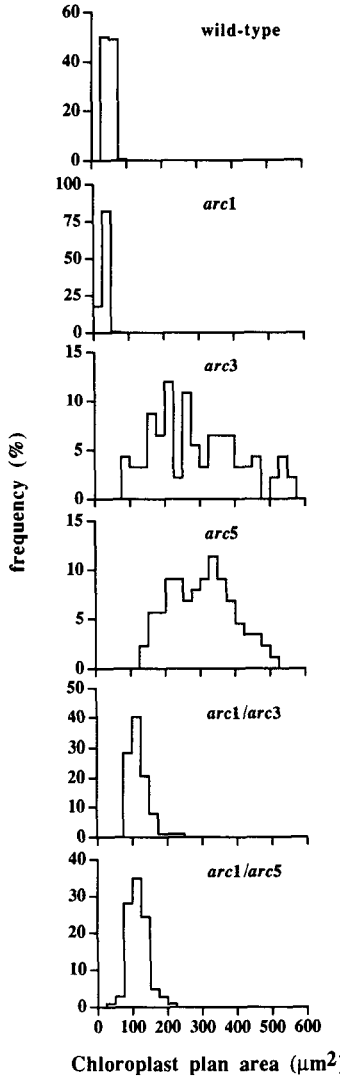

Figure 5. Frequency distributions of chloroplast size, measured as plan area, for chloroplasts from fully expanded leaves of three *arc* mutants and two double *arc* mutants. At least 150 chloroplasts were measured from at least 30 different mesophyll cells of each line. The mean cell plan area of the cells in which chloroplasts were measured was not significantly different from the mean for a large sample of cell sizes (150) and was a representative sample of the population.

reduced population of chloroplasts that have expanded beyond the size at which division can normally occur, as is found in *arc*5. The mean number of 13 chloroplasts present in *arc*5 cells reflects the allocation of proplastids into postmitotic mesophyll cells prior to chloroplast differentiation in this mutant and is similar to the value of 14 estimated for proplastid number in other *arc* mutants (Pyke and Leech, 1992). We have no evidence at present about whether the *arc*5 gene product may be associated with the constriction process itself, in which an actomyosin contractile system has been implicated (Whatley, 1988; Boffey, 1992), or whether it may act as a control factor.

The identification of plants that are mutant at two different *arc* loci has allowed further analysis of the *arc* mutant phenotypes. The phenotypes of both *arc*1/*arc*3 and *arc*1/*arc*5 double mutants suggest that neither *arc*3 nor *arc*5 mutations cause complete loss of the chloroplast's ability to divide. The increase in the number of successful chloroplast divisions in both of the double mutants compared with *arc*3 and *arc*5 alone suggests that both *arc*3 and *arc*5 mutations probably cause a limited reduction in the ability of daughter chloroplasts to separate during the chloroplast division process. The double-mutant phenotypes result from an interaction between *arc* loci, and the nature of this interaction will be the aim of future studies.

In both wild type and *arc* mutants of *Arabidopsis* there is a highly regulated mutual compensation between chloroplast division and chloroplast expansion. The 70% chloroplast cover of the cell surface in the wild-type plants is maintained throughout cell expansion (Pyke and Leech, 1992), and in the *arc* mutants, which exhibit a wide range of chloroplast number and size, this high proportion of chloroplast cover is also evident. When grown in our standardized growth conditions (Pyke and Leech, 1992), the *arc*3 and *arc*5 extreme mutant phenotypes do not show any major reduction in vigor, but their survival under conditions of environmental stress in which the wild-type plant survives is unknown. A comparison of the relative survival of wild-type and mutant plants in a wide range of environmental conditions could give very valuable information about the importance of chloroplast number/size relationships to plant survival.

The analysis of chloroplast-containing leaf cell types other than mesophyll cells shows that the *arc* mutations affect chloroplast development in all of the leaf tissues but to varying degrees. It is particularly interesting that there are fewer larger chloroplasts in both guard cells and epidermal cells of *arc*5 compared to wild type, characteristics typical of the *arc*5 mesophyll chloroplast phenotype. This would suggest that normally some chloroplast divisions do occur in both guard cells and epidermal cells of wild-type leaves. The lack of a reduction in size in parenchyma sheath chloroplasts associated with the *arc*1 mutation indicates a change in tissue-specific expression in this mutant.

The identification of *arc* loci in *Arabidopsis*, mutations at which have a major phenotypic effect on chloroplast division characteristics, and the recent isolation of putative transferred-DNA insertional mutants with *arc*-like phenotypes (K.A. Pyke, S. Rutherford, and R.M. Leech, unpublished data) should facilitate a detailed dissection of the molecular controls on chloroplast division in higher plants.

Table III. *Mean chloroplast number per cell and mean chloroplast plan area for chloroplasts from guard cells and epidermal cells of wild type and three arc mutants of A. thaliana*

Guard cell and epidermal chloroplasts were counted in at least 50 individual cells from fully expanded leaves. Number of chloroplasts is to the nearest whole number. Mean chloroplast plan area is a mean of at least 50 chloroplasts from 30 different cells. SE values are shown in parentheses.

Genotype	Guard Cell		Epidermal Cell	
	Chloroplasts	Mean chloroplast plan area	Chloroplasts	Mean chloroplast plan area
	mean no. per guard cell pair	*μm²*	*mean no. per cell*	*μm²*
Wild type	10	9.2	8	15.9
	(0.2)	(0.3)	(0.3)	(0.4)
*arc*1	9	8.4	9	16.2
	(0.1)	(0.2)	(0.4)	(0.4)
*arc*3	10	12.1	8	23.4
	(0.2)	(0.5)	(0.3)	(0.7)
*arc*5	5	10.0	5	29.9
	(0.2)	(0.4)	(0.2)	(1.8)

Table IV. *Mean chloroplast plan areas from mesophyll and parenchyma sheath cells of fully expanded first leaves of wild type, arc mutants, and double arc mutants of A. thaliana*

Mesophyll chloroplast plan areas are means of at least 150 chloroplasts from 30 different mesophyll cells. Parenchyma sheath chloroplasts plan areas are means of at least 50 chloroplasts from 30 different parenchyma sheath cells. SE values are shown in parentheses.

Genotype	Mean Chloroplast Plan Area	
	Mesophyll cell chloroplasts	Parenchyma sheath cell chloroplasts
	μm²	*μm²*
Wild type	50	36
	(0.5)	(1)
*arc*1	31	46
	(0.5)	(0.9)
*arc*3	294	262
	(13)	(22)
*arc*5	312	193
	(11)	(14)
Double mutants		
*arc*1/*arc*3	118	224
	(5)	(15)
*arc*1/*arc*5	80	209
	(5)	(7)

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LITERATURE CITED

Boffey SA (1992) Chloroplast replication. *In* N Baker, H Thomas, eds, Crop Photosynthesis: Spatial and Temporal Determinants. Elsevier, Amsterdam, pp 361–379

Boffey SA, Ellis JR, Sellden G, Leech RM (1979) Chloroplast division and DNA synthesis in light-grown wheat leaves. Plant Physiol **64:** 502–505

Boffey SA, Leech RM (1982) Chloroplast DNA levels and the control of chloroplast division in light-grown wheat leaves. Plant Physiol **69:** 1387–1391

Butterfass T (1983) A nucleotypic control of chloroplast reproduction. Protoplasma **118:** 71–74

Ellis JR, Leech RM (1985) Cell size and chloroplast size in relation to chloroplast replication in light-grown wheat leaves. Planta **165:** 120–125

Hashimoto H, Possingham JV (1989) Effect of light on the chloroplast division cycle and DNA synthesis in cultured leaf discs of spinach. Plant Physiol **89:** 1178–1183

Leech RM, Pyke KA (1988) Chloroplast division in higher plants with particular reference to wheat. *In* SA Boffey, D Lloyd, eds, The Division and Segregation of Organelles. Cambridge University Press, Cambridge, UK, pp 39–62

Leech RM, Thomson WW, Platt-Aloia KA (1981) Observations of the mechanism of chloroplast division in higher plants. New Phytol **87:** 1–9

Marrison JL, Leech RM (1992) Co-immunolocalization of topoisomerase II and chloroplast DNA in developing, dividing and mature wheat chloroplasts. Plant J **2:** 783–790

Possingham JV (1980) Plastid replication and development in the life cycle of higher plants. Annu Rev Plant Physiol **31:** 113–129

Possingham JV, Hashimoto H, Oross J (1988) Factors that influence plastid division in higher plants. *In* SA Boffey, D Lloyd, eds, Division and Segregation of Organelles. Cambridge University Press, Cambridge, UK, pp 1–20

Possingham JV, Smith JW (1972) Factors affecting chloroplast replication in spinach. J Exp Bot **23:** 1050–1059

Pyke KA, Leech RM (1987) The control of chloroplast number in wheat mesophyll cells. Planta **170:** 416–420

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

Pyke KA, Leech RM (1992) Nuclear mutations radically alter chloroplast division and expansion in *Arabidopsis thaliana*. Plant Physiol **99:** 1005–1008

Saurer W, Possingham JV (1970) Studies on the growth of spinach leaves (*Spinacea oleracea*). J Exp Biol **21:** 151–158

Scott NS, Possingham JV (1980) Chloroplast DNA in expanding spinach leaves. J Exp Bot **31:** 1081–1092

Susek RE, Chory J (1992) A tale of two genomes: role of a chloroplast signal in coordinating nuclear and plastid genome expression. Aust J Plant Physiol **19:** 387–399

Taylor WC, Barkan A, Martienssen RA (1987) Use of nuclear mutants in the analysis of chloroplast development. Dev Genet **8:** 305–320

Whatley JM (1988) Mechanisms and morphology of plastid division. *In* SA Boffey, D Lloyd, eds, The Division and Segregation of Organelles. Cambridge University Press, Cambridge, UK, pp 63–83