Plastid division

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Received: 7 July 2010; Returned for revision: 19 August 2010; Accepted: 28 September 2010; Published: 5 October 2010

Citation details: Pyke KA. 2010. Plastid division. AoB PLANTS 2010: plq016, doi:10.1093/aobpla/plq016

Abstract

Background and aims

Plastids undergo a process of binary fission in order to replicate. Plastid replication is required at two distinct stages of plant growth: during cell division to ensure correct plastid segregation, and during cell expansion and development to generate large populations of functional plastids, as in leaf mesophyll cells. This review considers some of the recent advances in the understanding of how plastids undergo binary fission, a process which uses several different proteins, both internal and external to the plastid, which have been derived from the original endosymbiont's genome as well as new proteins that have been recruited from the host genome.

Key points

Several of the proteins currently used in this process in higher plants have homologues in modern-day bacteria. An alternative mode of replication by a budding-type mechanism also appears to be used in some circumstances. The review also highlights how most of our knowledge of plastid division is centred on the chloroplast developing in leaf mesophyll cells and a role for plastid division during the development of other plastid types is poorly understood. Whilst models for a protein-based mechanism have been devised, exactly how the division process is controlled at the plastid level and at the plastid population level is poorly understood.

Introduction

Plastids form a group of organelles found in the cells of higher and lower plants, which originally evolved from prokaryotic ancestors around 2 billion years ago, when an endosymbiotic event took place, namely the uptake of a free-living photosynthetic prokaryote into a eukaryotic protozoan (McFadden, 1999, 2001). Through the course of subsequent evolution, plastids have become a defining feature of plants and contribute a very significant number of properties to plant function (Pyke, 2009). Foremost among these is the process of photosynthesis, enabling plants to increase in biomass and synthesize complex organic molecules and polymers from simple building molecules of carbon dioxide and water. In order for a functional endosymbiotic relationship to evolve, as is seen today in extant green plants, the original prokaryote had to adapt to the internal cellular environment of the eukaryote. Since the eukaryotic cell will have undergone cell divisions, the endosymbiotic prokaryote will have been required to divide as well, in order to remain resident within the cell. The result of this requirement in modern-day plants is that plastids have the ability to divide inside their host plant cells, giving rise to, in cell types such as leaf mesophyll cells, large populations of plastids within individual cells (Pyke, 1997). Another plastid trait which has evolved as higher and lower plants became multicellular organisms with defined cell types, was for the

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AoB PLANTS Vol. 2010, plq016, doi:10.1093/aobpla/plq016, available online at www.aobplants.oxfordjournals.org
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plastid to become differentiated into different plastid types in different types of plant cell. This trait arose for the purpose of storing different types of molecules or for the benefit of performing different types of biochemical activity in different cell types (Pyke, 2007). The end result of this process is that in modern-day green plants, there are several different distinct types of plastids, which reside in different types of cells. The most important, and certainly the best understood in terms of its biology, is the chloroplast, the green-pigmented plastid found in cells of leaves, stems and other green parts of plants.

Another important type of plastid is that found in meristem cells and young parts of plant tissues, such as embryos, which are called proplastids (Chaley and Possingham, 1981; Robertson et al., 1995; Gunning, 2007). Since the meristem cells give rise ultimately to all of the cells within the body of the plant, proplastids act as the progenitor plastid for all types of plastids found throughout the plant. The fact that proplastids reside in cells which are undergoing rapid cell division, with a short cell cycle, requires them to divide and be segregated to ensure continuity of plastids within each new daughter cell (Sheahan et al., 2004; Pyke, 2007). In addition, in cells where large populations of a defined plastid type are required, as in chloroplast-containing leaf mesophyll cells, division of differentiated plastid types is also required to give rise to such populations. Leaf mesophyll cells contain large populations of chloroplasts, the population size being determined largely by the size of the cell and the average size of the individual chloroplasts. Mesophyll cells generally contain between 50 and 200 mature green chloroplasts which are able to move around in the cytoplasm according to the photoenvironment that the cell experiences. It is thought that large populations of small plastids in mesophyll cells are better adapted to such relocations within the cell compared with a cell with a small population of giant chloroplasts (Jeong et al., 2002; Koniger et al., 2008).

Thus, there are two distinct phases of plastid division in higher plants: proplastid division within the cells in the meristem and chloroplast division within developing leaf mesophyll cells. Among the several other types of plastids in the plant such as starch-containing amyloplasts and pigmented chromoplasts, plastids may divide to differing degrees depending on the physiological and developmental status of the cell, although the nature of plastid division in these plastid types is poorly characterized to date.

The way in which plastids divide was first characterized by observing young developing leaf cells, in which chloroplasts could be observed with central constrictions, which result eventually in the production of two equally sized daughter plastids. These daughter plastids then need to grow in size before division can occur again. Extensive analysis of constricted chloroplasts in the context of cell expansion and an increase in the number of plastids per cell showed how this dynamic division process, termed binary fission, leads to an increase in chloroplast number (Leech et al., 1981; Ellis and Leech, 1983; Pyke and Leech, 1992; Robertson et al., 1996; Pyke, 1997). Proplastids are thought to divide in the same basic way, although they are much more difficult to observe, residing in small meristematic cells. Electron micrographs, however, do show centrally constricted proplastids in these cells, which are considered to be undergoing plastid division (Chaley and Possingham, 1981; Robertson et al., 1995, 1996). Their morphology, however, is much more heterogeneous than that seen in chloroplast division, with the constriction often extending to produce a long thin isthmus, joining the two plastid bodies.

The challenge of the last 20 years has been to elucidate the molecular machinery that drives this chloroplast division process, to understand how it works and how it is powered, and to work out how such a division apparatus is controlled in its activity, i.e. what tells chloroplasts to start to divide and what tells them to stop dividing. Much progress has been made in defining the molecules that participate in the division process in chloroplasts, primarily through the characterization of genes encoding proteins involved in the process, which are revealed as conveying mutant chloroplast phenotypes when mutants of Arabidopsis thaliana are systematically screened. The original mutant screen that produced arc mutants of A. thaliana (Pyke and Leech, 1992; 1994) and other screens since (Miyagishima et al. 2006) have been highly productive in revealing plastid division genes. An alternative approach has been to search for genes involved in prokaryotic cell division in genomes of higher plants (Osteryoung and Vierling, 1995; Colletti et al., 2000).

Interestingly, these approaches have revealed that proteins involved in the constriction process have originated by two different routes. One group of proteins were originally involved in the division of the free-living prokaryote, which invaded the eukaryotic cell and are prokaryotic in nature, whereas another group have been recruited from the eukaryotic genome or have been hijacked from other genes during the course of evolution. Thus, what is known currently of the plastid division machinery reveals a complex mechanism with a variety of different functional proteins.
Endosymbiont-derived plastid division proteins

Those proteins encoded by genes which were originally present in the endosymbiont’s own genome are prokaryotic in nature and function internally within the chloroplast during the division process. However, even though these proteins reside in the chloroplast stroma and inner envelope membranes, in higher plants their genes no longer reside in the organelle genome, but have moved to the cell’s nuclear genome. Consequently, these proteins are translated on cytosolic ribosomes and each contains transit peptide sequences, enabling them to be targeted back to the plastid organelle. The evolutionary transfer of genes from the prokaryotic genome to the cell’s nuclear genome is clearly shown by the fact that many of these genes are present in current-day photosynthetic bacteria, and several are present on the plastid genomes in algae, such as *Chlorrella*, which represent a stepping stone along the evolutionary pathway (Wakasugi et al., 1997; Colletti et al., 2000).

The major protein in the chloroplast division process is FtsZ (Osteryoung and Vierling, 1995), which has the ability to form protofilaments, which eventually form a ring of FtsZ molecules in the stroma of the chloroplasts, tightly associated with the inner envelope membrane (Vitha et al., 2001). Plant FtsZ has GTPase activity which is utilized in protofilament formation (El-Kafafi et al., 2005; Olson et al., 2010). Two types of FtsZ occur in higher plants, FtsZ1 and FtsZ2, both of which are nuclear-encoded (McAndrew et al., 2001; Stokes and Osteryoung, 2003; El-Kafafi et al., 2005). They have the ability to form filaments spontaneously on their own, but appear to interact together in a defined ratio to form the FtsZ ring, comprised of both FtsZ1 and FtsZ2 molecules (Yoder et al., 2007; McAndrew et al., 2008). The FtsZ ring so formed has contractile properties, with the ability to pull in the plastid membrane as it contracts (Yoshida et al., 2006). In order to fulfil this function, it needs to be tethered to the inner envelope membrane in order to pull it inwards as the ring contracts. This tethering appears to be carried out by the ARC6 protein, originally characterized from the *arc6* mutant of Arabidopsis (Pyke and Leech, 1994; Robertson et al., 1995; Fig. 1), which resides in the inner envelope membrane and has an extrinsic stromal region, which interacts specifically with FtsZ2 (Vitha et al., 2003; Maple et al., 2005; McAndrew et al., 2008). It appears likely that not only does ARC6 act as a tether for the FtsZ ring, but it also participates in organizing self-assembled polymerized filaments of FtsZ into a functional ring. ARC6 proteins form a ring within the inner envelope membrane, which underlies the FtsZ ring on the stromal surface (McAndrew et al., 2008).

A major feature of the division of plastids, and chloroplasts in particular, is that they divide centrally along their long axis, giving rise to two fairly equally sized daughter plastids. Thus, these organelles have defined poles at either end of the organellar body. Consequently, the division apparatus including the FtsZ ring has to be positioned midway between the two poles of the organelle in order for division to occur correctly. The positioning mechanism involves Min proteins, which are homologous to those Min proteins used to position sites of division in modern-day bacteria (Margolin, 2005). Higher plants contain nuclear genes, which encode two of the three Min proteins present in bacteria: MinD (Colletti et al., 2000) and MinE (Maple et al., 2002). These two
proteins regulate the positional polymerization of FtsZ proteins in that MinD inhibits FtsZ filament formation at the poles of the plastid, whereas MinE supports and maintains FtsZ filamentation in an antagonistic manner with MinD (Maple and Møller, 2007; Fujiwara et al., 2008). It remains unclear whether MinC, the third Min component from bacteria, functions in chloroplast division, since the gene is apparently missing from genomes of higher and lower plants, although a MinC-like sequence has been identified in a moss genome (Yang et al., 2008). Interestingly, if the bacterial MinC gene is overexpressed in Arabidopsis, then the chloroplast division process is seriously perturbed, suggesting that the prokaryotic MinC protein still has the ability to interact with the chloroplast division mechanism (Tavva et al., 2006). It has been suggested that another plastid division protein, ARC3 (Pyke and Leech, 1992; Shimada et al., 2004), may have replaced the functionality of MinC in chloroplast division (Maple et al., 2007). The ARC3 protein appears to have evolved specifically in higher plants and contains regions bearing similarities to an FtsZ sequence and the sequence of the enzyme phosphatidylinositol-4-phosphate 5-kinase.

Chaperonin proteins, apparently of a prokaryotic descent, also function in chloroplast division. ARC2 was originally identified as an Arabidopsis mutant, and the ARC2 gene together with another, BRO4, are both members of the plastid chaperonin family Cpn60: ARC2 is ptCpn60α and BRO4 is ptCpn60β (Suzuki et al., 2009). It is likely that their role is to correctly fold plastid division proteins and/or regulate FtsZ polymerization dynamics.

A further protein Giant Chloroplast 1 (GC1), also called AtSulA and described in two different studies (Maple et al., 2004; Raynaud et al., 2004), also functions in chloroplast division although its role is unclear. Its sequence bears weak homology to a prokaryotic factor SulA that is involved in the inhibition of FtsZ polymerization in prokaryotic cells (Raynaud et al., 2004) so it could conceivably be involved in FtsZ polymerization dynamics inside the chloroplast.

Host-derived plastid division protein

In order for the host eukaryotic cell to control effectively the division of the plastids within its cytoplasm, it was necessary for genes derived from the host’s nuclear genome to be recruited or to evolve new roles, such that the modern-day plastid division machinery in higher plants comprises groups of proteins derived originally from both the endosymbiont and the host.

Foremost among the host-derived genes is ARC5 (Gao et al., 2003; Miyagishima et al., 2003), which encodes a member of the dynamin family of proteins, a group which have GTPase activity and are involved in membrane remodelling and pinching of membranes. ARC5 was originally identified as a mutant in which chloroplasts accumulate in dumbbell shapes but fail to divide properly (Pyke and Leech, 1994; Robertson et al., 1996). ARC5 proteins form a punctuated ring around the outside of the dividing chloroplast, which overlays the FtsZ ring internal to the organelle (Miyagishima et al., 2006). The ARC5 protein does not possess a plastid targeting sequence so does not enter into the chloroplast but probably forms an array around the outside of the chloroplast by interacting with a specific membrane-bound protein, which recruits ARC5 proteins to the outer surface. There are two such binding proteins, called PDV1 and PDV2 (Miyagishima et al., 2006), and both reside in the outer envelope membrane of the chloroplast and both interact with ARC5 proteins by their N-termini, which are exposed to the cytosol. Exactly how the PDV proteins recruit ARC5, either directly or indirectly, is unclear and surprisingly PDV1 proteins form a punctate-type ring in the outer envelope membrane, in a similar fashion to ARC5 on the surface, whereas PDV2 forms a continuous ring within the outer envelope membrane (Miyagishima et al., 2006; Glynn et al., 2008). The localization of the PDV2 ring within the outer envelope membrane appears to be directed by the ARC6 protein on the inner envelope since both proteins have a C-terminus which enters in the luminal space between the outer and inner plastid envelope membranes. Thus, within this intermembrane space, ARC6 dictates the spatial localization of the PDV2 ring (Glynn et al., 2008). In this way, a coordinated chain of events is put in place laying down a precise position for the chloroplast division machinery to form at the midpoint of the plastid, from the internal FtsZ ring, the positioning of which is determined by the Min genes, although ARC6 and PDV1/2 to the external ARC5 ring.

This is a neat story, which has been figured out using a combination of Arabidopsis mutants and by making genetic comparisons with bacterial cell division systems. Models to describe how the basic mechanical array of proteins discovered fit together, as described above, produce a reasonable model of how the basic chloroplast mechanism might be built (Glynn et al., 2007; Yang et al., 2008). Unfortunately, the system is more complex. Several other proteins have recently been shown to be involved in the chloroplast division process in some way, and these will need to be assimilated into a functional working model of the process.

FtsZ assembly and the recruitment of PDV1 to the division ring are controlled by PARC6, also known as CDP1 (Zhang et al., 2009), a eukaryotically derived protein, which only appears to be present in vascular plant
MinD (Nakanishi et al., 2009) is involved with FtsZ ring position by interacting with MCD1, a eukaryotically derived protein, which is also observed in chloroplast division process by controlling the site of ring placement by interacting with the Min system. MCD1 is a eukaryotically derived protein, which is involved with FtsZ ring position by interacting with MinD (Nakanishi et al., 2009). It is clear that the system that the chloroplast now uses for division ring positioning, although originally based on the prokaryotic MinC, D, E system, has evolved and diverged significantly and that chloroplast-specific proteins now drive a system still containing MinD and MinE, but somewhat different to that which functions in modern-day bacteria.

The plastid dividing rings

In addition to the ring systems so far discussed, other rings have also been described as being functional in plastid division, and indeed observation of them far predates those revealed by modern molecular genetics approaches. Plastid dividing (PD) rings were seen originally as fuzzy opaque bands around the isthmus of dividing plastids in the electron microscope, and indeed were the only molecular structure that could be seen to be associated with plastid division (Leech et al., 1981; Oross and Possingham, 1989; Robertson et al., 1996). It was clearly shown that one PD ring resides in the stroma, one PD ring occurs on the cytoplasmic surface of the outer envelope, and occasionally a third PD ring is seen between the two envelope membranes, in the intermembrane space (Kuroiwa et al., 1998). Plastid dividing rings have been imaged precisely and even isolated (Miyagishima et al., 2001b), but the conclusion from these studies is that they are a distinct and different set of rings to those constructed from FtsZ and ARC5 (Miyagishima et al., 2001a; Kuroiwa et al., 2002). To date, no clear demonstration of the constituents of the PD rings has been made and it remains unclear exactly how the PD rings relate to the FtsZ and dynamin rings in a division mechanism. Moreover, it does seem an odd evolutionary quirk for a mechanism to require four concentric rings, together with a host of additional proteins, in order to pull in, under constriction, a double-membrane structure, this aside from the positional control, functional energy control or final membrane fusion in the process.

How is the chloroplast division machinery controlled?

Thus far, a model of the basic structure of the chloroplast division machinery has been developed by molecular genetic experiments in several different laboratories, but the next question is how it works in terms of functional mechanism and control. Certainly, it has been shown that the FtsZ ring and the ARC5 ring both have GTPase activity and that the division apparatus can be isolated and manipulated in vitro (Yoshida et al., 2006). Such elegant experiments with optical tweezers show that the power generation for the constriction of the apparatus comes from the ARC5 ring rather than the FtsZ ring itself.

How the activity of division is controlled by the cell and how the extent of cellular chloroplast division is controlled are still unclear, although some elements of the process may have been identified. For example, PDV1 and PDV2 appear to have some regulatory role as to the rate at which chloroplasts divide since manipulation of different levels of PDV proteins results in differently sized chloroplast populations in cells (Okazaki et al., 2009). In addition, in Arabidopsis, levels of PDV protein and the extent of resulting chloroplast division appear to be controlled by cytokinin, an observation that may be critical in exploring how the cell controls the extent of its chloroplast division in relation to other cellular processes.

Another discovery, which may open up our knowledge of how chloroplast division is controlled, is that of two mechanosensory proteins in the plastid envelope membrane. These proteins, called MSL2-1 and MSL3-1, are closely related to proteins in the bacterial membrane, which monitor osmotic shock (Haswell and Meyerowitz, 2006). When mutated in Arabidopsis, chloroplast division is severely perturbed, implying a role for MSL proteins in chloroplast division. More especially, MSL proteins co-localize on the inner envelope membrane with the MinE protein. Thus, mechanosensing could be a link between the extent of plastid division and the control of plastid size and number in a cell (Pyke, 2006).

Doing it a different way—plastid budding

Analysis of a variety of mutant and transgenic plants with altered plastid division genes has been very successful in identifying genes involved in plastid division. However, one overriding feature of these plants suggests that other mechanisms to divide chloroplasts may exist. In spite of the severely altered numbers of chloroplasts in leaf cells, resulting in large populations of small chloroplasts or small populations of large chloroplasts,
such plants grow in a near-normal fashion. More particularly, somehow these mutant plants must be able to allocate proplastids to daughter cells during cell division in the meristem since all cells in these mutant plants contain plastids and the plants are relatively healthy and fertile. This observation is especially true for the arc6 mutant of Arabidopsis, in which the mesophyll cells contain only one or two giant chloroplasts, yet such plastids must get replicated in some way during cell division (Pyke and Leech, 1994; Robertson et al., 1995). In addition, it has been shown that FtsZ1 and FtsZ2 are functionally dispensable, in that in an Arabidopsis plant with mutation in all three FtsZ genes, the chloroplasts can still divide and the plant is viable and fertile (Schmitz et al., 2009). This suggests that an alternative mechanism of plastid division may occur in plant cells. Such an alternative mechanism involving plastid budding, rather than binary fission, was implied from observations of the tomato suffulta mutant (Forth and Pyke, 2006). In this mutant, the chloroplasts in the mesophyll cells are giant and very few in number, yet during tomato fruit ripening in which green chloroplasts differentiate into red chromoplasts, the large chloroplasts fragment in a budding-like way to yield large numbers of normal-sized chromoplasts. Thus, it appears that budding of this nature may be a way in which such large plastids replicate. Furthermore, when green fluorescent protein (GFP) is targeted to the plastid compartment in both arc6 and suffulta cells which contain apparently no plastids, small green fluorescent bodies are observed, which may well be the result of budded vesicles from the main plastid body (Forth and Pyke, 2006; Chen et al., 2009). The budding of plastids in leaf mesophyll cells has been reported before in scanning electron micrographs of chloroplasts of Bryophyllum pinatum, in which chloroplasts appear to bud, much like the appearance of budding yeast (Kulandaivelu and Gnanam, 1985). The budding process, in which a small plastid is separated from the larger main plastid body, is quite similar to highly asymmetric divisions of chloroplasts when the division ring localization system breaks down, as in min gene mutants. In the minD mutant, arc11, the plastid population consists of a highly heterogeneous mix of large and small plastids, resulting from various asymmetric divisions, including the production of very small plastids (Marrison et al., 1999; Colletti et al., 2000; Fujiwara et al., 2004, 2008). Whether any type of budding mechanism involves the plethora of plastid division proteins that apparently function in normal binary division is not known, but certainly budding may represent an alternative strategy to plastid replication in certain situations during plastid development.

**Division of other plastid types**

Our knowledge of plastid division mechanisms as described here is almost entirely based on the chloroplast, despite the fact that there are several different types of plastids present in different cell types in a higher plant. The question remains as to how do plastid types other than chloroplasts undergo division and whether they use the same or similar mechanisms to those used by chloroplasts to divide.

The division of proplastids is fundamental to plastid continuity in cell lineages derived from the meristems of the plant and it is generally assumed that proplastids use the same mechanisms as chloroplasts, although no studies have addressed this directly. Indeed ARC proteins appear to have differing roles in proplastid and chloroplast division, since whilst mutation in the ARC6 protein alters proplastid numbers and size in meristem cells in Arabidopsis seedlings (Robertson et al., 1995), mutation in the ARCS protein has no effect on proplastid number or size, suggesting that ARCS does not function in the proplast division process (Robertson et al., 1996). Therefore, it remains to be determined whether the precise division machinery in proplastids is the same as that used by chloroplasts. Proplastids are much smaller than chloroplasts and their division control needs integrating into the cell cycle of the rapidly dividing meristem cells to ensure segregation of at least one proplast into the two daughter cells at cytokinesis.

Little is known about other types of plastids and their potential for division. Amyloplasts accumulate starch and are found in a variety of plant storage tissues, especially in the endosperm of cereal and the storage tubers of potatoes. During endosperm development in cereals, amyloplasts differentiate from proplastids and appear to divide by a similar process to chloroplasts involving central constriction (Langeveld et al., 2000; Bechtel and Wilson, 2003). However, in rice endosperm, multiple constriction sites are observed in dividing amyloplasts, producing a bead on a string-type appearance. Amyloplasts also appear to undergo budding, resulting in a small budded amyloplast on the surface of a larger one (Yun and Kawagoe, 2009). In potato tubers, the situation appears different again in that ‘mother’ amyloplasts appear to give rise to plastids, which presumably differentiate back into amyloplasts at a later stage (Sagisaka, 2008).

During fruit development in tomato, chromoplasts differentiate from chloroplasts and accumulate pigment, whilst degrading chlorophyll. Chromoplast populations in cells of ripe tomato fruit are very large, containing hundreds of chromoplasts. Although it is possible that chromoplasts are produced in part by a budding-type
mechanism, as mentioned previously (Forth and Pyke, 2006; Fig. 2), most of the plastid population increase takes place during the development of the green fruit, resulting in large chloroplast populations, which then differentiate into chromoplasts (Cookson et al., 2003). Indeed chromoplast sizes can be manipulated by altering levels of FtsZ transgenically, which alters chloroplast number and size, and can lead to the production of giant chromoplasts (P. Cook and K. Pyke, unpublished results).

By using fluorescent proteins such as GFP to visualize other plastid types which lack pigmentation (Fig. 3), it should be possible to learn more about whether the plastid division machinery of the chloroplast also functions to divide other types of plastid. This approach has been successful in pollen, where GFP-labelled plastids were shown to use FtsZ as part of their division mechanism (Tang et al., 2009).

Conclusions and forward look

Our understanding of plastids and how they divide has come a long way in the last 15 years, and we now know something of the basic mechanism that chloroplasts use to perform binary fission. Even so, there are many unanswered questions about the process and a whole raft of questions when we consider plastid types other than chloroplasts, and cell types other than leaf mesophyll cells. Central to a further understanding must be elucidation of the mechanism whereby a cell controls how many plastids it contains. It is apparent that in some cell types, such as epidermal cells and root cells, little, if any, plastid division occurs since plastids in these cells are similar in number to the proplastid number in the originator meristem cells (Pyke, 2009).

In addition, a consideration of the role of plastid division in the context of dynamic plastid morphology is needed. Many plastids, including many non-green plastids, show highly irregular morphologies and produce thin stroma-filled tubules from the plastid body, termed stromules (Hanson and Sattarzadeh, 2008). The role of stromules is unclear, although they have yet to be implicated specifically in plastid division itself. However, long thin isthmuses that develop between dividing plastids in some cell types are very stromule-like. Also, stromules break and can give rise to

Fig. 2 Green chloroplasts in the pericarp cells of ripening tomato fruit containing a plastid-targeted GFP and imaged by confocal microscopy. These chloroplasts have distinct vesicle-like structures containing GFP, which may be derived from stromules as well as showing heterogeneity in the shape of the plastid body. Although plastids divide by binary fission during the development of these cells, plastid population growth as a result of budding or vesicle production appears to occur as an alternative mode of replication.

Fig. 3 Imaging of plastids in a group of cultured tomato cells reveals a complex array of plastids. This cluster of about 12 cells are from a cell culture derived originally from a transgenic tomato seedling contain a transgene which targets GFP to the plastid and revealed by confocal fluorescence microscopy. Plastids routinely congregate around the nucleus (arrowed n) and are also dispersed throughout the cytoplasm in a heterogeneous array of shapes and sizes, with many congregating on the cross-cell walls joining cells together (arrowed cw). Larger plastids produce stromule tubules (arrowed s), whilst many small bodies containing GFP are also present. The detailed dynamics of such a population of plastids is largely unknown. Image by Linda Cholerton.
plastid-derived vesicles, in a mode not unlike a mode for plastid budding. Exactly how stromule dynamics and plastid budding relate to each other remains to be elucidated. Furthermore, alterations in plastid division genes have been shown to have effects in other cell types and plastid types in relation to plastid morphology and stromule incidence (Holzinger et al., 2008). This interesting piece of plant cell biology certainly has a long way to go before we fully understand how plastids divide.

**Conflicts of interest statement**

None declared.

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