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Plastid Division and Development

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INTRODUCTION

Plastids are an important group of plant cellular organelles and comprise one of the primary features that distinguish plant cells from those of other eukaryotes. Plastids are thought to have arisen as a result of an endosymbiotic event in which an early photosynthetic prokaryote invaded a primitive eukaryotic host (Margulis, 1970; Gray, 1992). Subsequently, plastids have evolved to become essential components for plant cell function. The central role of plastids in plant cell biology derives primarily from the chloroplast's ability to supply the cell with fixed carbon and energy as a result of photosynthetic carbon assimilation. Moreover, portions of several major plant metabolic pathways, such as lipid biosynthesis and amino acid metabolism, occur in plastids (Galili, 1995; Ohlrogge and Browse, 1995).

All plastids are derived initially from small, undifferentiated plastids termed proplastids, which are found in dividing cells in meristems. During cell differentiation, proplastids differentiate into particular plastid types according to the type of cell in which they reside. By far the best studied of these plastid differentiation pathways is the biogenesis of chloroplasts during leaf mesophyll cell differentiation, and recent reviews have considered the molecular biology and biochemistry of this differentiation process (Barkan et al., 1995; Mache et al., 1997).

The emphasis on chloroplasts has overshadowed a variety of other plastid differentiation pathways that also occur in specific cell types in plants. These include plastid differentiation during root cell development (Whatley, 1983), amyloplast differentiation during seed and tuber formation (Thomson and Whatley, 1980), chromoplast formation in fruits and flowers (Marano et al., 1993), and leucoplast formation in petals (Pyke and Page, 1998). Indeed, although plastids have long been classified into different types by virtue of their storage components and internal structures (Kirk and Tilney-Bassett, 1978), plastids may better be described as a continuous spectrum of types; precise categorization is often difficult and may not always be biologically meaningful.

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CELL BIOLOGY OF PLASTID DIVISION

Cell Biology of Plastid Division in Higher Plants

Although proplastids are the progenitor plastid type, the cell biology of their division has been studied only sparsely. This is principally because proplastids are small and colorless and because the meristem cells in which they reside are themselves small and difficult to access. Clearly, to ensure that plastid lineages are maintained through the segregation of plastids into each of the two daughter cells, proplastids must divide before meristematic cells do so. Indeed, from studies in several species, it is estimated that meristematic cells contain 10 to 20 proplastids (Juniper and Clowes, 1965; Cran and Possingham, 1972; Lyndon and Robertson, 1976). Furthermore, electron microscopical observation of these proplastids reveals central constrictions that have been interpreted to represent stages in proplastid division (Figure 1A; Chaly and Possingham, 1981).

Chloroplasts are by far the best studied of the diverse array of plastid types that have been described in different plant cells (Kirk and Tilney-Bassett, 1978). However, confirmation that chloroplasts could divide inside developing plant cells was only made in the late 1960s (Possingham and Saurer, 1969). Evidence for chloroplast division arose mainly as a result of improvements in electron microscopy techniques that facilitated the production of images of young chloroplasts with central constrictions (Boasson et al., 1972; Platt-Aloia and Thomson, 1977). These structures were interpreted to be chloroplasts undergoing division by a process of binary fission, and careful ultrastructural analysis subsequently confirmed that dividing chloroplasts can be found in a variety of tissue types (Leech et al., 1981). Although the actual division of a chloroplast seems to have been observed rarely, this event has been reported in living cells (Honda et al., 1971) and in hanging drops (Ridley and Leech, 1970).

The population dynamics of chloroplasts have been analyzed in detail in expanding mesophyll cells of young wheat leaves (Boffey et al., 1979; Boffey and Leech, 1982; Dean and Leech, 1982; Ellis et al., 1983; Ellis and Leech, 1985) and spinach leaves (Possingham and Saurer, 1969; Saurer and Possingham, 1970; Cran and Possingham, 1972; Possingham and Smith, 1972; Possingham et al., 1975). These studies showed a correlation between the existence

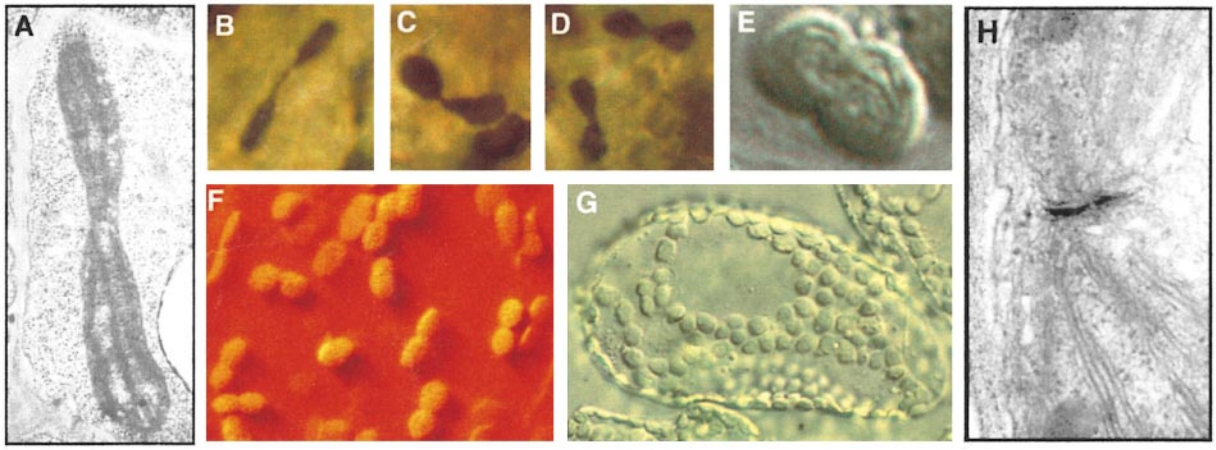

Figure 1. Dividing and Mature Plastids in Arabidopsis.

(A) Dividing proplastid from a cell in the shoot apical meristem.
(B) to **(D)** Division conformations of epidermal cell chloroplasts stained with silver nitrate from young expanding cotyledons.
(E) Typical chloroplast in early division, from an expanding leaf mesophyll cell.
(F) Confocal topographic view of dumbbell-shaped plastids in the base of petals in the Arabidopsis *arc5* mutant.
(G) A mature Arabidopsis mesophyll cell showing part of the monolayer of chloroplasts over the internal cell surface with a large hole in the monolayer representing where the cell was previously attached to a neighboring cell.
(H) An electron micrograph of *arc5* petal chloroplasts showing an electron-dense plastid dividing ring at the narrow isthmus of a chloroplast in division (photograph courtesy of K. Hagley).

of dumbbell-shaped dividing chloroplasts in cells and a subsequent increase in chloroplast number per cell. As a result of these studies, it became clear that chloroplast division is an integral part of normal chloroplast development and an important process in plant cell development.

The process of plastid division has been characterized morphologically from careful analysis of light and electron microscopy images (Leech et al., 1981; Oross and Possingham, 1989; Robertson et al., 1996). It is initiated by a constriction in the middle of the plastid, which narrows further and, in the later stages of division, can form a thin isthmus that joins the two daughter plastids (Figures 1A to 1E). Once the central constriction is narrowed, the daughter plastids can move independently and can rotate about the isthmus. The internal membranes of the daughter chloroplasts appear to be separated at this time, although in early stages of isthmus formation, thylakoid membranes that still join the two daughter plastids can be seen. This narrow isthmus eventually breaks, and the envelope membranes of the daughter plastids reseal.

Two hypotheses have been postulated concerning the motive force that drives plastid division. Greenspan (1977) initially suggested that daughter plastids could separate as a result of membrane thermodynamics. However, another body of evidence suggests that the constriction process in plastid division is caused by a contractile ring. This electron-dense ring, which is often called the plastid-dividing ring (PD), becomes visible by electron microscopy only in the latter stages of the process. Nevertheless, PDs have been observed in dividing chloroplasts and proplastids from several different species (Figure 1H; Susuki and Ueda, 1975; Leech et al., 1981; Oross and Possingham, 1989; Robertson et al., 1996). Moreover, in some cases PDs appear to form a torus, with one component on the inside of the plastid and one component on the outside (Hashimoto, 1986).

Identifying the components of the PD would greatly enhance our understanding of the molecular basis of plastid division. Unfortunately, attempts to localize specific proteins to the PD in higher plant chloroplasts have proved inconclusive. However, a similar structure is observed in dividing chloroplasts of algae, and the localization of rhodamine phalloidin fluorescence to the isthmus of dividing plastids of *Closterium ehrenbergii* implies that actin plays a role in the division process (Hashimoto, 1992). A role for actin in the division of higher plant plastids has not been established, and even though actin gene families have been well characterized in plants (Meagher, 1991), no individual actin isomer that is specifically associated with plastid division has been identified.

Although binary fission is the most well-characterized type of chloroplast division, other mechanisms have been reported. For example, chloroplast division by partition involving the growth of a membranous baffle across the center of the chloroplast has been described in several species (Modrusan and Wrischer, 1990; Miyake and Taniguchi, 1995). Furthermore, a budding mode of chloroplast division

has been reported in a *Bryophyllum* spp (Kulandaivelu and Gnanam, 1985). The relative importance of these alternative division morphologies is unclear and requires further study.

Plastid Division in Lower Green Plants

Many studies of plastid division have been conducted in a wide variety of lower green plants, particularly algae (Mita and Kuroiwa, 1988; Hashimoto, 1992; Kuroiwa and Uchida, 1996), ferns (Duckett and Ligrone, 1993), and mosses (Tewinkel and Volkmann, 1987; Abel et al., 1989; Reski et al., 1992; Rother et al., 1994). The variety of organisms in which plastid division has been studied has led to a rather confused picture, and the direct relevance of findings in these organisms to the controls of higher plant plastid division is unclear. Certainly the advantages of much clearer observation of plastids in single-celled or simple multicellular organisms are obvious, and these features have facilitated the localization of actin filaments in association with dividing plastids (Mita and Kuroiwa, 1988; Hashimoto, 1992).

Genetic analyses are also feasible in some of these organisms. For example, the characterization of a *Physcomitrella patens* mutant defective in chloroplast division (Abel et al., 1989; Rother et al., 1994) has shown that cytokinin and blue light can functionally complement the mutation (Reski et al., 1991), implying a role for cytokinin and blue light in chloroplast division. However, it is quite feasible that lower plants may use different signaling controls to initiate chloroplast division than do higher plants, particularly because chloroplast division and cell division are more intimately associated in lower plants than they are in higher plants. Indeed, there is no clear evidence for hormonal and/or specific light effects on the higher plant chloroplast division process, although the molecular and genetic technologies that are necessary to address these issues are available.

MOLECULES INVOLVED IN PLASTID DIVISION

The *ARC* Genes

A genetic approach to dissecting chloroplast division and development has always appeared attractive. However, mutations that affect distinct cellular processes but have a pleiotropic effect on chloroplast division have made the interpretation of plastid mutant phenotypes difficult. Despite these potential complexities, the most recent progress in our understanding of plastid division mechanisms in higher plants has come from the use of Arabidopsis and its molecular genetic resources. Microscope-based screens have led to the identification of a collection of Arabidopsis mutants with altered numbers of chloroplasts per cell (Pyke and Leech, 1991, 1992, 1994; Pyke, 1997). The characterization of accumulation and replication of chloroplasts (*arc*) mutants has shown that specific nuclear genes must play specific roles both in the chloroplast division process itself as well as in the control of chloroplast population size within a cell during its development.

Foremost among this collection of mutants is *arc6*, which contains an average of two enlarged chloroplasts per mesophyll cell instead of the usual >100 chloroplasts per cell (Pyke et al., 1994). In addition, proplastids in both shoot and root meristems of *arc6* are reduced in number and are larger in size (Robertson et al., 1995), although redifferentiation from the resulting large chloroplasts into leucoplasts in petal cells is unaffected (Pyke and Page, 1998). The perturbations in proplastid division in this mutant result in altered plastid phenotypes in all the cells of the *arc6* plant that have been studied to date. Thus, the *ARC6* gene product appears to be of major importance to the initiation of both proplastid and chloroplast division. Another mutant, *arc12*, which is not allelic to *arc6* but has a similar chloroplast phenotype, has also been identified recently (E.A. Kinsman and K.A. Pyke, unpublished data).

A particularly novel feature of the arc6 and arc12 phenotypes is the presence of guard cells that lack plastids (Robertson et al., 1995). This phenotype appears to be guard cell specific and may result from abnormal plastid segregation during the series of asymmetric cell divisions that occur during stomatal biogenesis (Larkin et al., 1997). Because every other cell type in these mutants appears to contain at least one plastid, there must be some mechanism by which proplastids segregate into each daughter cell during cytokinesis. One possibility is that proplastid segregation simply results from breakage of the plastid during cell plate formation.

Two other mutations, *arc3* (Pyke and Leech, 1992) and *arc5* (Pyke and Leech, 1994), appear to specifically affect chloroplast division, resulting in ~15 chloroplasts per leaf mesophyll cell. *ARC3* seems to be involved in the initiation of chloroplast division, whereas in *arc5* mesophyll cells, chloroplasts enter division but appear to stop when they become centrally constricted, unable to proceed through the separation stage. These *arc5* chloroplasts then increase in size, resulting in large mature chloroplasts that retain the dumbbell shape typical of younger smaller chloroplasts (Robertson et al., 1996). Green chloroplasts in the base of *arc5* petal cells show this phenotype dramatically, with virtually all the chloroplasts in each cell exhibiting the dumbbell-shaped morphology (Figure 1F). Interestingly, a large proportion of the green chloroplasts at the base of petals in wild-type plants is also dumbbell shaped (Pyke and Page, 1998) and does not appear to complete the separation stage of division. It is therefore tempting to speculate that the *ARC5* gene product may be required to complete the separation process and that this gene is expressed in a cell-specific manner.

Other *arc* mutants show a variety of different types of chloroplast division phenotypes and numbers per mesophyll

cell. Both the *arc1* mutant (Pyke and Leech, 1992) and the *arc7* mutant (Rutherford, 1996) have a larger number of smaller chloroplasts per cell than does the wild type, and both have pale leaves that are slow to green. Because both of these mutations are recessive, it seems most likely that the primary lesions are in aspects of chloroplast development per se, rather than division control, and that the increased number of chloroplasts results from compensation to reduced chloroplast growth. The *arc10* mutant is of particular interest because its mesophyll cell chloroplasts are highly heterogenous in size within a single cell (Rutherford, 1996). This size heterogeneity may be caused by the presence of a subpopulation of chloroplasts that does not divide or by some other form of abnormal chloroplast division, such as asymmetric division.

Plastid Division and Bacterial Cell Division

The binary fission process of plastid division is morphologically similar to bacterial cell division. Although the possibility of a similar genetic control for the two processes was suggested several years ago (Possingham et al., 1988), such ideas have come to fruition only recently with the development of Arabidopsis molecular genetics. Division of *Escherichia coli* cells is controlled by a set of genes (Donachie, 1993), the most important members of which are the *fts* (for filamentous temperature-sensitive) genes, so called because when mutant bacteria are grown at the permissive temperature, they form filaments. *FtsZ* is among the more interesting of these genes (Bramhill, 1997; Erickson, 1997) because its product is able to form a filamentous ring around the center of the bacterial cell (Bi and Lutkenhaus, 1991; Levin and Losick, 1996; Ma et al., 1996). This ring contracts during cell division to separate the two daughter cells. Links between the FtsZ ring and the bacterial cell membrane appear to be mediated by a membrane protein, termed ZipA, which specifically binds to FtsZ (Hale and de Boer, 1997).

The structure of FtsZ and its ability to form filaments suggested that it may represent an ancient prokaryotic tubulin-like molecule. This hypothesis has received a boost from the recently solved crystal structure of a bacterial FtsZ, which exhibits close similarity to the structure of tubulin (Lowe and Amos, 1998). Moreover, an Arabidopsis expressed sequence tag clone showing sequence similarity to bacterial *FtsZ* has been identified, a finding that has led to the major discovery of *FtsZ* gene homologs in Arabidopsis (Osteryoung and Vierling, 1995). One of these Arabidopsis *FtsZ* genes contains a chloroplast transit peptide that is known to be cleaved during chloroplast uptake experiments. This strongly suggests that FtsZ molecules are present inside higher plant chloroplasts and, by implication, that they are likely to be involved in the chloroplast division process.

Further evidence that Arabidopsis homologs of *FtsZ* play a role in chloroplast division come from experiments showing that transgenic plants carrying antisense *AtFtsZ* constructs expressed under the control of a constitutive promoter have fewer enlarged chloroplasts in their mesophyll cells as compared with the wild type (Osteryoung et al., 1998). This phenotype is similar to those of the *arc6* and *arc12* mutants. In addition, an *FtsZ* knockout in the moss *P. patens* also results in few enlarged chloroplasts per cell (Strepp et al., 1998), although studies using antibiotic inhibitors suggest that there are differences between moss and higher plants in the protein components of the presumed division complex (Kasten and Reski, 1997).

The sequencing of the *Chorella vulgaris* chloroplast genome has also contributed evidence that supports the evolutionary conservation of bacterial and chloroplast division processes. For example, the *Chorella* chloroplast genome contains two adjacent genes, *minD* and *minE*, which are arranged in the same order as their homologs in *E. coli* (Wakasugi et al., 1997). The *E. coli* genes form part of the *E. coli minicell* locus, which consists of three *min* genes that are required for the correct positioning of a septal division site in the bacterial cell. *min* gene homologs have been found in higher plant chloroplast genomes, suggesting either that they have moved to the nuclear genome during evolution or that other nuclear-encoded proteins have taken over their function. A minD homolog has been reported in the Arabidopsis nuclear genome (Osteryoung and Pyke, 1998).

Overall, these recent developments in the molecular analysis of chloroplast division have shown clearly that a mechanism that has evolved from bacterial cell division most likely operates in higher plant chloroplasts. FtsZ would appear to be a prime candidate for the portion of the PD ring that resides inside the plastid. It may be that in the plant cell, such a system interacts with an actin-based component in the cytosol, but confirmation of this hypothesis must await a clear characterization of a chloroplast FtsZ-based division system and its associated proteins.

THE CONTROL OF PLASTID DIVISION

Cellular Factors Controlling Plastid Population Size

Although considerable progress has been made in determining the molecular mechanisms that underlie plastid binary fission, an understanding of how cells control their plastid numbers is completely lacking. In several plant species, it is clear that the size of a leaf mesophyll cell is a primary determinant of the number of chloroplasts within it (Lamppa et al., 1980; Ellis and Leech, 1985; Pyke and Leech, 1992). In addition, the increase in chloroplast number during mesophyll cell expansion appears to be strictly regulated such that the relationship between chloroplast number and cell size is constant as the cell enlarges.

A characterization of variation in the chloroplast number–size relationship for large numbers of noncultivated plant

species is lacking, although there appear to be no reports of natural species with greatly enlarged chloroplasts that are comparable to those observed in Arabidopsis mutants. A report that leaf cells of cocoa (*Theobroma cacao*) contain only two or three chloroplasts per cell (Baker and Hardwick, 1973) has been confirmed, but these chloroplasts are of normal size and the mesophyll cells are very small (Figure 2; N. Salmon and K.A. Pyke, unpublished data). Indeed, analysis of a small number of dicotyledonous species shows that in spite of variation in cell size, plastid size, and plastid number, the total chloroplast area in cells is strictly related to cell size over a 10-fold range of cell sizes from different species (Figure 2).

During the development of mesophyll cells in dicotyledonous leaves, a monolayer of chloroplasts is maintained over a large proportion of the mesophyll cell surface (Figure 1G), and as the cell expands, individual chloroplasts undergo division and subsequent expansion to maintain this coverage. The factors that signal individual chloroplasts to divide in this way are not known, but chloroplast density in relation to the size of the cell seems to be involved.

How the signal is transmitted is also a matter for speculation. It may be that chloroplasts can monitor their packing density within the cell. This is because in most mesophyll cells, chloroplasts are densely packed with an individual chloroplast in contact with several neighbors. Furthermore, signals coordinating plastid division may pass among neighboring chloroplasts along membranous tubule connections that link individual plastids and that appear to be capable of protein transfer (Kohler et al., 1997). Alternatively, the degree of compression of a chloroplast may provide a means by which subsequent divisions are initiated. There is also evidence that plastids in cells undergoing rapid expansion appear to initiate divisions, almost to the point where all plastids show dumbbell phenotypes (Leech and Pyke, 1988; Pyke, 1997; Pyke and Page, 1998). This can be interpreted as a rapid decline in plastid density providing a major "on" signal to initiate division.

Another major factor that dictates the number of plastids within a cell is the cell type. Indeed, the differentiation status of a cell can often be characterized by the number and type of plastids that it contains. This is an often-overlooked aspect of plastid division biology, because chloroplast division in mesophyll cells has come to dominate the subject. All other cell types in a plant will contain plastids that are capable of division, yet practically nothing is known about the basic population dynamics of these systems, let alone the cell-specific mechanisms that control them.

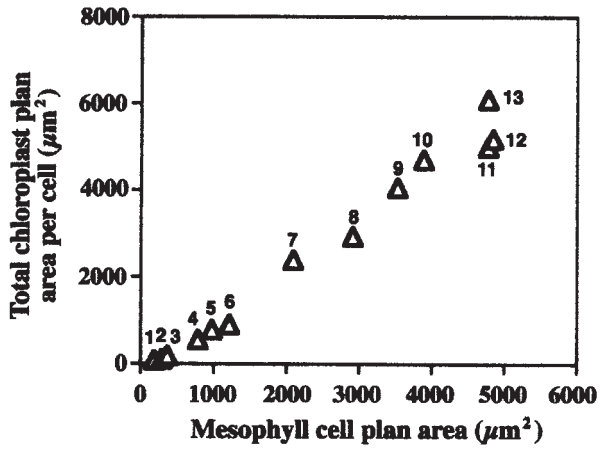

Figure 2. Chloroplast Cover and Mesophyll Cell Size in Different Dicotyledonous Species.

The relationship between mesophyll cell size, measured as plan area, and the total plan area of chloroplasts per mesophyll cell, measured as chloroplast number per cell multiplied by mean chloroplast plan area per cell. The data plotted are for eight different dicotyledonous species and several *arc* mutants of Arabidopsis. The species are (1) *T. cacao*, (2) *Citrus sinensis*, (3) *Quercus thureyi*, (4) *Hedera helix*, (5) *Gossypium arboreum*, (6) *Bouganvillea glabra*, (7) Arabidopsis *arc1* mutant, (8) *Lycopersicon esculentum*, (9) Arabidopsis *arc5* mutant, (10) Arabidopsis *arc3-1* mutant, (11) Arabidopsis *arc2* mutant, (12) Arabidopsis *arc3-2* mutant, and (13) Arabidopsis wild type (Landsberg *erecta*). Species data are courtesy of N. Salmon.

Developmental Factors Controlling Plastid Division

Several detailed studies of chloroplast populations during development have suggested that chloroplasts must attain a certain size before they can divide (Boffey and Leech, 1982; Ellis et al., 1983), and it is obvious that newly divided chloroplasts must subsequently increase in size before another round of division can occur. It is possible that chloroplasts can divide only when they are within a "size window," but clarification of this concept will probably require an in vitro chloroplast division system. In *P. patens*, application of cytokinin appears able to initiate divisions in greatly enlarged chloroplasts (Kasten et al., 1997), but such studies are largely missing in higher plant systems.

Although the idea of a chloroplast division cycle is convenient, the close control by the cell of chloroplast divisions, particularly in the context of a chloroplast population, suggests that a free-running chloroplast division cycle does not exist. Although the chloroplast genome does not appear to be directly involved in the control of chloroplast division, there is close interaction between chloroplast DNA replication and chloroplast division. Indeed, it is well established that chloroplast DNA replication results in an increase in chloroplast genome copy number before chloroplast division (Boffey et al., 1979; Boffey and Leech, 1982), with the genome copies segregated into the two daughter chloroplasts.

Although chloroplast division is obviously a normally integrated part of chloroplast development, abolishing divisions,

as occurs in some *arc* mutants, seemingly does not affect continued chloroplast development, and all studies on these greatly enlarged chloroplasts suggest that their development and internal structure are largely normal (Robertson et al., 1995, 1996). Consequently, it appears that division and development, particularly in terms of chloroplast size, are independent but mutually compensating processes (Pyke, 1997).

Why Do Cells Contain So Many Small Chloroplasts?

The fact that developing chloroplasts normally divide during mesophyll cell expansion implies that a division event is an integral part of normal chloroplast development and that plant cells have evolved to contain many small chloroplasts rather than a few large ones. Because Arabidopsis *arc* mutants with just a few enlarged chloroplasts per cell are indistinguishable from wild-type plants in terms of their overall morphology and growth rate, the question arises as to why higher plant chloroplasts need to divide rather than simply to expand.

One potential answer to this question comes from studies of chloroplast positioning in Arabidopsis. These investigations show that plastid positioning within the cell is controlled by light conditions (Trojan and Gabrys, 1996) and that chloroplasts appear to locate preferentially on cell surfaces exposed to air spaces in the leaf (K.A. Pyke, unpublished data). These features can be visualized easily in separated fixed Arabidopsis mesophyll cells, in which empty patches in the monolayer of chloroplasts tightly appressed to the cell wall represent areas where two cells were originally joined in the leaf anatomy (Figure 1G). Presumably, if a cell had only a few enlarged chloroplasts, the capacity for those chloroplasts to resolve their placement appropriately would be greatly compromised.

PLASTID DIVISION—THE FUTURE?

Although this article has highlighted several recent developments in the understanding of plastid division, it has also revealed the paucity of knowledge about many aspects of the process. The discovery of bacterial cell division gene homologs strongly suggests that higher plant plastid division is based on a system that has evolved from that utilized in prokaryotic cells. It remains to be seen whether novel plastid-specific components of this process have evolved independently in plants.

With the characterization of genes that control aspects of plastid division, efforts to manipulate genetically the size of the plastid compartment will become feasible. It is likely that such efforts will be successful only if the mechanisms that allow compensation between plastid number and plastid size are broken, either by mutation or by other genetic manipulations. Finally, considering the progress that has been made in understanding the biology of higher plant plastid division, it would be pleasing to see plant cells represented in today's biology textbooks with a substantial number of chloroplasts in their cytoplasm.

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