

REVIEW ARTICLE

Stromules: a characteristic cell-specific feature of plastid morphology*

Senthil Kumar A. Natesan, James A. Sullivan[†] and John C. Gray[‡]

Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK

Received 20 August 2004; Accepted 23 November 2004

Abstract

Stromules (stroma-filled tubules) are highly dynamic structures extending from the surface of all plastid types examined so far, including proplastids, chloroplasts, etioplasts, leucoplasts, amyloplasts, and chromoplasts. Stromules are usually 0.35–0.85 µm in diameter and of variable length, from short beak-like projections to linear or branched structures up to 220 µm long. They are enclosed by the inner and outer plastid envelope membranes and enable the transfer of molecules as large as Rubisco (~560 kDa) between interconnected plastids. Stromules occur in all cell types, but stromule morphology and the proportion of plastids with stromules vary from tissue to tissue and at different stages of plant development. In general, stromules are more abundant in tissues containing non-green plastids, and in cells containing smaller plastids. The primary function of stromules is still unresolved, although the presence of stromules markedly increases the plastid surface area, potentially increasing transport to and from the cytosol. Other functions of stromules, such as transfer of macromolecules between plastids and starch granule formation in cereal endosperm, may be restricted to particular tissues and cell types.

Key words: Amyloplast, chloroplast, chromoplast, GFP, leucoplast, plastid, proplastid, stromule.

Introduction

Stromules (stroma-filled tubules) have been recognized as an important feature of plastid morphology over the past few years, mainly due to the ease with which they can be

visualized in plants containing plastid-located green fluorescent protein (GFP; Köhler *et al.*, 1997, 2000; Tirlapur *et al.*, 1999; Köhler and Hanson, 2000). Stromules are highly dynamic structures emanating from the plastid surface; they are usually 0.3–0.85 µm in diameter (Köhler *et al.*, 1997; Tirlapur *et al.*, 1999; Shiina *et al.*, 2000; Arimura *et al.*, 2001) and are enclosed by the outer and inner plastid envelope membranes (Gray *et al.*, 2001). Stromules are extremely variable in length and they continuously and rapidly change length and shape (Wildman *et al.*, 1962; Köhler and Hanson, 2000; Gunning, 2004b). They range from short beak-like projections to linear or branched structures up to 220 µm in length (Waters *et al.*, 2004), occasionally interconnecting different plastids in the same cell. Although the transfer of GFP between plastids via stromules has been demonstrated by photobleaching experiments (Köhler *et al.*, 1997, 2000; Tirlapur *et al.*, 1999), the function of stromules, particularly those that do not interconnect plastids, is still a matter of conjecture. There are still many unanswered questions concerning the structure, function, and motility of stromules. The aim of this review is primarily to consider current knowledge of the distribution of stromules in different cell types and on different plastid types in higher plants; this information may give some clues to the function of stromules. This article should complement the recent review of Kwok and Hanson (2004c) and provide an update on advances in our understanding of stromules since the last review (Gray *et al.*, 2001).

Visualization of stromules

Although structures that would now be recognized as stromules were observed by light microscopy using

* This article is dedicated to the memory of Sam Wildman, who died on 16 August 2004 aged 92. He produced the first real-time moving images of stromules in 1964 and made many seminal contributions to our understanding of chloroplast structure and function.

[†] Present address: Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

[‡] To whom correspondence should be addressed. Fax: +44 1223 333953. E-mail: jcg2@mole.bio.cam.ac.uk

Abbreviations: CaMV, cauliflower mosaic virus; DIC, differential interference contrast; GFP, green fluorescent protein; *gfp*, gene encoding GFP; TMV, tobacco mosaic virus; YFP, yellow fluorescent protein.

bright-field illumination over 100 years ago (Gray *et al.*, 2001), the introduction of GFP and its detection by fluorescence microscopy have revolutionized the detection and visualization of stromules. Köhler *et al.* (1997) observed stromules in transgenic tobacco and petunia plants containing GFP targeted to the plastids by the transit peptide of the RecA protein. Subsequently, GFP has been observed in stromules in many other studies using several different expression systems to produce plastid-located GFP. Stromules have been observed in stable transformants obtained either by nuclear transformation with a chimeric gene construct producing a plastid-targeted GFP (Table 1) or by plastid transformation with *gfp* under the control of a plastid promoter (Gray *et al.*, 1999; Shiina *et al.*, 2000; Reed *et al.*, 2001; Waters *et al.*, 2004). Alternatively, stromules have been observed following transient expression of chimeric *gfp* constructs introduced into developing wheat seeds, leaves of various plants, or onion epidermal peels by microprojectile bombardment (Table 1).

More recently, stromules have been observed following expression of cDNA-*gfp* fusions from a *tobacco mosaic virus* vector (Escobar *et al.*, 2003). This system was introduced as a high-throughput method of identifying proteins targeted to different subcellular compartments in leaves of *Nicotiana benthamiana*. Infection of leaves with the TMV vector containing fusions of random cDNAs to the 5' or 3' ends of a *gfp* reporter gene resulted in chloroplast-targeting of the encoded proteins from about 5% of the 5' fusions and 0.2% of the 3' fusions. Some of these GFP fusion proteins were shown to be present in stromules in mesophyll cells (Escobar *et al.*, 2003). Expression of a cDNA encoding aspartate aminotransferase resulted in the visualization of long stromules, whereas expression of a cDNA encoding an unidentified protein produced small beak-like projections (Escobar *et al.*, 2003).

This system offers the prospect of identifying proteins associated with specific structural features of stromules.

Most of the targeting sequences used for directing GFP to plastids, either in stable transformants or in transient expression systems (Table 1), result in a stromal location of GFP. Expression of *gfp* from the *psbA* or *rrn* promoters in chloroplasts of transplastomic tobacco (Gray *et al.*, 1999; Shiina *et al.*, 2000; Reed *et al.*, 2001; Waters *et al.*, 2004) also resulted in GFP located in the stroma. GFP targeted to the plastid envelope membranes has provided evidence that both the inner and the outer envelope membranes are present in stromules. Gray *et al.* (2001) reported the presence of stromules in cells expressing chimeric *gfp* constructs encoding fusions to the outer membrane protein OEP14 (Li *et al.*, 1991) and to the presequence and inner-envelope-targeting domain of the phosphate translocator (Knight and Gray, 1995). Stromules have also been observed in onion epidermal cells bombarded with a chimeric gene construct encoding GFP fused to the plastid envelope-located long-chain acyl CoA synthetase LACS9 (Schnurr *et al.*, 2002). The presence of these proteins in stromules suggests that stromule membranes carry out a normal range of plastid functions, and are not composed exclusively of membrane proteins specific to stromules.

Although most current studies on stromules use fluorescence microscopy to locate plastid-located GFP, the use of differential interference contrast (DIC or Nomarski) microscopy allows the identification of stromules in cells in the absence of GFP (Köhler *et al.*, 1997; Gunning, 2004a, b; Kwok and Hanson, 2004a). Video time-lapse images of tomato trichomes, sub-epidermal tissue of petals of *Iris unguicularis*, and epidermal tissue of arabidopsis hypocotyls clearly demonstrate the dynamic nature of stromules in these cells (Gunning, 2004a, b; Kwok and Hanson, 2004a). Stromules grow out from plastids along tracks

Table 1. Plant expression systems for detecting GFP in stromules

Origin of plastid-targeting sequence	Plant source	Expression system ^a	Recipient plant or tissue	Reference
AgpL, AgpS2	<i>Perilla frutescens</i>	Stable	Tobacco suspension cells	Choi <i>et al.</i> (2001)
Asp5	Arabidopsis	Stable	Tobacco	Kwok and Hanson (2004b)
CPS1	Arabidopsis	Transient	Tobacco leaves	Helliwell <i>et al.</i> (2001)
FtsZ1	Arabidopsis	Stable	Arabidopsis	Vitha <i>et al.</i> (2001)
	Arabidopsis	Transient	Tobacco leaves	Fujiwara and Yoshida (2001)
GBSS	Wheat	Transient	Wheat grain	Langeveld <i>et al.</i> (2000)
InfA	Arabidopsis	Transient	Arabidopsis leaves	Millen <i>et al.</i> (2001)
KS1	Arabidopsis	Transient	Tobacco leaves	Helliwell <i>et al.</i> (2001)
LACS9	Arabidopsis	Transient	Onion epidermis	Schnurr <i>et al.</i> (2002)
MAP1B,C,D	Arabidopsis	Transient	Onion epidermis	Gigliione <i>et al.</i> (2000)
PAC	Arabidopsis	Stable	Arabidopsis	Tirlapur <i>et al.</i> (1999)
TPT	Tobacco	Stable	Tobacco	This paper
RbcS	Pea	Stable	Tobacco suspension cells	Kwok and Hanson (2004b)
RbcS	Tobacco	Transient	Arabidopsis leaf, onion bulb	Gray <i>et al.</i> (2001)
RecA	Arabidopsis	Stable	Petunia, tobacco	Köhler <i>et al.</i> (1997)
			Tomato	Pyke and Howells (2002)
Rpl12-1	Rice	Transient	Tobacco leaves, rice leaves, <i>Commelina communis</i> leaves	Arimura <i>et al.</i> (2001)

^a Stable, transgenic plant with chimeric gene inserted in nucleus; Transient, microprojectile bombardment of tissue sample.

marked by linear streaming of other cell organelles, with stops and starts and transient retractions. Stromules usually grow in the direction of the cytoplasmic streaming, but have also been observed growing upstream, against the flow (Gunning, 2004a, b). In iris petal tissue, rates of stromule extension and retraction of $0.23 \mu\text{m s}^{-1}$ and $1.16 \mu\text{m s}^{-1}$, respectively, have been recorded (Gunning, 2004b). Retracting stromules were observed to form loops at the plastid surface in tomato trichomes and iris petals (Pyke and Howells, 2002; Gunning, 2004a, b).

Although the overwhelming impression of stromules in many tissues is their mobility, DIC microscopy has defined regions of stromules that are anchored or tethered to other, largely undefined, subcellular structures (Gunning, 2004b). Anchoring can occur at the tip of the stromule, or at other points along the length of the stromule, and may be to cytoskeletal elements or to cellular membranes (Gunning, 2004b). Kwok and Hanson (2004d) have suggested interactions of stromules with the inner surface of the plasma membrane, but the nature and function of these interactions is unknown.

The release of vesicles from the ends of stromules has been observed by phase contrast or DIC microscopy (Wildman *et al.*, 1962; Hongladarom *et al.*, 1964; Gunning, 2004a, b) and has recently been called tip-shedding (Gunning, 2004b). The release of the terminal part of the

stromule as a spherical vesicle is accompanied by the instant recoil of the rest of the stromule (Hongladarom *et al.*, 1964; Gunning, 2004a). This behaviour has not so far been observed by fluorescence microscopy of stromules marked with GFP, but small GFP-containing vesicles, separate from plastid bodies or stromules, have been observed in tobacco leaf epidermal cells (Fig. 1; Arimura *et al.*, 2001) and in tomato fruit mesocarp cells (Pyke and Howells, 2002; Waters *et al.*, 2004). The inner mesocarp of tomato fruit contains the longest stromules so far reported (up to $220 \mu\text{m}$, with a mean length of nearly $40 \mu\text{m}$) and it has been suggested that vesicles may be formed following the collapse of long stromules (Waters *et al.*, 2004). The vesiculation of a long stromule ($\sim 8 \mu\text{m}$ long) has been observed on a tobacco mesophyll chloroplast over a short period of time (2–3 min; Fig. 1A–C). The segmentation of stromules into vesicles has also been observed in onion bulb epidermal cells (Guilliermond, 1934; diagram reproduced in Gray *et al.*, 2001). Figure 1 (D, E) shows the vesiculation of a long stromule in an onion bulb epidermal cell treated with 2,3-butanedione 2-monoxime (BDM), a myosin ATPase inhibitor. This vesiculation was observed very rarely and its physiological basis is unclear. However, the formation of vesicles by fragmentation appears to represent a different phenomenon to tip-shedding (Gunning, 2004b).

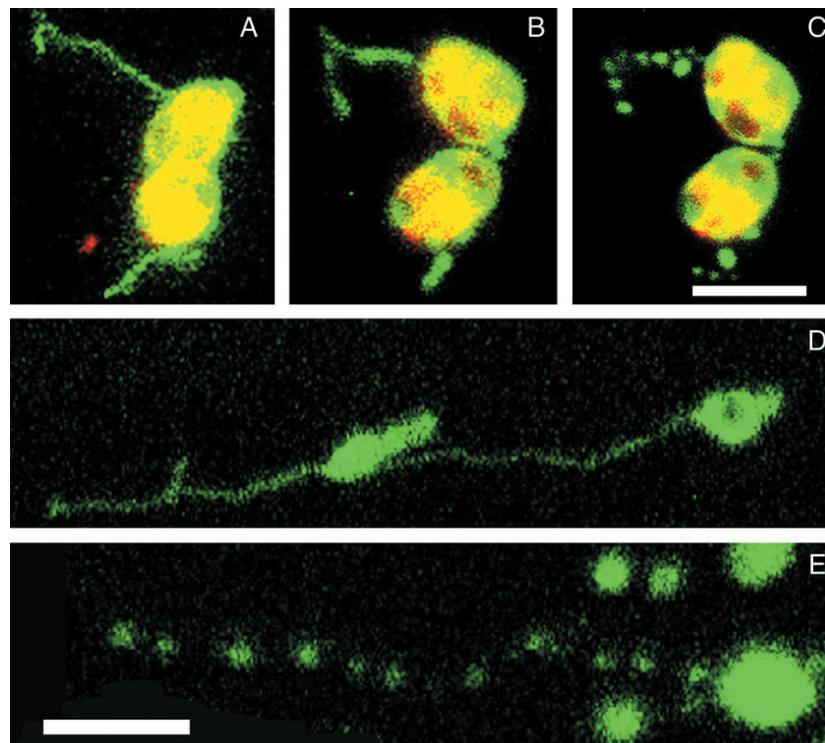


Fig. 1. GFP-containing vesicles in tobacco and onion. (A, B, C) Confocal images, taken at 2 min intervals, showing vesiculation of a stromule protruding from a tobacco mesophyll chloroplast containing GFP expressed from *gfp* integrated in the plastid genome. The merged images contain the green (GFP) and red (chlorophyll) channels. (D, E) Confocal images, taken 30 min apart, showing vesiculation of a long stromule in an onion bulb epidermal cell containing inner-envelope-targeted YFP following treatment with 1 mM 2,3-butanedione 2-monoxime. Scale bars, $5 \mu\text{m}$.

Femtosecond-pulsed near-infrared laser scanning microscopy (NIR-LSM) has been introduced as a novel method for non-invasive high-resolution imaging of chloroplasts in non-transformed tissue (Tirlapur and König, 2001) and has produced stunning images of stromules in epidermal, mesophyll, and bundle sheath cells of arabidopsis. Lasers tuned to 740–800 nm provide improved penetration into strongly scattering plant tissues with reduced photobleaching and the resulting oxidative stress (Tirlapur and König, 2001). This offers another method for visualization of stromules in plants or tissues in which transformation with a *gfp* construct may be problematic.

Distribution of stromules

Stromules are widespread in higher and lower plants (Gray *et al.*, 2001; Gunning, 2004b). Since the introduction of GFP as a marker to facilitate the observation of stromules, investigations of stromule distribution and morphology have been carried out in a range of plants. GFP has been observed in stromules in petunia, tobacco, arabidopsis, wheat, onion, rice, *Commelina communis*, and tomato (Table 1). Stromules have been observed in all plants examined for their presence, and there seems to be no reason to believe that they are not a normal feature of all plant species.

Most studies of stromules have described their presence and morphology in only a relatively restricted set of organs or tissues. An exception was the study of Köhler and Hanson (2000) who examined a wide range of tissue and cell types in transgenic tobacco containing plastid-targeted GFP for the presence of stromules. This produced important information on the distribution of stromules in tobacco plants. Unfortunately, the presence of stromules in some tissues or cell types was not investigated, or stromules could not be detected due to low levels of GFP fluorescence (Köhler and Hanson, 2000). An attempt has been made to carry out a comprehensive investigation of the distribution of stromules in transgenic tobacco plants containing either a nuclear transgene encoding yellow fluorescent protein (YFP) fused to the RbcS transit peptide under the control of the CaMV 35S promoter, or a plastid transgene encoding GFP under the control of the tobacco *rrn* promoter (Newell *et al.*, 2003). The plastid-located *rrn-gfp* construct produced GFP fluorescence in all parts of the plants except the roots where the GFP signal was low. By contrast, the nuclear transformants containing the CaMV 35S *RbcS-yfp* construct showed bright fluorescence in all parts of the plant, and were used specifically to examine plastids in root tissues.

Stromules in tobacco seedlings

Stromules were present in the epidermal cells of the plumule and radicle on emergence from the testa; clumps of plastids interconnected with stromules 8–10 µm long

were especially prevalent in the root epidermis. In the shoot meristem, small proplastids, 1–2 µm in diameter, were arranged in a network of interconnecting stromules (Fig. 2A). In the cotyledons, stromules were prevalent in the epidermal cells, where chloroplasts were closely packed together and appeared linked by stromules (Fig. 2B). Stromules have been observed in guard cells of cotyledons (Kwok and Hanson, 2004c), although stromules were rarely seen on the large chloroplasts in the mesophyll cells of the cotyledons (Kwok and Hanson, 2004c). In the petioles of cotyledons, plastids were clustered around the nucleus with long stromules of 20–30 µm extending to the cell periphery (Kwok and Hanson, 2004c, d).

In the hypocotyl region of 7-d-old tobacco seedlings, extensive networks of interconnected stromules were observed in epidermal cells (Fig. 2C). This has become a favoured region of young seedlings for observation of stromule morphology and behaviour (Kwok and Hanson, 2003, 2004b, d; Waters *et al.*, 2004). In the lower part of the hypocotyl region, the plastids were preferentially arranged around the nucleus and long stromules of up to 100 µm extend to the cell periphery (Fig. 2C). Numerous long stromules were observed throughout the cells of the root, including root hairs, of young tobacco seedlings.

Stromules have been observed on etioplasts in epidermal cells and guard cells of cotyledons of dark-grown seedlings (Shiina *et al.*, 2000). Stromules were also observed on almost all the plastids in hypocotyl epidermal cells in dark-grown tobacco seedlings (Kwok and Hanson, 2004b, d). Waters *et al.* (2004) have shown that stromule number is inversely proportional to plastid density in hypocotyl epidermal cells at various stages of development. In general, stromules were more abundant in cell types present at the seedling stage compared with similar cell types in mature plants.

Stromules in mature tobacco plants

Although stromules were observed in all cell types of mature tobacco plants, there was wide variation between cell types in stromule morphology and in the proportion of plastids with stromules. In general, stromules were abundant in tissues that contain chlorophyll-free plastids, such as petals and roots, but were much less abundant in tissues containing chloroplasts (Köhler and Hanson, 2000).

Leaves and stems: Stromules tend to be relatively rare in cell types such as mesophyll or guard cells that contain large chloroplasts. Stromules were usually seen as beak-like projections on the closely packed chloroplasts of spongy and palisade mesophyll, although longer stromules have been observed occasionally (Köhler *et al.*, 1997; Shiina *et al.*, 2000; Köhler and Hanson, 2000). Leaf epidermal cells, with smaller chloroplasts, often contained long stromules interconnecting several chloroplasts (Arimura *et al.*, 2001). Fewer stromules were seen in guard cells

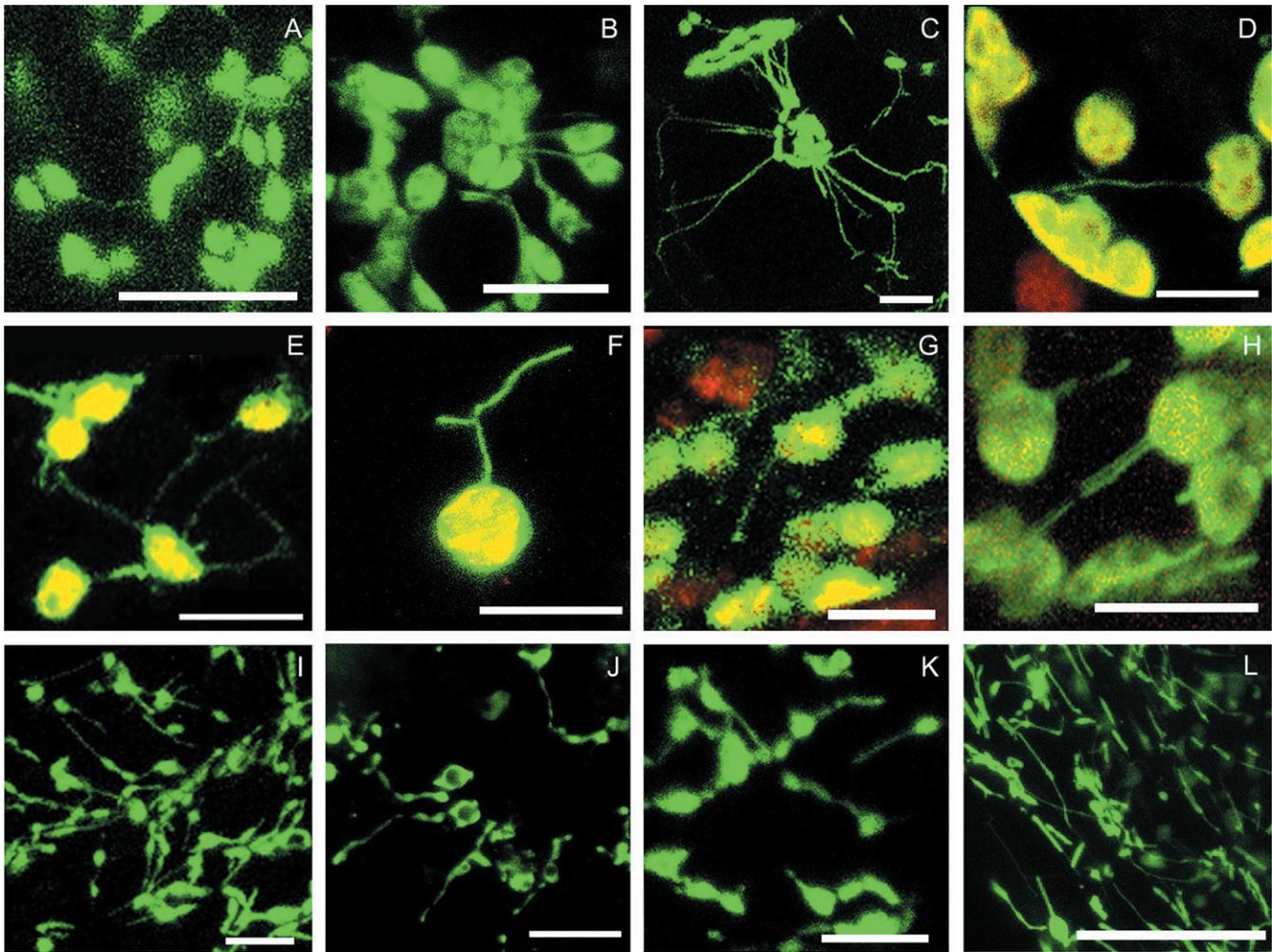


Fig. 2. Stromules on plastids in different tissues of transgenic tobacco plants. Confocal images of GFP or YFP in plastids of plants containing either stromal GFP expressed from *gfp* integrated in the plastid genome (A–H, J, K) or inner-envelope-located YFP expressed from a nuclear gene (I, L). (A) Proplastids in a shoot meristematic cell of a 7-d-old seedling; (B) chloroplasts in a cotyledon epidermal cell of a 7-d-old seedling; (C) leucoplasts in a hypocotyl epidermal cell of a 7-d-old seedling; (D) chloroplasts in a stomatal guard cell of a mature leaf; (E) chloroplasts in the basal cell of a leaf trichome; (F) branched stromule on a vascular cortical cell of a mature leaf; (G) chloroplasts in a bundle sheath cell in the vascular region of a mature leaf; (H) chloroplasts in a stem epidermal cell; (I) leucoplasts in a root epidermal cell; (J) chloroplasts in a placental cell in a developing seed pod; (K) chromoplasts in a nectary cell; (L) chromoplasts in a hypocotyl epidermal cell of a 7-d-old seedling treated with CPTA. All scale bars, 10 μ m.

(Shiina *et al.*, 2000; Köhler and Hanson, 2000), but occasionally stromules up to 20 μ m long interconnecting chloroplasts were observed (Fig. 2D). Leaf trichomes were a favoured subject for cinephotomicroscopic investigation of stromules (Wildman *et al.*, 1962; Hongladarom *et al.*, 1964). The basal cells of the trichome contained a complex network of stromules (Fig. 2E), whereas the apical cells contained numerous linear stromules. Long branched stromules were seen in the vascular cortical cells (Fig. 2F), where chloroplasts were relatively few in number and most showed stromules. In bundle sheath cells surrounding the vascular tissue, stromules up to 10 μ m in length interconnecting adjacent chloroplasts were observed; small beak-like projections were also present on most bundle sheath chloroplasts (Fig. 2G). In stem epidermal cells, some chloroplasts were interconnected by long stromules, but

most chloroplasts showed small beak-like stromules (Fig. 2H). Stem parenchyma cells contained more plastids than epidermal cells, but showed only occasional short stromules on the plastids.

Roots: Stromules in tobacco roots have been well documented (Köhler and Hanson, 2000). They were present in all cell types in different parts of the root, although they differed in appearance, length, and intracellular distribution depending on the cell type and the stage of development (Köhler and Hanson, 2000). In general, plastids in root cells, including cells of the epidermis, root tip, root cap, root hair, and the vascular tissues, contained more stromules than plastids in other parts of the plant. Cells of the root cap contained stromules approximately twice the length of the plastid body on most plastids. Root epidermal

cells had more stromules than cells in other parts of root. Multiple stromules protruded from most plastids in epidermal cells and formed a complex network (Fig. 2I). Root hair cells contained fewer plastids than other root cells, but most plastids in root hairs showed stromules that interconnected with adjacent plastids. The root vascular parenchyma had long stromules up to 40 μm on their leucoplasts.

Flowers and fruit: In tobacco flowers, stromules in the epidermal and guard cells of the sepals were similar to those in the equivalent leaf cells. Few stromules were detectable in the mesophyll cells of the sepals. Cells in the white and pink regions of the flower petals showed a complex network of stromules (Kwok and Hanson, 2004b, c), resembling the distribution seen in the hypocotyl epidermal cells of seedlings (Fig. 2C). Plastids in petal cells were often arranged in a circular network around the nucleus and stromules extended out to interconnect with peripheral plastids (Kwok and Hanson, 2004b, c). In the bright orange nectaries, long stromules interconnecting the chromoplasts were frequently observed (Fig. 2J). Plastids in epidermal cells of the filament of the stamen and in the cells of anther walls contained beak-like projections and longer branched stromules interconnecting plastids. Hair-like structures arising from the anther walls were also found to contain long interconnecting networks of stromules. Stromules were also observed in epidermal cells of the style and the stigma. Placental cells of the ovaries nurturing developing seeds also contained stromules interconnecting plastids (Fig. 2K). The developing seeds contained plastids reminiscent of amyloplasts in cereal endosperm (Langeveld *et al.*, 2000) with stromules extending from plastid bodies.

Stromules on different plastid types

Stromules have been recorded on all major plastid types, including proplastids, chloroplasts, etioplasts, leucoplasts, amyloplasts, and chromoplasts. Stromules have been observed on proplastids in both the shoot and root meristems of tobacco seedlings (Köhler and Hanson, 2000; Fig. 2A), although the proplastids appear to have different morphologies. In the shoot meristem, the proplastids are part of a network apparently interconnected by short stromules (Fig. 2A), whereas the proplastids cluster around the nucleus with long stromules extending toward the cell periphery in the root meristem (Köhler and Hanson, 2000).

The presence of stromules on chloroplasts is inversely correlated with the size and number of chloroplasts in the cell. Stromules are more abundant in cells with few, widely distributed chloroplasts, such as epidermal pavement cells and the basal cells of trichomes in tobacco (Köhler and Hanson, 2000; Vitha *et al.*, 2001), rather than in leaf mesophyll cells and guard cells, where stromules are rarely observed on the large closely packed chloroplasts. A careful quantitative analysis has established a clear negative

correlation of stromule length and chloroplast density in epidermal cells of tobacco hypocotyls (Waters *et al.*, 2004).

Leucoplasts are non-green plastids located in roots and non-photosynthetic tissues of plants. They may become specialized for bulk storage of starch, lipid or protein and are then known as amyloplasts, elaioplasts, or proteinoplasts (Schnepf, 1980). However, in many cell types, leucoplasts do not have a major storage function and are present to provide a wide range of essential biosynthetic functions, including the synthesis of fatty acids, many amino acids, and tetrapyrrole compounds such as haem. In general, leucoplasts are much smaller than chloroplasts and have a variable morphology, often described as amoeboid. Extensive networks of stromules interconnecting leucoplasts have been observed in epidermal cells of roots, hypocotyls and petals, and in callus and suspension culture cells of tobacco (Köhler and Hanson, 2000). In some cell types at certain stages of development, leucoplasts are clustered around the nucleus with stromules extending to the cell periphery, as observed for proplastids in the root meristem (Köhler and Hanson, 2000). Amyloplasts in wheat endosperm show extensive stromules that are the site of B- and C-type starch granule formation (Buttrose, 1963; Parker, 1985; Langeveld *et al.*, 2000; Bechtel and Wilson, 2003). The presence of stromules on elaioplasts and proteinoplasts requires further study. Faull (1935) described elaioplasts in rhizomes of *Iris* species, and found that elaioplasts were pleiomorphic in the meristematic region of the rhizome. Later, the elaioplasts developed into more spherical organelles, some containing starch granules, but the presence of stromules on mature elaioplasts was not mentioned (Faull, 1935). However, Faull (1935) clearly described tubular protrusions from plastids in root tips of *Iris pallida* indicating that she was familiar with this feature of plastid morphology in other tissues.

Stromules on chromoplasts in tomato fruit tissue have been extensively documented (Pyke and Howells, 2002; Waters *et al.*, 2004). Stromules are more abundant and much longer in the inner mesocarp cells, which contain fewer plastids than the outer mesocarp cells. Stromules up to 220 μm long were observed in inner mesocarp cells (Waters *et al.*, 2004). Stromules on chromoplasts have also been observed in tobacco nectaries (Fig. 2J), the only tissue in tobacco normally containing chromoplasts. However, treatment of tobacco seedlings with CPTA [2-(4-chlorophenyl) thioether) triethylamine], an inhibitor of methyl-ketone carotenoid synthesis (Yokoyama *et al.*, 1971), is able to promote the accumulation of lycopene and the development of chromoplasts (JA Sullivan and JC Gray, unpublished data). The chromoplasts in CPTA-treated tobacco seedlings show abundant stromules (Fig. 2L).

With the exception of elaioplasts and proteinoplasts, which have not been well studied, all plastid types have been clearly shown to produce stromules. Stromules must,

therefore, be regarded as a natural feature of plastid morphology. However, stromule morphology and occurrence are extremely variable between cell types and at different stages of development within a single cell type. It is not yet possible to define the underlying basis for these differences.

Developmental changes in stromule morphology

Changes in stromule number and morphology in specific cell types have been observed during plant development in several different plants, including tobacco, tomato, maize, and wheat. Köhler and Hanson (2000) have previously documented the changes in plastid and stromule morphology along the length of individual tobacco roots. Alterations in stromule morphology during four other developmental changes are outlined below.

Tobacco hypocotyl epidermal cells

The epidermal cells of hypocotyls of tobacco seedlings provide an easily accessible tissue for studies of stromules (Kwok and Hanson, 2003, 2004b, d; Waters *et al.*, 2004). During seedling development, the hypocotyl epidermal cells elongate and this is accompanied by changes in plastid and stromule morphology and distribution. Stromules are abundant in all epidermal cells and essentially all plastids have stromules extending in the direction of cell elongation, parallel to the lateral cell walls, probably corresponding to the orientation of microfilament strands. In 6-d-old seedlings the mean length of stromules is 6 μm , and this increases as the cells elongate, such that the average stromule length is 14 μm in hypocotyl epidermal cells of 8-d-old seedlings. Waters *et al.* (2004) have shown a strong negative correlation between stromule length and plastid density. The position within the hypocotyl also appears to influence morphology of plastids and stromules in epidermal cells. Near the junction with the cotyledons, where the hypocotyl cells are shorter, the plastids are often clustered around the nucleus and stromules extend laterally to the cell periphery, as well as parallel to the long dimension of the cell. Light also appears to influence stromule morphology with fewer long stromules in hypocotyl epidermal cells of light-grown seedlings than in dark-grown seedlings (Kwok and Hanson, 2004b, d).

Tomato fruit mesocarp

Waters *et al.* (2004) have examined stromule frequency and length on plastids in the mesocarp of ripening tomato fruits. In full-sized unripe green fruit, the chloroplasts in the outer mesocarp cells are small with relatively short stromules (mean length $\sim 6 \mu\text{m}$), whereas the chloroplasts in the inner mesocarp are much larger, contain prominent starch grains and have much longer stromules (mean length $\sim 14 \mu\text{m}$). In ripening fruit, chloroplasts develop into chromoplasts and there are large changes in stromule number and morphology,

particularly in the inner mesocarp cells. Stromules up to 220 μm have been observed in inner mesocarp cells 3 d after ripening starts; the average stromule length is $\sim 38 \mu\text{m}$ and many chromoplasts have more than one stromule. Increased stromule length correlates with the transition from chloroplast to chromoplast in inner mesocarp cells. By contrast, there is little change in the length of stromules in outer mesocarp cells during ripening. However, fruit development in the dark results in an increased frequency of stromules on plastids in outer mesocarp cells, but no significant change in frequency in inner mesocarp cells. In fruit of the *ripening inhibitor (rin)* mutant, chloroplasts lose chlorophyll but do not accumulate lycopene and do not develop into chromoplasts. The differences between the plastids in the outer and inner mesocarp largely disappear and long stromules are not present. The mean length of the stromules is about 2.5 μm , with the longest stromules about 5 μm , and these often form loops, where both ends of the stromule appears to be attached to the same plastid body, as also observed in tomato trichomes (Pyke and Howells, 2002; Gunning, 2004a). These dramatic effects on stromule morphology are suggested to be an indirect result of a block in plastid development (Waters *et al.*, 2004).

Arbuscule development in roots

The colonization of plant roots by symbiotic arbuscular mycorrhizal fungi leads to the formation of the arbuscule, an intracellular symbiotic interface between the host plant and the fungus. Enormous changes in plastid morphology occur in root cortical cells of tobacco and maize on inoculation with *Glomus intraradices* (Fester *et al.*, 2001; Hans *et al.*, 2004). In uninfected cells, plastids tend to be localized around the nucleus with long stromules (up to 25 μm) extending into the cytosol. On infection, plastid number increases and a dense extended network of plastids with interconnecting stromules forms around the fungal arbuscule. Unusually long curved plastids, curled around the arbuscular branches, had previously been observed by electron microscopy (Dexheimer *et al.*, 1990). It is assumed that the close proximity of the plastids and arbuscules is necessary for a functioning symbiosis, most probably for the transfer of the numerous products of plastid metabolism (Fester *et al.*, 2001; Hans *et al.*, 2004).

Amyloplast development in cereal endosperm

All starch is synthesized in plastids. Starch granules of several size classes are found in the endosperm of cereals such as wheat. The large A-type granules are located in the amyloplast body, whereas smaller B-type granules are located initially in membrane-bounded plastid protrusions, which would now be recognized as stromules (Buttrose, 1963; Parker, 1985; Langeveld *et al.*, 2000; Bechtel and Wilson, 2003). Bechtel and Wilson (2003) examined amyloplast formation and starch granule development in

wheat using transmission electron microscopy. They have identified two phases of stromule formation in cells of the developing endosperm. Large A-type granules appear to be initiated in undifferentiated plastids in the coenocytic endosperm tissue at 2–4 d after anthesis. Following cellularization, the formation of additional B-type granules is initiated in stromules protruding from amyloplasts containing A-type granules in the subaleurone layer at 7–8 d after anthesis. Very small starch granules were observed in long stromules that branched and formed a network in the cytosol. These stromules disappeared by 14 d after anthesis and amyloplasts containing single starch granules predominated, suggesting the growth of the B-type granules and the fragmentation of the stromules. Stromules reappeared in the subaleurone cells at 17 d after anthesis, and appear responsible for the initiation of a third round of starch granule formation. Eventually, three size classes of starch granules, each the result of a separate round of initiation, were present in the subaleurone layer of the seed: large A-type granules (>15 µm), medium B-type granules (5–15 µm) and small C-type granules (<5 µm). Similar development of starch granules and stromules was observed in cells of the central endosperm, although the two phases of stromule formation were delayed by several days compared with the subaleurone layer (Bechtel and Wilson, 2003).

These observations on changes in stromule number and morphology during cell development suggest that stromules are intimately involved in a variety of cell and developmental processes.

Movement of stromules

Stromules are extremely dynamic structures (Wildman *et al.*, 1962; Kwok and Hanson, 2003; Gunning, 2004a, b) suggesting the involvement of motility systems for force generation and utilization. Experiments with inhibitors of microfilament- and microtubule-based movement suggested that stromules move along actin microfilaments powered by the ATPase activity of myosin motors (Gray *et al.*, 2001). Close association of stromules and microfilaments has been observed by DIC microscopy (Gunning, 2004a, b; Kwok and Hanson, 2004a) and by fluorescence microscopy of hypocotyl epidermal cells of arabidopsis containing GFP-talin to decorate microfilaments (Kwok and Hanson, 2004a). Disruption of actin microfilaments by treatment with cytochalasin D or latrunculin B resulted in inhibition of stromule movement and decreased stromule length in tobacco and onion epidermal cells (Gray *et al.*, 2001; Kwok and Hanson, 2003). The myosin ATPase inhibitor 2,3-butanedione 2-monoxime also resulted in decreased stromule movement and occasional fragmentation (Fig. 1; Gray *et al.*, 2001).

The involvement of myosin in stromule movement suggests an interaction, direct or indirect, between the

C-terminal tail domain of myosin and the plastid outer envelope. Myosin has been located by immunofluorescence of the surface of chloroplasts from several plants, including *Lemna* and maize (La Claire, 1991; Malec *et al.*, 1996; Wang and Pesacreta, 2004). Detailed analysis with an antibody to a peptide from the tail region of a subclass of maize myosins indicated an interaction of myosin XI with mitochondria and chloroplasts, but not peroxisomes, Golgi or endoplasmic reticulum, in maize leaves (Wang and Pesacreta, 2004). Myosin XI proteins have been implicated in organelle movement in tobacco and arabidopsis (Yokota *et al.*, 2001; Holweg and Nick, 2004). Arabidopsis contains 13 genes encoding myosin XI proteins (Reddy and Day, 2001). These proteins have highly variable C-terminal tail domains, which are proposed to act as cargo-binding domains, suggesting that different proteins may show different binding specificities for different organelles. A gene-knockout of the arabidopsis *MYA2* gene encoding a myosin XI protein resulted in decreased cytoplasmic streaming of organelles tentatively identified as endoplasmic reticulum (Holweg and Nick, 2004). The identification of specific myosin proteins involved in stromule movement would provide a starting point for dissecting the molecular basis of stromule movement.

There is apparently contradictory evidence for the role of microtubules in stromule movement. Gray *et al.* (2001) reported that the microtubule inhibitors oryzalin and amiprothosmethyl had no effect on stromule movement in tobacco and onion epidermal cells, whereas Kwok and Hanson (2003) reported decreased stromule length (to 75% of the starting length) on incubation of tobacco hypocotyl epidermal cells with amiprothosmethyl or oryzalin. Furthermore, these treatments increased plastid mobility by 50%, compared to plastids in untreated seedlings, suggesting that microtubules may normally constrain plastid movement (Kwok and Hanson, 2003). More detailed analysis is necessary to resolve the role of microtubules in stromule and plastid movement.

Functions of stromules

A definitive description of the function of stromules is not yet possible. A number of suggestions of possible functions of stromules have been made based primarily on observations of plastid and stromule morphology, but there have been few experiments designed to examine functional properties. A key observation on stromule function was made using fluorescence recovery after photobleaching (FRAP) experiments on GFP-labelled plastids in petunia, tobacco, and arabidopsis (Köhler *et al.*, 1997, 2000; Tirlapur *et al.*, 1999). These experiments established that GFP, a 27 kDa globular protein, was able to move through stromules interconnecting two different plastids. Similar experiments with GFP-tagged proteins have recently shown

that aspartate aminotransferase (90 kDa) and Rubisco (560 kDa) are able to move through stromules interconnecting plastids (Kwok and Hanson, 2004b). Rubisco had previously been located in short protrusions of rice chloroplasts by immunogold labelling of ultrathin sections produced after high pressure freezing to preserve stromule structure for electron microscopy (Bourett *et al.*, 1999). These observations suggest that large macromolecules are able to enter and move through stromules. Köhler *et al.* (2000) have established that GFP moves through stromules predominantly by diffusion, although a slower directional active transport of batches of GFP also occurred. The presence of beads of GFP distributed evenly along very thin (>0.1 µm diameter) stromules has been observed in tomato trichome and mesocarp cells (Pyke and Howells, 2002). The possible movement of informational macromolecules, such as DNA, RNA, and ribosomes, through stromules has not yet been established.

The movement of macromolecules, and presumably lower molecular mass solutes, between plastids cannot be the primary function of stromules, because most stromules do not appear to interconnect plastids. The presence of GFP and other macromolecules in stromules may just be a consequence of the presence of stroma in the stromules. Freely diffusing solutes in the stroma may be able to move into the stromules and continue with their physiological functions. This could account for the initiation of starch granule formation in stromules in developing wheat endosperm (Buttrose, 1963; Parker, 1985; Langeveld *et al.*, 2000; Bechtel and Wilson, 2003). The large A-type starch granules occupy a large proportion of the amyloplast body and may physically displace the stroma into an extensive network of stromules, which provides the location for the next round of starch granule initiation. The idea that stromules are a normal site of plastid metabolism is supported by the observation that the long-chain acyl-CoA synthase LACS9 is located in stromule membranes (Schnurr *et al.*, 2002). It might therefore be expected that stromules contain all the normal metabolic activities of plastids, with the exception of those associated with the thylakoid membrane, which appears not to be present in stromules (Wildman *et al.*, 1962; Köhler *et al.*, 1997).

The presence of stromules provides a much larger surface area for the transport of metabolic intermediates into and out of the plastids. Metabolic processes in plastids are highly integrated with reactions taking place in the cytosol, mitochondria, peroxisomes, and endoplasmic reticulum. A close association of stromules with mitochondria has been observed by DIC microscopy in sub-epidermal cells of petals of *Iris unguicularis* (Gunning, 2004a, b) and by fluorescence microscopy with GFP-labelled plastids and mitochondria in tobacco (Kwok and Hanson, 2004a).

The pre-eminent role for stromules appears to be as a means of increasing the surface area of the plastid to enhance interactions with the surrounding cytosol and other

organelles. There is a marked inverse relationship between the size and number of plastids in a cell and the frequency and length of stromules (Köhler and Hanson, 2000; Waters *et al.*, 2004). Cells with large closely packed plastids, such as leaf mesophyll cells, appear to have relatively few short stromules. By contrast, cells with fewer and smaller plastids, such as in roots, in general show larger numbers of longer stromules. The formation of stromules provides a means of increasing the plastid surface area with only relatively small changes in volume. Measurements of a long stromule interconnecting two plastids in an arabidopsis leaf epidermal cell indicated that the stromule accounted for 50% of the plastid surface area, but only 15% of the plastid volume (Gray *et al.*, 2001). Similarly, Gunning (2004b) has calculated that the surface area of a 25 µm-long stromule is equivalent to about 35% of the surface area of an iris petal chloroplast. Stromule formation therefore provides the prospect of exchanging metabolites or signalling molecules over a much larger area. Metabolite exchange has been suggested as the reason for the large network of stromules associated with arbuscules in roots infected with mycorrhizal fungi (Fester *et al.*, 2001; Hans *et al.*, 2004). This would require that the stromule membranes contain an array of transporters necessary for metabolite exchange. The composition of the stromule membranes is so far unknown, but the ability to separate stromules from the plastid body in tomato fruit tissue (Pyke and Howells, 2002) may enable biochemical characterization of stromules.

A role for stromules in signalling has been suggested on the basis of close associations of stromules with nuclei and the plasma membrane in some cells (Kwok and Hanson, 2004d). Tirilapur *et al.* (1999) suggested that long stromules extending from plastids clustered around the nucleus towards the cell periphery provided a means of conveying electrical events at the plasma membrane to the nucleus. However, the nature of the interaction of stromules with the plasma membrane has not been defined. Similarly, interactions of stromules with nuclei are not well characterized. Kwok and Hanson (2004d) have observed stromules lying in grooves and invaginations of the nuclear membranes in tobacco hypocotyl epidermal cells. This presumably offers the possibility of improved signalling efficiency between plastids and nuclei in these cells. However, it is not clear which signals or molecules might be preferentially exchanged in such an interaction.

The possibility that stromules are merely a consequence of plastid division has been raised, and rejected (Menzel, 1994). The dynamic nature of stromules in many cell types suggests they are not just relict division structures. The presence of long stromules in leaf epidermal cells of the arabidopsis *arc6* mutant (Gray *et al.*, 2001), which has much reduced plastid division resulting in only a few plastids per cell (Pyke *et al.*, 1994; Vitha *et al.*, 2003), suggests that stromule formation is not directly related to plastid division. However, in developing wheat endosperm,

the initiation of starch granules in stromules may provide a means of producing new amyloplasts, because existing amyloplasts containing a single large A-type starch granule appear unable to divide (Bechtel and Wilson, 2003). In this case, the formation of stromules appears to be a key stage in amyloplast formation.

Conclusions

The use of plastid-located GFP has facilitated the description of stromule morphology and behaviour in a limited number of plants. There is now a good knowledge of stromules in different cells and tissues of tobacco, arabidopsis, and tomato, and this has led to suggestions of the possible functions of stromules. However, it is important to move from this essentially descriptive phase of research to experiments designed to understand the underlying molecular mechanisms of stromule behaviour. So far, relatively few experiments have investigated molecular mechanisms. The use of inhibitors has provided information on the movement of GFP through stromules (Köhler *et al.*, 2000) and on stromule movement (Gray *et al.*, 2001; Kwok and Hanson, 2003). However, biochemical and genetic approaches are needed to identify molecules involved in stromule function. It is necessary to define and address the key questions: What is the primary function of stromules? Are they essential for cell viability? How are stromules formed? How do stromules move? The next few years should see a change in direction away from phenomenology towards an understanding of molecular mechanisms.

Acknowledgements

We thank Julian Hibberd and Michael Hansen for helpful discussions, Brian Gunning, Mark Waters, and Kevin Pyke for providing manuscripts before publication, and Christine Newell for the gift of tobacco seeds containing *gfp* in the plastid genome. SKAN was supported by a scholarship from the Gates Cambridge Trust and an ORS award. This work was supported by a research grant from the UK Biotechnology and Biological Sciences Research Council (BBSRC).

References

- Arimura S-I, Hirai A, Tsutsumi N. 2001. Numerous and highly developed tubular projections from plastids observed in tobacco epidermal cells. *Plant Science* **160**, 449–454.
- Bechtel DB, Wilson JD. 2003. Amyloplast formation and starch granule development in hard red winter wheat. *Cereal Chemistry* **80**, 175–183.
- Bourett TM, Czymmek KJ, Howard RJ. 1999. Ultrastructure of chloroplast protuberances in rice leaves preserved by high-pressure freezing. *Planta* **208**, 472–479.
- Buttrose MS. 1963. Ultrastructure of the developing wheat endosperm. *Australian Journal of Biological Science* **16**, 305–317.
- Choi S-B, Kim K-H, Kavakli IH, Lee S-K, Okita TW. 2001. Transcriptional expression characteristics and subcellular local-

- ization of ADP-glucose pyrophosphorylase in the oil plant *Perilla frutescens*. *Plant and Cell Physiology* **42**, 146–153.
- Dexheimer J, Gérard J, Bougarda K, Jeanmaire C. 1990. Les plastides des cellules-hôtes des mycorrhizes à vésicules et arbuscules. *Canadian Journal of Botany* **68**, 50–55.
- Escobar NM, Haupt S, Thow G, Boevink P, Chapman S, Oparka K. 2003. High-throughput viral expression of cDNA-green fluorescent protein fusions reveals novel subcellular addresses and identifies unique proteins that interact with plasmodesmata. *The Plant Cell* **15**, 1507–1523.
- Faull AF. 1935. Elaioplasts in iris: a morphological study. *Journal of the Arnold Arboretum* **16**, 225–267.
- Fester T, Strack D, Hause B. 2001. Reorganization of tobacco root plastids during arbuscule development. *Planta* **213**, 864–868.
- Fujiwara M, Yoshida S. 2001. Chloroplast targeting of chloroplast division FtsZ2 protein in *Arabidopsis*. *Biochemical and Biophysical Research Communications* **287**, 462–467.
- Giglione C, Serero A, Pierre M, Biosson B, Meinel T. 2000. Identification of eukaryotic peptide deformylases reveals universality of N-terminal protein processing mechanisms. *EMBO Journal* **19**, 5916–5929.
- Gray JC, Hibberd JM, Linley PJ, Uijtewaal B. 1999. GFP movement between chloroplasts. *Nature Biotechnology* **17**, 1146.
- Gray JC, Sullivan JA, Hibberd JM, Hansen MR. 2001. Stromules: mobile protrusions and interconnections between plastids. *Plant Biology* **3**, 223–233.
- Guilliermond A. 1934. *Les constituants morphologiques du cytoplasme: le chondriome*. Paris: Hermann.
- Gunning BES. 2004a. Plant cell biology on CD. <http://www.plantcellbiologyonCD.com>
- Gunning BES. 2004b. Plastid stromules: video microscopy of their outgrowth, retraction, tensioning, anchoring, branching, bridging and tip-shedding. *Protoplasma* (in press).
- Hans J, Hause B, Strack D, Walter MH. 2004. Cloning, characterization, and immunolocalization of a mycorrhiza-inducible 1-deoxy-D-xylulose 5-phosphate reductoisomerase in arbuscule-containing cells of maize. *Plant Physiology* **134**, 614–624.
- Helliwell CA, Sullivan JA, Mould RM, Gray JC, Peacock WJ, Dennis ES. 2001. A plastid envelope location of *Arabidopsis* entkaurene oxidase links the plastid and endoplasmic reticulum steps of gibberellin biosynthesis pathway. *The Plant Journal* **28**, 201–208.
- Holweg C, Nick P. 2004. *Arabidopsis* myosin XI mutant is defective in organelle movement and polar auxin transport. *Proceedings of the National Academy of Sciences, USA* **101**, 10488–10493.
- Hongladarom T, Honda S, Wildman SG. 1964. Organelles in living plant cells. Videotape available from University of California Extension Center for Media and Independent Learning, 2000 Center St., Berkeley, CA 94704, USA.
- Knight JS, Gray JC. 1995. The N-terminal hydrophobic region of the mature phosphate translocator is sufficient for targeting to the chloroplast inner envelope membrane. *The Plant Cell* **7**, 1421–1432.
- Köhler RH, Cao J, Zipfel WR, Webb WW, Hanson MR. 1997. Exchange of protein molecules through connections between higher plant plastids. *Science* **276**, 2039–2042.
- Köhler RH, Schwillie P, Webb WW, Hanson MR. 2000. Active protein transport through plastid tubules: velocity quantified by fluorescence correlation spectroscopy. *Journal of Cell Science* **113**, 3921–3930.
- Köhler RH, Hanson MR. 2000. Plastid tubules of higher plants are tissue-specific and developmentally regulated. *Journal of Cell Science* **113**, 81–89.

- Kwok EY, Hanson MR.** 2003. Microfilaments and microtubules control the morphology and movement of non-green plastids and stromules in *Nicotiana tabacum*. *The Plant Journal* **35**, 16–26.
- Kwok EY, Hanson MR.** 2004a. *In vivo* analysis of interactions between GFP-labeled microfilaments and plastid stromules. *BMC Plant Biology* **4**, 2.
- Kwok EY, Hanson MR.** 2004b. GFP-labeled Rubisco and aspartate aminotransferase are present in plastid stromules and traffic between plastids. *Journal of Experimental Botany* **55**, 595–604.
- Kwok EY, Hanson MR.** 2004c. Stromules and the dynamic nature of plastid morphology. *Journal of Microscopy* **214**, 124–137.
- Kwok EY, Hanson MR.** 2004d. Plastids and stromules interact with the nucleus and cell membranes in vascular plants. *Plant Cell Reports* **23**, 188–195.
- La Claire JW.** 1991. Immunolocalization of myosin in intact and wounded cells of the green alga *Ernodesmis verticillata* (Kützinger) Børgesen. *Planta* **184**, 189–195.
- Langeveld SMJ, van Wijk R, Stuurman N, Kijne JW, dePater S.** 2000. B-type granule containing protrusions and interconnections between amyloplasts in developing wheat endosperm revealed by transmission electron microscopy and GFP expression. *Journal of Experimental Botany* **51**, 1357–1361.
- Li H-M, Moore T, Keegstra K.** 1991. Targeting of proteins to the outer envelope membrane uses a different pathway than transport in to chloroplasts. *The Plant Cell* **3**, 709–717.
- Malec P, Rinaldi RA, Gabrys H.** 1996. Light-induced chloroplast movement in *Lemna trisulca*. Identification of the motile system. *Plant Science* **120**, 127–137.
- Menzel D.** 1994. An interconnected plastidom in *Acetabularia*: implications for the mechanism of chloroplast motility. *Protoplasma* **179**, 166–171.
- Millen RS, Olmstead RG, Adams KL, et al.** 2001. Many parallel losses of *infA* from chloroplast DNA during angiosperm evolution with multiple independent transfers to the nucleus. *The Plant Cell* **13**, 645–658.
- Newell CA, Birch-Machin I, Hibberd JM, Gray JC.** 2003. Expression of green fluorescent protein from bacterial and plastid promoters in tobacco chloroplasts. *Transgenic Research* **12**, 631–634.
- Parker ML.** 1985. The relationship between A-type and B-type starch granules in the developing endosperm of wheat. *Journal of Cereal Science* **3**, 271–278.
- Pyke KA, Howells CA.** 2002. Plastid and stromule morphogenesis in tomato. *Annals of Botany* **90**, 559–566.
- Pyke K, Rutherford SM, Robertson EJ, Leech RM.** 1994. *arc6*, a fertile *Arabidopsis* mutant with only two mesophyll cell chloroplasts. *Plant Physiology* **106**, 1169–1177.
- Reddy ASN, Day IS.** 2001. Analysis of the myosins encoded in the recently completed *Arabidopsis thaliana* genome sequence. *Genome Biology* **2**, 1–17.
- Reed ML, Wilson SK, Sutton CA, Hanson MR.** 2001. High-level expression of a synthetic red-shifted GFP coding region incorporated into transgenic chloroplasts. *The Plant Journal* **27**, 257–265.
- Schnepp E.** 1980. Types of plastids: their development and inter-conversions. In: Reinert J, ed. *Chloroplasts*. Berlin: Springer, 1–27.
- Schnurr JA, Shockey JM, deBoer G-J, Browse JA.** 2002. Fatty acid export from the chloroplast. Molecular characterization of a major plastidial acyl-coenzyme A synthetase from *Arabidopsis*. *Plant Physiology* **129**, 1700–1709.
- Shiina T, Hayashi K, Ishii N, Morikawa K, Toyoshima Y.** 2000. Chloroplast tubules visualized in transplastomic plants expressing green fluorescent protein. *Plant and Cell Physiology* **41**, 367–371.
- Tirlapur UK, Dahse I, Reiss B, Meurer J, Oelmüller R.** 1999. Characterization of the activity of a plastid-targeted green fluorescent protein in *Arabidopsis*. *European Journal of Cell Biology* **78**, 233–240.
- Tirlapur UK, König K.** 2001. Femtosecond near-infrared lasers as a novel tool for non-invasive real-time high-resolution time-lapse imaging of chloroplast division in living bundle sheath cells of *Arabidopsis*. *Planta* **214**, 1–10.
- Vitha S, Froehlich JE, Koksharova O, Pyke KA, van Erp H, Osteryoung KW.** 2003. ARC6 is a J-domain plastid division protein and an evolutionary descendant of the cyanobacterial cell division protein Ftn2. *The Plant Cell* **15**, 1918–1933.
- Vitha S, McAndrew RS, Osteryoung KW.** 2001. FtsZ ring formation at the chloroplast division site in plants. *Journal of Cell Biology* **153**, 111–119.
- Wang ZY, Pesacreta TC.** 2004. A subclass of myosin XI is associated with mitochondria, plastids, and the molecular chaperone subunit TCP-1a in maize. *Cell Motility and the Cytoskeleton* **57**, 218–232.
- Waters MT, Fray RG, Pyke KA.** 2004. Stromule formation is dependent upon plastids size, plastid differentiation status and the density of plastids within the cell. *The Plant Journal* **39**, 655–667.
- Wildman SG, Hongladarom T, Honda SI.** 1962. Chloroplasts and mitochondria in living plant cells: cinephotomicrographic studies. *Science* **138**, 434–436.
- Yokota E, Sonobe S, Orii H, Yuasa T, Inada S, Shimmen T.** 2001. The type and the localization of 175 kDa myosin in tobacco cultured cells BY-2. *Journal of Plant Research* **114**, 115–116.
- Yokoyama H, Coggins CW, Henning GL.** 1971. The effect of 2-(4-chlorophenylthioether) triethylamine hydrogen chloride on the formation of carotenoids in citrus. *Phytochemistry* **10**, 1831–1834.