Title

Visualizing the dynamics of plant energy organelles.

Journal

Biochemical Society Transactions, 51(6)

Authors

Koenig, Amanda

Liu, Bo

Hu, Jianping

Publication Date

2023-12-20

DOI

10.1042/BST20221093

Peer reviewed

Biochemical Society Transactions (2023) **51** 2029–2040
https://doi.org/10.1042/BST20221093

Review Article

Visualizing the dynamics of plant energy organelles

Amanda M. Koenig[1], Bo Liu[2] and Jianping Hu[1]

[1]Michigan State University-Department of Energy Plant Research Laboratory, Michigan State University, East Lansing, MI, U.S.A.; [2]Department of Plant Biology, University of California, Davis, CA, U.S.A.

Correspondence: Jianping Hu (huji@msu.edu)

Plant organelles predominantly rely on the actin cytoskeleton and the myosin motors for long-distance trafficking, while using microtubules and the kinesin motors mostly for short-range movement. The distribution and motility of organelles in the plant cell are fundamentally important to robust plant growth and defense. Chloroplasts, mitochondria, and peroxisomes are essential organelles in plants that function independently and coordinately during energy metabolism and other key metabolic processes. In response to developmental and environmental stimuli, these energy organelles modulate their metabolism, morphology, abundance, distribution and motility in the cell to meet the need of the plant. Consistent with their metabolic links in processes like photorespiration and fatty acid mobilization is the frequently observed inter-organellar physical interaction, sometimes through organelle membranous protrusions. The development of various organelle-specific fluorescent protein tags has allowed the simultaneous visualization of organelle movement in living plant cells by confocal microscopy. These energy organelles display an array of morphology and movement patterns and redistribute within the cell in response to changes such as varying light conditions, temperature fluctuations, ROS-inducible treatments, and during pollen tube development and immune response, independently or in association with one another. Although there are more reports on the mechanism of chloroplast movement than that of peroxisomes and mitochondria, our knowledge of how and why these three energy organelles move and distribute in the plant cell is still scarce at the functional and mechanistic level. It is critical to identify factors that control organelle motility coupled with plant growth, development, and stress response.

Introduction

Plant cells are often envisioned as the classic textbook cartoon: static organelles spaced indiscriminately in the cytoplasm within the confines of rigid cell walls. However, organelles are highly dynamic, as their quantities fluctuate via biogenesis, fission, fusion, and degradation and their morphologies shift among a variety of sizes and shapes. Organelles traverse the cell by cytoplasmic streaming and organelle-specific, targeted motility along the cytoskeleton, driven by molecular motors that travel along the tracks of the cytoskeletal filaments.

Eukaryotic cells employ the cytoskeletal motors of kinesins, dyneins, and myosins that hydrolyze ATP to propel organelle transport directionally along polarized microtubule and actin tracks, whose orientation dictates the direction of travel. Active motor-mediated movement can trigger other modes of transportation, including cytoplasmic streaming and 'hitchhiking', in which organelles are transported indirectly by associating with cargoes already being carried by motors [1].

In animal cells, the microtubule-based cytoskeleton, along with kinesin and dynein motors, are predominantly responsible for vesicle and organelle trafficking [2–4]. In contrast, actin filaments comprise the major intracellular highway system in plants for long-range organelle trafficking, driven primarily by the class XI myosin proteins [5–9]. Plant microtubules and their associated motors, kinesins, mainly direct short-range organelle movement, pauses, and orientation in coordination with the actin cytoskeleton [10–14]. Additionally, the endoplasmic reticulum (ER) is intricately involved in

Received: 28 September 2023
Revised: 6 November 2023
Accepted: 8 November 2023

Version of Record published:
17 November 2023

Biochemical Society Transactions (2023) **51** 2029–2040
https://doi.org/10.1042/BST20221093

motor-assisted organelle motility. For example, the ER is associated with the dynamic cytoskeletal network through tethering and anchoring mechanisms, most especially at ER-plasma membrane contact sites [6,15]. Furthermore, proximity and interactions among organelles correlate with fluctuations in ER morphology, which suggests that the ER mediates the positioning and movements of organelles, including chloroplasts, mitochondria and peroxisomes [16,17].

A number of studies testing organelle motility in *Arabidopsis* myosin mutants and myosin localization in plant cells indicate functional redundancy in the Myosin XI protein family and targeting of the same myosin motor to multiple types of organelles [5,18–22]. The apparent promiscuity in the interactions between myosin motors and organelles beckons for regulatory mechanisms of organelle movement that is possibly governed by organelle-specific receptors/adaptors, which recruit designated Myosin XI motors [23].

These molecular motors and their associated cytoskeleton facilitate the movement and dynamics of the nucleus and endomembrane system, including ER, Golgi, and vesicles, which have been expertly reported in several recent papers [24–27]. In this review, we focus on the motility of plant energy organelles — chloroplasts, mitochondria, and peroxisomes — which work independently as well as in concert during energy metabolism. These organelles modulate not only their metabolism and morphology but also their distribution and motility in accordance with developmental stages and environmental conditions. Here, we introduce the collaborative nature of these three energy organelles in photosynthetic organisms and methods used to study their motilities and discuss the factors that trigger and modulate their movement and distribution. We apologize to researchers whose publications cannot be cited due to space limitation.

Organelle functions

Chloroplasts, enclosed by double envelopes and containing their own genome, house the photosynthetic machinery that performs light capture and carbon assimilation, along with many other vital cellular functions beyond direct energy production, such as the biosynthesis of lipids, phytohormones and other key molecules [28–31]. Mitochondria are double-membraned, DNA-containing organelles whose primary role is cellular respiration while also performing plant-specific functions such as malate oxidation and photorespiration [32]. Peroxisomes are single-membraned and DNA-less organelles involved in fatty acid degradation and detoxification of reactive oxygen species, along with plant-specific functions such as phytohormone production and photorespiration [33–35].

Several of the above-mentioned processes require the collaboration of chloroplasts, mitochondria, and/or peroxisomes [36–38]. Namely, the photorespiratory pathway requires these three organelles to recover misassimilated carbon produced due to the oxygenase activity of the photosynthetic enzyme Rubisco, protecting the plant cell from toxic byproducts [39,40]. Besides being essential to the survival of organisms performing oxygenic photosynthesis in ambient air, photorespiration also has a demonstrated role in immunity [41]. In addition, during the metabolism of fatty acids, triacylglycerol stored in oil bodies can be degraded in peroxisomes via β-oxidation, after which the glyoxylate cycle, also housed in peroxisomes, produces metabolites that are exported to mitochondria for the tricarboxylic acid (TCA) cycle to ultimately release the stored energy [33,42]. Moreover, lipid transfer can occur directly between chloroplasts and mitochondria during phosphate starvation [43]. As a final example, jasmonate (JA) biosynthesis requires chloroplasts to produce the precursor 12-oxo-phytodienoic acid (OPDA), which is then imported into the peroxisome to be converted to JA via β-oxidation [33,44,45].

Consistent with their metabolic connections, these organelles are often observed to be in close physical proximity. Mitochondrial associations with chloroplasts increase under high light in the diatom *Phaedactylum*, as well as during mixotrophy (compared with phototrophy) in the alga *Nannochloropsis* [46]. In *Arabidopsis*, the proximity area between these organelles expands in light compared with dark [47]. Chloroplast-peroxisome-mitochondrion complexes are formed more frequently in light than in dark, and light-induced triple organelle interaction is suggested to be regulated by photosynthesis and facilitated by ER-chloroplast nexuses [47–49]. ER reorganization is also proposed to contribute to the formation of tubular protrusions from chloroplasts, peroxisomes, and mitochondria, termed stromules, peroxules, and matrixules, respectively [50]. During high light irradiation, small mitochondria cluster around peroxules [49]. While proximity among all three energy organelles increases in mesophyll in light [47,48] and stromule formation is more frequent in light-treated epidermal pavement cells (Figure 1A), similar numbers of mitochondria and peroxisomes interact with both the chloroplast body and stromules in these pavement cells [51]. These organelle protrusions very likely provide a platform for inter-organellar interaction and metabolite exchange among the organelles.

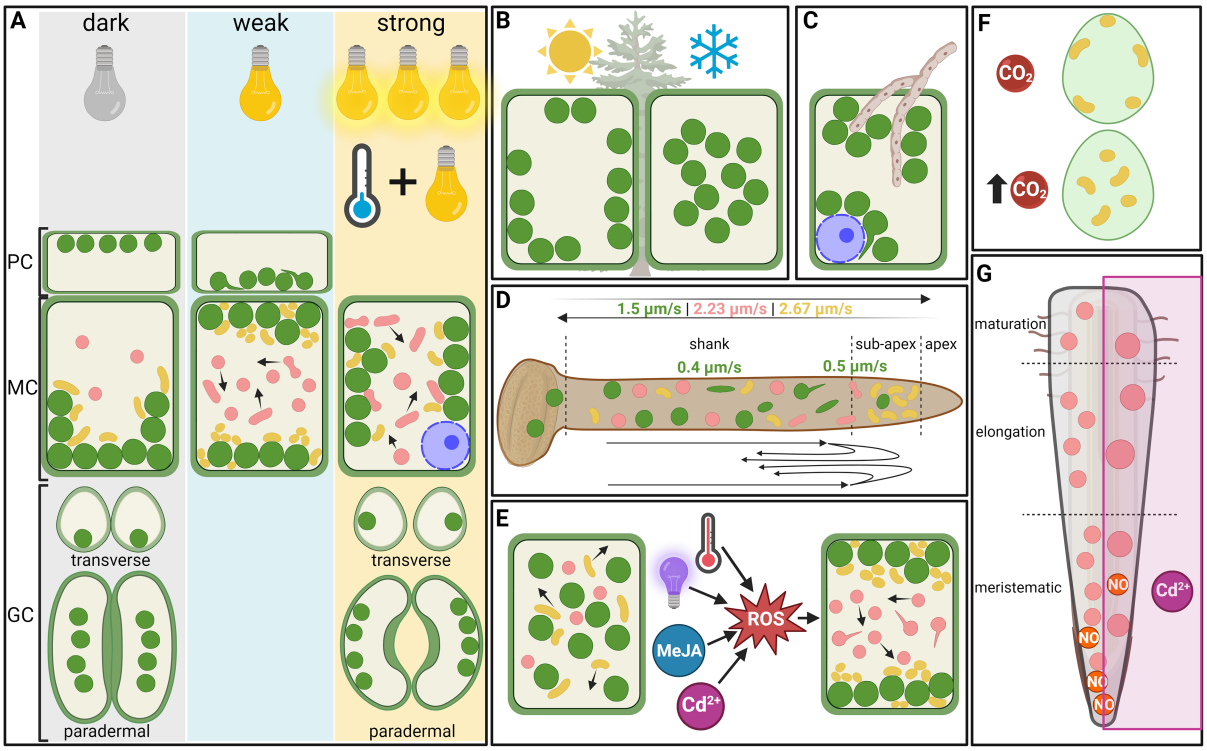

Figure 1. Examples of energy organelle dynamics in photosynthetic organisms during environmental and developmental changes.

Changes in the distribution and movement of chloroplasts/plastids (green), mitochondria (yellow), and peroxisomes (pink) are shown. (**A**) Chloroplasts and mitochondria exhibit photorelocation and cold positioning in *Arabidopsis* and tobacco pavement cells (PC), mesophyll cells (MC), and guard cells (GC). Peroxisome proliferation and motility increase in light. Light-induced morphological changes include mitochondrial aggregation, peroxisome elongation, and chloroplast stromule formation. (**B**) Seasonal rearrangement of chloroplasts occurs in conifer species. (**C**) Chloroplasts redistribute during pathogen infection and immune response in *Arabidopsis* and tobacco leaves. (**D**) The three energy organelles are differentially distributed and vary their motility during pollen tube development in flowering plants. (**E**) Dynamics of the three energy organelles shift in response to reactive oxygen species (ROS) in *Arabidopsis* leaves. Mitochondria slow and aggregate while peroxisomes speed up and form peroxules. (**F**) Mitochondria slow down, redistribute under fluctuating carbon dioxide (CO_2) levels in Chlamydomonas. (**G**) Peroxisomes are more concentrated in the meristematic zone in root tips and their morphology is altered in response to cadmium (Cd^{2+}) toxins in roots. Arrows in (**A**), (**D**) and (**E**) represent general motility patterns. MeJA, methyl jasmonate; NO, nitric oxide. The nucleus (blue) is only depicted when its interaction with energy organelles is involved. Created with BioRender.com.

Methods for organelle and cytoskeletal visualization

Chloroplasts, due to their superiorly large size and chlorophyll autofluorescence, can be readily visualized through light and fluorescence microscopy [52].

In early studies, detections of peroxisomes and mitochondria were largely dependent on organelle-specific enzymes or distinct properties of their membranes. For example, using fluorescence microscopy, plant peroxisomes were detected by immunofluorescence using antibodies against catalase [53] while mitochondria were probed by dyes like rhodamine 123 that binds to P-glycoproteins [54,55]. Due to disadvantages associated with rhodamine 123 like challenges of sample washes and photostability, it has largely been replaced by synthetic hydrophobic dyes commonly known as MitoTrackers, which bind to mitochondrial membranes and can be conjugated with different fluorophores [56] to track mitochondria in living plant cells [57].

Lately, fluorescent protein (FP) tags introduced by recombinant DNA techniques have been widely adopted to monitor the dynamic behavior of peroxisomes and mitochondria, when either stable transformation or

Biochemical Society Transactions (2023) **51** 2029–2040
https://doi.org/10.1042/BST20221093

transient expression is applicable in the host cells. Besides the founding green fluorescent protein (GFP), numerous FPs may be chosen nowadays for their features of color, brightness, quantum yield, maturation property, pKa, and lifetime, which are frequently updated by the research community (https://www.fpbase.org/). When covalently attached to proteins that are unique to the respective organelle at either the organelle membranes or lumens after translation, these FPs faithfully report the intracellular positions of the organelles by fluorescence microscopy. Moreover, organelle and cytoskeletal FP marker collections have expanded beyond model plants and are now optimized for uses in crop plants like maize [58], rice [59,60], and barley [61]. Several excellent articles have summarized typical examples [62–64].

To label peroxisomes, a 3-amino acid canonical peroxisomal targeting signal 1 (PTS1) sequence, such as Ser-Lys-Leu (SKL) or Ser-Arg-Leu (SRL), is usually translationally conjugated to the C-terminus of an FP [63,65]. Peroxisomes can also be illuminated using the fusion of the N-terminus of a peroxisome matrix protein containing the PTS2 nonapeptide, such as that from the 3-ketoacyl-CoA thiolase or the glyoxysomal malate dehydrogenase, and the FP [66]. When an FP-PTS1 or PTS2-FP fusion protein is expressed under constitutively active promoters like the viral 35S promoter, it is in high abundance so that peroxisomes are brightly labeled for convenient observation by confocal microscopy.

Similarly, mitochondria can be labeled by attaching their targeting sequence at the N-terminus of FPs. A widely used targeting sequence in plants is a 29-amino acid peptide derived from the yeast cytochrome *c* oxidase IV (COX4) [63]. Mitochondrial-specific proteins like the γ-subunit of F1-ATPase serve the purpose equally well in plant cells [64]. Although they require transcription and translation in host cells, such FP-based probes are often favored over lipophilic dyes because of their organelle specificity.

The motility as well as polarized distribution of plant organelles including peroxisomes and mitochondria is dependent primarily on actin microfilaments and employs the myosin motors [18,53]. Live-cell imaging of actin filaments has evolved from the application of the fluorescent dye-conjugated fungal toxin of phalloidin to FP-tagged polypeptides of various actin-binding proteins [67,68]. Earlier adoption of the actin-binding protein talin often resulted in the formation of thick actin bundles [68]. As a replacement, ABD2, an actin-binding domain of the *Arabidopsis* fimbrin 1 protein, or the 17-amino acid Lifeact peptide derived from the yeast actin-binding protein Abp140 [69], is often used these days, both of which can significantly reduce the bundling and aggregation of actin microfilaments.

The ever-growing diversity of FPs and highly sophisticated microscopes with exceptional resolution enable synchronous visualization of multi-organellar and cytoskeletal topology in living cells. For example, peroxisomes, mitochondria, and actin labeled with multicolored FP-fusion biomarkers as described above, along with autofluorescent chloroplasts, can be monitored simultaneously in living tobacco epidermal cells (Figure 2). This concurrent visualization enables direct comparisons of organelles in the context of one another. Moreover, intricate visualization techniques have vast potential to further illuminate organelle dynamics and motility by rapid capture of organelles in action (Supplementary Movie 1).

FP-tagged probes are also used to detect the dynamic microtubule network in plant cells. The microtubule-binding domain (MBD) derived from the mammalian microtubule-associated protein 4 (MAP4) decorates cortical microtubules in fava bean epidermal cells upon transient expression [70]. This GFP-MBD renders superior brightness, possibly resulted from induced microtubule bundling. Alternatively, an MBD derived from the atypical casein kinase CKL6 has also been used to mark microtubules in living plant cells [71]. To minimize the potential impact of ectopically expressing an MBD in plant cells, one wishes to fuse FP with tubulins so that microtubules are labeled when the FP-tubulin fusion is incorporated into polymerized microtubules, which often requires stable transformation as demonstrated first by a GFP-α-tubulin in *Arabidopsis* [72]. The β-tubulin isoform *TUB6* was later chosen because of its minimal toxicity after ectopic expression [73]. Stable expression of TUB6 under its native promoter in *Arabidopsis* results in neglectable disturbance to the dynamic remodeling of microtubule arrays and plant growth [74].

Progressively advanced, specific, and tunable visualization techniques equip researchers to uncover the nuances of energy organelle dynamics and expand our current knowledge of chloroplastic/plastidial, mitochondrial, and peroxisomal distribution and motility.

Chloroplasts

As light-harvesting apparatus, chloroplasts optimize photon capture by shifting their positions in the cell, a process termed photorelocation [75–78] (Figure 1A). In the dark, chloroplasts are distributed along the bottom

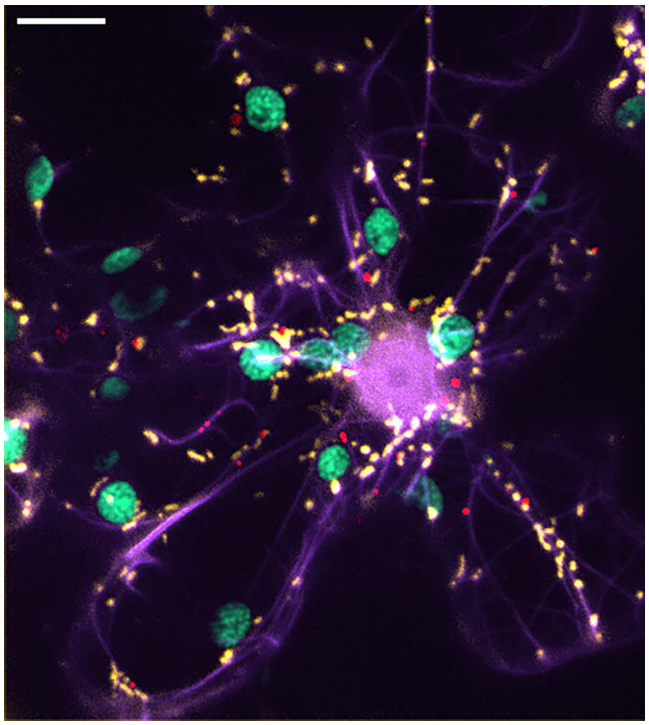

Figure 2. Simultaneous visualization of plant energy organelles and actin in tobacco cells.
Chloroplasts (chlorophyll autofluorescence, green), mitochondria (COXIV^{29aa}-eYFP, yellow), peroxisomes (mScarlet-I-SRL, red), and actin (Lifeact-eGFP, purple) in *Nicotiana tabacum* were imaged using a 3i spinning disk confocal microscope with excitation wavelengths 640 nm, 515 nm, 561 nm, and 488 nm, respectively. The emission capture range for each fluorophore is as follows: 672–712 nm for chlorophyll, 528.5–555.5 nm for eYFP, 580.5–653.5 nm for mScarlet-I, and 510–540 nm for eGFP. Scale bar represents 10 μm.

of mesophyll cells and the top of pavement cells [51,79]. However, in shaded or low-light environments, chloroplasts accumulate across the peri-clinal cortex to increase light absorbance and energy capture, and pavement cell chloroplasts relocate to the bottom of the cell nearer to mesophyll chloroplasts. Conversely, when light intensity exceeds the plant's photosynthetic capacity, chloroplasts reduce photodamage by employing an avoidance mechanism in which they stack along the anti-clinal walls of the cell [51,80] (Figure 1A). During blue-light avoidance, nuclei move concordantly with chloroplasts by hitchhiking as passengers of chloroplasts to protect DNA from UV damage [81,82] (Figure 1A). While photorelocation is predominantly described in mesophyll cells where photosynthesis is most active, evidence suggests that chloroplasts are repositioned in an actin-dependent manner in guard cells in the epidermis as well. Shifts in light modulate stomatal aperture, which is also regulated by phytohormones produced in the chloroplast. Chloroplasts in tobacco guard cells redistribute from the inner peri-clinal walls to the dorsal walls at the center of guard cells during white light-induced stomatal opening (Figure 1A), a process proposed to be mediated by stochastic dynamics of actin filaments [83,84].

Because light availability fluctuates, chloroplasts must readily initiate photorelocation routinely. Blue-light receptors (phototropins) trigger the reorganization of specialized chloroplast actin filaments (cp-actin) via actin-bundling proteins, while Chloroplast Unusual Positioning Protein 1 (CHUP1) mediates interactions among chloroplasts, cp-actin, and the plasma membrane [77,79,85–87]. Although some data suggests the involvement of myosin proteins in chloroplast photorelocation [88], the process predominantly depends on actin reorganization itself in which cp-actin aggregates at the leading edge of chloroplasts to direct movement towards anti- or peri-clinal cell walls [83,87,89].

Critical to the cold acclimation of photosynthesis, the chloroplast avoidance response can be triggered by weaker light intensities at low temperatures in *Arabidopsis* (Figure 1A), suggesting the modulation of photoprotective mechanisms upon the onset of dark when temperatures drop [90,91]. Since cold positioning was first

Biochemical Society Transactions (2023) **51** 2029–2040
https://doi.org/10.1042/BST20221093

described in ferns [92], researchers have further elaborated on the links between photorelocation and cold acclimation in bryophytes [90,91] and flowering plants [90]. Phototropins act as thermosensors via blue-light regulated autophosphorylation [95] and subcellular re-localization [96–98], modulating the actin-dependent repositioning of chloroplasts as unfused aggregates in response to cold [99–101]. Seasonal rearrangement of chloroplasts was also observed in the mesophyll of two conifer species, in which chloroplasts move from the periphery along the cell wall in the summer to a more internal location in the cell in the winter (Figure 1B), a process that involves the vacuole and cytoplasmic strands [102].

Chloroplasts cluster around the nucleus during innate immune response. Oppositional movement of nuclei and chloroplasts is proposed to contribute to the formation of stromules enriched in proximity to the nucleus (Figure 1C), possibly to facilitate the exchange of defense signals [103–107]. Independent of nuclear movement, chloroplasts accumulate at the pathogen interface (Figure 1C) during *Phytophthora infestans* infection in *Nicotiana benthamiana* [108]. They even employ some components of the photorelocation mechanism for motility in *Arabidopsis* epidermal cells during fungal infections [109,110]. Immunity-related repositioning observed in tobacco is directed by the coordinated efforts of actin, microtubules, and stromules [103], all of which have dynamic roles in chloroplast motility and environmental stress response [111].

During pollen tube elongation, plastids exhibit differential motility patterns that are loosely correlated with a variety of plastid morphologies, where spherical plastids correspond with less streaming and elongated plastids and stromules with higher rates of streaming. Specifically, plastids move long-distances from pole-to-pole at high velocities (~1.5 μm/s), with slower motions (~0.4 μm/s) and arrests predominating in the middle region of the tube, and short vibrations or fluctuations at the tip end of the shank with a velocity of ~0.5 μm/s [112,113] (Figure 1D).

Mitochondria

Early characterizations of mitochondrial dynamics describe the organelle behavior and morphology as highly heterogeneous [114], which is further emphasized by the mitochondrial diversity recently observed across microalgae [46], indicating its multipotent functions in photosynthetic organisms. Mitochondria traverse plant cells along actin filaments in a variety of patterns, including short oscillations, bidirectional long-range movement, and turnaround motions facilitated by circular actin bundles [115,116]. Their morphology and motility are influenced by development, cell-type, proximity to other organelles, and environmental conditions, as described below.

The fundamental regulation of mitochondrial movement is likely combinatorial and collaborative, involving actin and myosins for various speeds and patterns of motion as well as microtubules and likely kinesins for positioning [116,117]. This translocation and positioning infrastructure enables the mitochondria to meet the energy demands of the cell. For example, lily mitochondria are concentrated at the sub-apex of the pollen tube (Figure 1D), where energy is required for elongation [14,118,119]. The pollen tube exhibits a characteristic reverse fountain-like pattern of cytoplasmic streaming in which organelles, including mitochondria, move through the shank towards the apex (~2.67 μm/s) but then reverse course (Figure 1D) as the actin structure shifts from long filaments to shorter parallel bundles referred to as the actin fringe [118,120,121]. This fast forward movement followed by slowing down in the sub-apical region is attributed to actin/myosins and microtubules/kinesins, respectively [122]. In fact, specific motors Kinesin-like Protein 1 [123] and Myosin XI-C2 are associated with mitochondria, and Myosin XI-C2 contributes to mitochondrial as well as peroxisomal and Golgi movement in *Arabidopsis* pollen tubes [124].

ROS accumulation acts as a trigger for mitochondrial redistribution during heat stress-, UV- and methyl jasmonate (MeJA)-induced programmed cell death, in which mitochondria aggregate in clusters, shift from elongated morphology to more swollen and spherical shapes, and decrease their overall motility [125–127] (Figure 1E). These morphological changes are believed to be pivotal early steps in the progression of programmed cell death.

The trend towards more spherical, shorter, or simplified shapes for mitochondria is also evident in the transition from dark to light in *Arabidopsis* mesophyll cells [47] (Figure 1A). Light conditions also influence mitochondrial distribution and mobility. In *Arabidopsis* mesophyll cells, weak and strong blue light illuminations cause differential localization of mitochondria compared with mesophyll cells in the dark, mirroring chloroplast accumulation and avoidance [128] (Figure 1A). Mitochondrial long-range motility is dependent on actin, whereas they become more static in the proximity of chloroplasts, adopting slower, actin-independent wiggling-

type motions [129]. In the green alga *Chlamydomonas*, mitochondria are closely associated with the plasma membrane in ambient CO_2 and more uniformly distributed throughout the cytoplasm under high CO_2 [130] (Figure 1F). These mitochondrial movements may facilitate necessary associations among chloroplasts, mitochondria, as well as peroxisomes during processes requiring their collaboration, such as photorespiration that is modulated by light and CO_2 levels [131].

Peroxisomes

Despite having a simple structure, peroxisomes are highly dynamic in size, number, morphology, and biochemistry [33]. For instance, light increases peroxisome abundance in dark-grown *Arabidopsis* seedlings (Figure 1A) through a phytochrome A-mediated signaling pathway involving the transcription factors Homolog (HYH) and (Forkheaded-Associated Domain 3) FHA3 and the peroxisome elongation factor Peroxin 11b (PEX11b) [132–134]. In addition, peroxisomes in *Arabidopsis* mesophyll are significantly larger in light compared with the dark, and as their volume increases, they become less spherical [47] (Figure 1A). Moreover, chloroplast-associated peroxisomes are more elliptical (Figure 1A), increasing surface area available for interorganellar interaction. This notion is further supported by stronger adhesion between these organelles in red/blue light, although these changes are not regulated by the red and blue light receptors phytochromes and phototropins [48]. In *Arabidopsis* root tips, peroxisomes are more intensely concentrated in the elongation and meristematic zones compared with the maturation zone, which is correlated with the finding that nitric oxide (NO), which exists in the peroxisome, distributes mostly in the primary and lateral root apices [135] (Figure 1G). Interestingly, cadmium (Cd^{2+})-imposed ROS increases peroxisome proliferation in *Arabidopsis* leaves and induces peroxule formation [136,137] (Figure 1E), while Cd^{2+} decreases the number of peroxisomes in primary roots and increases peroxisome size (Figure 1G), suggesting increased organelle fusion [135].

Peroxisomes display various patterns of movement: vibrations or oscillations in place, short-range travel, and traversing longer distances [7]. While myosin drives fast and long-range movement [18,138,139], oscillatory patterns of peroxisome motion correlate with ER dynamics in *Arabidopsis* seedling epidermal cells, suggesting a possible concomitant regulation of ER-peroxisome motility [140]. Moreover, individual peroxisomes switch directions, speeds, and motions all within the same cell type and timeframe in *Arabidopsis* epidermal cells [7]. As such, peroxisomes are not simply passive passengers but rather are subject to yet undiscovered biological triggers that govern their movements.

Developmental stage and tissue type is suggested to have an impact on peroxisome motility. Brownian-type vibrations have been ascribed to peroxisomes in leaves, with slower velocities in younger leaves, whereas more rapid peroxisomal movement is described in *Arabidopsis* roots, trichomes, mature leaves, and pollen tubes [19,121,138,139,141]. However, these velocities are highly variable: <0.2 μm/s in seedling epidermis [141], ~2.23 μm/s in pollen tubes [121], up to 7 μm/s in seedling mesophyll [49], indicating that careful consideration regarding age, tissue, and cell type must be taken when evaluating peroxisome movements. Moreover, environmental factors likely influence peroxisomal motion. For example, significantly more peroxisomes are mobile in light compared with dark in *Arabidopsis* mesophyll cells (Figure 1A). Organelle mobility is maintained by actin filaments as peroxisome-chloroplast interactions increase, while peroxisome-chloroplast tethering is an actin-independent process involving peroxule formation [48,142]. Motility quickens during Cd^{2+}-induced oxidative stress, which is hypothesized to be regulated by increased peroxisomal levels of ROS and may involve calcium signaling [141] (Figure 1E). While overall motility increases 1–3 days following Cd^{2+} treatment, peroxisomes were previously reported to pause during peroxule formation within 15 min of exposure to Cd^{2+}, followed by an increase in proliferation [136,141]. These data suggest peroxisomes may dwell when undergoing morphological changes or intra/inter-organelle contact, and the number and size of peroxisomes may affect their motility.

Perspectives

- Chloroplasts, mitochondria, and peroxisomes are essential organelles functioning independently as well as coordinately during plant energy metabolism and other key processes. The motility and distribution of these energy organelles are fundamentally important for plant physiology and defense.

Biochemical Society Transactions (2023) **51** 2029–2040
https://doi.org/10.1042/BST20221093

- Besides morphing between shapes, dividing and fusing, these energy organelles move throughout the cell and interact with one another and with other cell compartments to accomplish their roles efficiently. Most of what we know till now about their motility and distribution in photosynthetic organisms addresses general patterns and velocity changes in response to developmental and environmental cues, whereas the underlying mechanisms are largely unknown.

- Many questions remain to be addressed in future research. For example, how are the molecular motors recruited to the organelles selectively? Which signaling pathways trigger the various modes of organelle positioning, movement, and physical interaction? What functional role does organelle motility play in plant physiology and health? How do these organelles coordinate their physical interaction and movement along the cytoskeletal tracks while performing collaborative metabolic functions?

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
This work was supported by funding from The National Science Foundation to J.H. (MCB 2148206) and B.L. (MCB 2148207).

Author Contributions
A.M.K., B.L. and J.H. co-wrote the manuscript. A.M.K. generated the figures.

Abbreviations
CHUP1, Chloroplast Unusual Positioning Protein 1; COX4, cytochrome c oxidase IV; ER, endoplasmic reticulum; FP, fluorescent protein; GFP, green fluorescent protein; JA, jasmonate; MBD, microtubule-binding domain; MeJA, methyl jasmonate; NO, nitric oxide; PTS1, peroxisomal targeting signal 1; ROS, reactive oxygen species; SKL, Ser-Lys-Leu; SRL, Ser-Arg-Leu.

References
1 Christensen, J.R. and Reck-Peterson, S.L. (2022) Hitchhiking across kingdoms: cotransport of cargos in fungal, animal, and plant cells. *Annu. Rev. Cell Dev. Biol.* **38**, 155–178 https://doi.org/10.1146/annurev-cellbio-120420-104341
2 Olenick, M.A. and Holzbaur, E.L.F. (2019) Dynein activators and adaptors at a glance. *J. Cell Sci.* **132**, jcs227132 https://doi.org/10.1242/jcs.227132
3 Logan, C.M. and Menko, A.S. (2019) Microtubules: evolving roles and critical cellular interactions. *Exp. Biol. Med. (Maywood)* **244**, 1240–1254 https://doi.org/10.1177/1535370219867296
4 Jongsma, M.L.M., Bakker, N. and Neefjes, J. (2023) Choreographing the motor-driven endosomal dance. *J. Cell Sci.* **136**, jcs259689 https://doi.org/10.1242/jcs.259689
5 Sparkes, I.A., Teanby, N.A. and Hawes, C. (2008) Truncated myosin XI tail fusions inhibit peroxisome, Golgi, and mitochondrial movement in tobacco leaf epidermal cells: a genetic tool for the next generation. *J. Exp. Bot.* **59**, 2499–2512 https://doi.org/10.1093/jxb/ern114
6 Perico, C. and Sparkes, I. (2018) Plant organelle dynamics: cytoskeletal control and membrane contact sites. *New Phytol.* **220**, 381–394 https://doi.org/10.1111/nph.15365
7 Mathur, J., Mathur, N. and Hulskamp, M. (2002) Simultaneous visualization of peroxisomes and cytoskeletal elements reveals actin and not microtubule-based peroxisome motility in plants. *Plant Physiol.* **128**, 1031–1045 https://doi.org/10.1104/pp.011018
8 Ueda, H., Tamura, K. and Hara-Nishimura, I. (2015) Functions of plant-specific myosin XI: from intracellular motility to plant postures. *Curr. Opin. Plant Biol.* **28**, 30–38 https://doi.org/10.1016/j.pbi.2015.08.006
9 Nebenfuhr, A. and Dixit, R. (2018) Kinesins and Myosins: molecular motors that coordinate cellular functions in plants. *Annu. Rev. Plant Biol.* **69**, 329–361 https://doi.org/10.1146/annurev-arplant-042817-040024
10 Liang, Y.J. and Yang, W.X. (2019) Kinesins in MAPK cascade: how kinesin motors are involved in the MAPK pathway? *Gene* **684**, 1–9 https://doi.org/10.1016/j.gene.2018.10.042
11 Ali, I. and Yang, W.C. (2020) The functions of kinesin and kinesin-related proteins in eukaryotes. *Cell Adh. Migr.* **14**, 139–152 https://doi.org/10.1080/19336918.2020.1810939
12 Ali, I. and Yang, W.C. (2020) Why are ATP-driven microtubule minus-end directed motors critical to plants? An overview of plant multifunctional kinesins. *Funct. Plant Biol.* **47**, 524–536 https://doi.org/10.1071/FP19177

13 Cai, G. (2022) The legacy of kinesins in the pollen tube 30 years later. *Cytoskeleton (Hoboken)* **79**, 8–19 https://doi.org/10.1002/cm.21713
14 Cai, G., Parrotta, L. and Cresti, M. (2015) Organelle trafficking, the cytoskeleton, and pollen tube growth. *J. Integr. Plant Biol.* **57**, 63–78 https://doi.org/10.1111/jipb.12289
15 Zang, J., Kriechbaumer, V. and Wang, P. (2021) Plant cytoskeletons and the endoplasmic reticulum network organization. *J. Plant Physiol.* **264**, 153473 https://doi.org/10.1016/j.jplph.2021.153473
16 Mathur, J. (2020) Review: morphology, behaviour and interactions of organelles. *Plant Sci.* **301**, 110662 https://doi.org/10.1016/j.plantsci.2020.110662
17 Mathur, J., Kroeker, O.F., Lobbezoo, M. and Mathur, N. (2022) The ER is a common mediator for the behavior and interactions of other organelles. *Fron. Plant Sci.* **13**, 846970 https://doi.org/10.3389/fpls.2022.846970
18 Avisar, D., Prokhnevsky, A.I., Makarova, K.S., Koonin, E.V. and Dolja, V.V. (2008) Myosin XI-K Is required for rapid trafficking of Golgi stacks, peroxisomes, and mitochondria in leaf cells of *Nicotiana benthamiana*. *Plant Physiol.* **146**, 1098–1108 https://doi.org/10.1104/pp.107.113647
19 Prokhnevsky, A.I., Peremyslov, V.V. and Dolja, V.V. (2008) Overlapping functions of the four class XI myosins in Arabidopsis growth, root hair elongation, and organelle motility. *Proc. Natl Acad. Sci. U.S.A.* **105**, 19744–9 https://doi.org/10.1073/pnas.0810730105
20 Sattarzadeh, A., Schmelzer, E. and Hanson, M.R. (2011) Analysis of organelle targeting by DIL domains of the Arabidopsis Myosin XI family. *Front. Plant Sci.* **2**, 72 https://doi.org/10.3389/fpls.2011.00072
21 Sattarzadeh, A., Schmelzer, E. and Hanson, M.R. (2013) Arabidopsis myosin XI sub-domains homologous to the yeast myo2p organelle inheritance sub-domain target subcellular structures in plant cells. *Front. Plant Sci.* **4**, 407 https://doi.org/10.3389/fpls.2013.00407
22 Li, J.F. and Nebenfuhr, A. (2008) The tail that wags the dog: the globular tail domain defines the function of myosin V/XI. *Traffic* **9**, 290–298 https://doi.org/10.1111/j.1600-0854.2007.00687.x
23 Perico, C., Gao, H., Heesom, K.J., Botchway, S.W. and Sparkes, I.A. (2021) Arabidopsis thaliana myosin XIK is recruited to the Golgi through interaction with a myoB receptor. *Commun. Biol.* **4**, 1182 https://doi.org/10.1038/s42003-021-02700-2
24 Wada, M. (2018) Nuclear movement and positioning in plant cells. *Semin. Cell Dev. Biol.* **82**, 17–24 https://doi.org/10.1016/j.semcdb.2017.10.001
25 Kriechbaumer, V. and Brandizzi, F. (2020) The plant endoplasmic reticulum: an organized chaos of tubules and sheets with multiple functions. *J. Microsc.* **280**, 122–133 https://doi.org/10.1111/jmi.12909
26 Renna, L. and Brandizzi, F. (2020) The mysterious life of the plant trans-Golgi network: advances and tools to understand it better. *J. Microsc.* **278**, 154–163 https://doi.org/10.1111/jmi.12881
27 Zhang, R., Xu, Y., Yi, R., Shen, J. and Huang, S. (2023) Actin cytoskeleton in the control of vesicle transport, cytoplasmic organization and pollen tube tip growth. *Plant Physiol.* **193**, 9–25 https://doi.org/10.1093/plphys/kiad203
28 Sabater, B. (2018) Evolution and function of the chloroplast. Current investigations and perspectives. *Int. J. Mol. Sci.* **19**, 3095 https://doi.org/10.3390/ijms19103095
29 Song, Y., Feng, L., Alyafei, M.A.M., Jaleel, A. and Ren, M. (2021) Function of chloroplasts in plant stress responses. *Int. J. Mol. Sci.* **22**, 3464 https://doi.org/10.3390/ijms222413464
30 Foyer, C.H. and Hanke, G. (2022) ROS production and signalling in chloroplasts: cornerstones and evolving concepts. *Plant J.* **111**, 642–661 https://doi.org/10.1111/tpj.15856
31 Li, M. and Kim, C. (2022) Chloroplast ROS and stress signaling. *Plant Commun.* **3**, 100264 https://doi.org/10.1016/j.xplc.2021.100264
32 Moller, I.M., Rasmusson, A.G. and Van Aken, O. (2021) Plant mitochondria - past, present and future. *Plant J.* **108**, 912–959 https://doi.org/10.1111/tpj.15495
33 Pan, R., Liu, J., Wang, S. and Hu, J. (2020) Peroxisomes: versatile organelles with diverse roles in plants. *New Phytol.* **225**, 1410–1427 https://doi.org/10.1111/nph.16134
34 Hu, J., Baker, A., Bartel, B., Linka, N., Mullen, R.T., Reumann, S. et al. (2012) Plant peroxisomes: biogenesis and function. *Plant Cell* **24**, 2279–2303 https://doi.org/10.1105/tpc.112.096586
35 Kaur, N., Reumann, S. and Hu, J. (2009) Peroxisome biogenesis and function. *Arabidopsis Book* **7**, e0123 https://doi.org/10.1199/tab.0123
36 He, C., Berkowitz, O., Hu, S., Zhao, Y., Qian, K., Shou, H. et al. (2023) Co-regulation of mitochondrial and chloroplast function: molecular components and mechanisms. *Plant Commun.* **4**, 100496 https://doi.org/10.1016/j.xplc.2022.100496
37 Gomez-Casati, D.F., Barchiesi, J. and Busi, M.V. (2022) Mitochondria and chloroplasts function in microalgae energy production. *PeerJ* **10**, e14576 https://doi.org/10.7717/peerj.14576
38 Van Aken, O. and Van Breusegem, F. (2015) Licensed to kill: mitochondria, chloroplasts, and cell death. *Trends Plant Sci.* **20**, 754–766 https://doi.org/10.1016/j.tplants.2015.08.002
39 Timm, S., Florian, A., Fernie, A.R. and Bauwe, H. (2016) The regulatory interplay between photorespiration and photosynthesis. *J. Exp. Bot.* **67**, 2923–2929 https://doi.org/10.1093/jxb/erw083
40 Sunil, B., Talla, S.K., Aswani, V. and Raghavendra, A.S. (2013) Optimization of photosynthesis by multiple metabolic pathways involving interorganelle interactions: resource sharing and ROS maintenance as the bases. *Photosynth. Res.* **117**, 61–71 https://doi.org/10.1007/s11120-013-9889-z
41 Jiang, X., Walker, B.J., He, S.Y. and Hu, J. (2023) The role of photorespiration in plant immunity. *Front. Plant Sci.* **14**, 1125945 https://doi.org/10.3389/fpls.2023.1125945
42 Li, N., Xu, C., Li-Beisson, Y. and Philippar, K. (2016) Fatty acid and lipid transport in plant cells. *Trends Plant Sci.* **21**, 145–158 https://doi.org/10.1016/j.tplants.2015.10.011
43 Michaud, M., Gros, V., Tardif, M., Brugiere, S., Ferro, M., Prinz, W.A. et al. (2016) Atmic60 is involved in plant mitochondria lipid trafficking and is part of a large complex. *Curr. Biol.* **26**, 627–639 https://doi.org/10.1016/j.cub.2016.01.011
44 Pan, R., Reumann, S., Lisik, P., Tietz, S., Olsen, L.J. and Hu, J. (2018) Proteome analysis of peroxisomes from dark-treated senescent Arabidopsis leaves. *J. Integr. Plant Biol.* **60**, 1028–1050 https://doi.org/10.1111/jipb.12670
45 Wasternack, C. and Strnad, M. (2018) Jasmonates: news on occurrence, biosynthesis, metabolism and action of an ancient group of signaling compounds. *Int. J. Mol. Sci.* **19**, 2539 https://doi.org/10.3390/ijms19092539
46 Uwizeye, C., Decelle, J., Jouneau, P.H., Flori, S., Gallet, B., Keck, J.B. et al. (2021) Morphological bases of phytoplankton energy management and physiological responses unveiled by 3D subcellular imaging. *Nat. Commun.* **12**, 1049 https://doi.org/10.1038/s41467-021-21314-0

47 Midorikawa, K., Tateishi, A., Toyooka, K., Sato, M., Imai, T., Kodama, Y. et al. (2022) Three-dimensional nanoscale analysis of light-dependent organelle changes in Arabidopsis mesophyll cells. *PNAS Nexus* **1**, pgac225 https://doi.org/10.1093/pnasnexus/pgac225
48 Oikawa, K., Matsunaga, S., Mano, S., Kondo, M., Yamada, K., Hayashi, M. et al. (2015) Physical interaction between peroxisomes and chloroplasts elucidated by in situ laser analysis. *Nat. Plants* **1**, 15035 https://doi.org/10.1038/nplants.2015.35
49 Jaipargas, E.A., Mathur, N., Bou Daher, F., Wasteneys, G.O. and Mathur, J. (2016) High light intensity leads to increased peroxule-mitochondria interactions in plants. *Front. Cell Dev. Biol.* **4**, 6 https://doi.org/10.3389/fcell.2016.00006
50 Mathur, J. (2021) Organelle extensions in plant cells. *Plant Physiol.* **185**, 593–607 https://doi.org/10.1093/plphys/kiaa055
51 Barton, K.A., Wozny, M.R., Mathur, N., Jaipargas, E.A. and Mathur, J. (2018) Chloroplast behaviour and interactions with other organelles in *Arabidopsis thaliana* pavement cells. *J. Cell Sci.* **131**, jcs202275 https://doi.org/10.1242/jcs.202275
52 Wada, M. (2013) Chloroplast movement. *Plant Sci.* **210**, 177–182 https://doi.org/10.1016/j.plantsci.2013.05.016
53 Collings, D.A., Harper, J.D.I. and Vaughn, K.C. (2003) The association of peroxisomes with the developing cell plate in dividing onion root cells depends on actin microfilaments and myosin. *Planta* **218**, 204–216 https://doi.org/10.1007/s00425-003-1096-2
54 Wu, F.S. (1987) Localization of mitochondria in plant-cells by vital staining with rhodamine-123. *Planta* **171**, 346–357 https://doi.org/10.1007/Bf00398680
55 Gambier, R.M. and Mulcahy, D.L. (1994) Confocal laser-scanning microscopy of mitochondria within microspore tetrads of plants using rhodamine-123 as a fluorescent vital stain. *Biotech. Histochem.* **69**, 311–316 https://doi.org/10.3109/10520299409106311
56 Poot, M., Zhang, Y.Z., Kramer, J.A., Wells, K.S., Jones, L., Hanzel, D.K. et al. (1996) Analysis of mitochondrial morphology and function with novel fixable fluorescent stains. *J. Histochem. Cytochem.* **44**, 1363–1372 https://doi.org/10.1177/44.12.8985128
57 Zheng, M.Z., Beck, M., Muller, J., Chen, T., Wang, X.H., Wang, F. et al. (2009) Actin turnover is required for myosin-dependent mitochondrial movements in Arabidopsis root hairs. *PLoS ONE* **4**, e5961 https://doi.org/10.1371/journal.pone.0005961
58 Wu, Q., Luo, A., Zadrozny, T., Sylvester, A. and Jackson, D. (2013) Fluorescent protein marker lines in maize: generation and applications. *Int. J. Dev. Biol.* **57**, 535–543 https://doi.org/10.1387/ijdb.130240qw
59 Chen, Z., Zheng, W., Chen, L., Li, C., Liang, T., Chen, Z. et al. (2019) Green fluorescent protein- and Discosoma sp. red fluorescent protein-tagged organelle marker lines for protein subcellular localization in rice. *Front. Plant Sci.* **10**, 1421 https://doi.org/10.3389/fpls.2019.01421
60 Liu, Z., Osterlund, I., Ruhnow, F., Cao, Y., Huang, G., Cai, W. et al. (2022) Fluorescent cytoskeletal markers reveal associations between the actin and microtubule cytoskeleton in rice cells. *Development* **149**, dev200415 https://doi.org/10.1242/dev.200415
61 Kaduchova, K., Marchetti, C., Ovecka, M., Galuszka, P., Bergougnoux, V., Samaj, J. et al. (2023) Spatial organization and dynamics of chromosomes and microtubules during barley mitosis. *Plant J.* **115**, 602–613 https://doi.org/10.1111/tpj.16355
62 Chen, T., Wang, X.H., von Wangenheim, D., Zheng, M.Z., Samaj, J., Ji, W.Q. et al. (2012) Probing and tracking organelles in living plant cells. *Protoplasma* **249**, 157–167 https://doi.org/10.1007/s00709-011-0364-4
63 Nelson, B.K., Cai, X. and Nebenfuhr, A. (2007) A multicolored set of *in vivo* organelle markers for co-localization studies in Arabidopsis and other plants. *Plant J.* **51**, 1126–1136 https://doi.org/10.1111/j.1365-313X.2007.03212.x
64 Komis, G., Novak, D., Ovecka, M., Samajova, O. and Samaj, J. (2018) Advances in imaging plant cell dynamics. *Plant Physiol.* **176**, 80–93 https://doi.org/10.1104/pp.17.00962
65 Reumann, S., Quan, S., Aung, K., Yang, P.F., Manandhar-Shrestha, K., Holbrook, D. et al. (2009) In-depth proteome analysis of Arabidopsis leaf peroxisomes combined with *in vivo* subcellular targeting verification indicates novel metabolic and regulatory functions of peroxisomes. *Plant Physiol.* **150**, 125–143 https://doi.org/10.1104/pp.109.137703
66 Schuhmann, H., Huesgen, P.F., Gietl, C. and Adamska, I. (2008) The DEG15 serine protease cleaves peroxisomal targeting signal 2-containing proteins in arabidopsis. *Plant Physiol.* **148**, 1847–1856 https://doi.org/10.1104/pp.108.125377
67 Zhang, D., Wadsworth, P. and Hepler, P.K. (1993) Dynamics of microfilaments are similar, but distinct from microtubules during cytokinesis in living, dividing plant cells. *Cell Motil. Cytoskel.* **24**, 151–155 https://doi.org/10.1002/cm.970240302
68 Wang, Y.S., Yoo, C.M. and Blancaflor, E.B. (2008) Improved imaging of actin filaments in transgenic Arabidopsis plants expressing a green fluorescent protein fusion to the C- and N-termini of the fimbrin actin-binding domain 2. *New Phytol.* **177**, 525–536 https://doi.org/10.1111/j.1469-8137.2007.02261.x
69 Dyachok, J., Sparks, J.A., Liao, F., Wang, Y.S. and Blancaflor, E.B. (2014) Fluorescent protein-based reporters of the actin cytoskeleton in living plant cells: fluorophore variant, actin binding domain, and promoter considerations. *Cytoskeleton (Hoboken)* **71**, 311–327 https://doi.org/10.1002/cm.21174
70 Marc, J., Granger, C.L., Brincat, J., Fisher, D.D., Kao, T., McCubbin, A.G. et al. (1998) A GFP-MAP4 reporter gene for visualizing cortical microtubule rearrangements in living epidermal cells. *Plant Cell* **10**, 1927–1940 https://doi.org/10.1105/tpc.10.11.1927
71 Ben-Nissan, G., Cui, W., Kim, D.J., Yang, Y., Yoo, B.C. and Lee, J.Y. (2008) Arabidopsis casein kinase 1-like 6 contains a microtubule-binding domain and affects the organization of cortical microtubules. *Plant Physiol.* **148**, 1897–1907 https://doi.org/10.1104/pp.108.129346
72 Ueda, K., Matsuyama, T. and Hashimoto, T. (1999) Visualization of microtubules in living cells of transgenic *Arabidopsis thaliana*. *Protoplasma* **206**, 201–206 https://doi.org/10.1007/BF01279267
73 Nakamura, M., Naoi, K., Shoji, T. and Hashimoto, T. (2004) Low concentrations of propyzamide and oryzalin alter microtubule dynamics in Arabidopsis epidermal cells. *Plant Cell Physiol.* **45**, 1330–1334 https://doi.org/10.1093/pcp/pch300
74 Liu, W., Wang, C., Wang, G., Ma, Y., Tian, J., Yu, Y. et al. (2019) Towards a better recording of microtubule cytoskeletal spatial organization and dynamics in plant cells. *J. Integr. Plant Biol.* **61**, 388–393 https://doi.org/10.1111/jipb.12721
75 Kasahara, M., Kagawa, T., Oikawa, K., Suetsugu, N., Miyao, M. and Wada, M. (2002) Chloroplast avoidance movement reduces photodamage in plants. *Nature* **420**, 829–832 https://doi.org/10.1038/nature01213
76 Kong, S.G. and Wada, M. (2014) Recent advances in understanding the molecular mechanism of chloroplast photorelocation movement. *Biochim. Biophys. Acta* **1837**, 522–530 https://doi.org/10.1016/j.bbabio.2013.12.004
77 Kong, S.G. and Wada, M. (2016) Molecular basis of chloroplast photorelocation movement. *J. Plant Res.* **129**, 159–166 https://doi.org/10.1007/s10265-016-0788-1
78 Kong, S.G. and Wada, M. (2011) New insights into dynamic actin-based chloroplast photorelocation movement. *Mol. Plant* **4**, 771–781 https://doi.org/10.1093/mp/ssr061

79 Wada, M. (2016) Chloroplast and nuclear photorelocation movements. *Proc. Jpn Acad. Ser. B Phys. Biol. Sci.* **92**, 387–411 https://doi.org/10.2183/pjab.92.387
80 Gotoh, E., Suetsugu, N., Yamori, W., Ishishita, K., Kiyabu, R., Fukuda, M. et al. (2018) Chloroplast accumulation response enhances leaf photosynthesis and plant biomass production. *Plant Physiol.* **178**, 1358–1369 https://doi.org/10.1104/pp.18.00484
81 Higa, T., Suetsugu, N., Kong, S.G. and Wada, M. (2014) Actin-dependent plastid movement is required for motive force generation in directional nuclear movement in plants. *Proc. Natl Acad. Sci. U.S.A.* **111**, 4327–4331 https://doi.org/10.1073/pnas.1317902111
82 Higa, T., Suetsugu, N. and Wada, M. (2014) Plant nuclear photorelocation movement. *J. Exp. Bot.* **65**, 2873–2881 https://doi.org/10.1093/jxb/ert414
83 Wang, X.L., Gao, X.Q. and Wang, X.C. (2011) Stochastic dynamics of actin filaments in guard cells regulating chloroplast localization during stomatal movement. *Plant Cell Environ.* **34**, 1248–1257 https://doi.org/10.1111/j.1365-3040.2011.02325.x
84 Chu, C.P., Liu, Z.H., Hu, Z.Y. and Wang, X.L. (2011) Tubular actin filaments in tobacco guard cells. *Plant Signal. Behav.* **6**, 1578–1580 https://doi.org/10.4161/psb.6.10.17095
85 Yuan, N., Mendu, L., Ghose, K., Witte, C.S., Frugoli, J. and Mendu, V. (2023) FKF1 interacts with CHUP1 and regulates chloroplast movement in Arabidopsis. *Plants (Basel)* **12**, 542 https://doi.org/10.3390/plants12030542
86 Aihara, Y., Tabata, R., Suzuki, T., Shimazaki, K. and Nagatani, A. (2008) Molecular basis of the functional specificities of phototropin 1 and 2. *Plant J.* **56**, 364–375 https://doi.org/10.1111/j.1365-313X.2008.03605.x
87 Wada, M. and Kong, S.G. (2018) Actin-mediated movement of chloroplasts. *J. Cell Sci.* **131**, jcs210310 https://doi.org/10.1242/jcs.210310
88 Paves, H. and Truve, E. (2007) Myosin inhibitors block accumulation movement of chloroplasts in *Arabidopsis thaliana* leaf cells. *Protoplasma* **230**, 165–169 https://doi.org/10.1007/s00709-006-0230-y
89 Suetsugu, N., Higa, T., Gotoh, E. and Wada, M. (2016) Light-induced movements of chloroplasts and nuclei are regulated in both Cp-actin-filament-dependent and -independent manners in *Arabidopsis thaliana*. *PLoS ONE* **11**, e0157429 https://doi.org/10.1371/journal.pone.0157429
90 Labuz, J., Hermanowicz, P. and Gabrys, H. (2015) The impact of temperature on blue light induced chloroplast movements in *Arabidopsis thaliana*. *Plant Sci.* **239**, 238–249 https://doi.org/10.1016/j.plantsci.2015.07.013
91 Kitashova, A., Schneider, K., Furtauer, L., Schroder, L., Scheibenbogen, T., Furtauer, S. et al. (2021) Impaired chloroplast positioning affects photosynthetic capacity and regulation of the central carbohydrate metabolism during cold acclimation. *Photosynth. Res.* **147**, 49–60 https://doi.org/10.1007/s11120-020-00795-y
92 Kodama, Y., Tsuboi, H., Kagawa, T. and Wada, M. (2008) Low temperature-induced chloroplast relocation mediated by a blue light receptor, phototropin 2, in fern gametophytes. *J. Plant Res.* **121**, 441–448 https://doi.org/10.1007/s10265-008-0165-9
93 Ogasawara, Y., Ishizaki, K., Kohchi, T. and Kodama, Y. (2013) Cold-induced organelle relocation in the liverwort *Marchantia polymorpha* L. *Plant Cell Environ.* **36**, 1520–1528 https://doi.org/10.1111/pce.12085
94 Yong, L.K., Tsuboyama, S., Kitamura, R., Kurokura, T., Suzuki, T. and Kodama, Y. (2021) Chloroplast relocation movement in the liverwort *Apopellia endiviifolia*. *Physiol. Plant* **173**, 775–787 https://doi.org/10.1111/ppl.13473
95 Fujii, Y., Tanaka, H., Konno, N., Ogasawara, Y., Hamashima, N., Tamura, S. et al. (2017) Phototropin perceives temperature based on the lifetime of its photoactivated state. *Proc. Natl Acad. Sci. U.S.A.* **114**, 9206–9211 https://doi.org/10.1073/pnas.1704462114
96 Hirano, S., Sasaki, K., Osaki, Y., Tahara, K., Takahashi, H., Takemiya, A. et al. (2022) The localization of phototropin to the plasma membrane defines a cold-sensing compartment in *Marchantia polymorpha*. *PNAS Nexus* **1**, pgac030 https://doi.org/10.1093/pnasnexus/pgac030
97 Sakata, M., Kimura, S., Fujii, Y., Sakai, T. and Kodama, Y. (2019) Relationship between relocation of phototropin to the chloroplast periphery and the initiation of chloroplast movement in *Marchantia polymorpha*. *Plant Direct* **3**, e00160 https://doi.org/10.1002/pld3.160
98 Fujii, Y., Ogasawara, Y., Takahashi, Y., Sakata, M., Noguchi, M., Tamura, S. et al. (2020) The cold-induced switch in direction of chloroplast relocation occurs independently of changes in endogenous phototropin levels. *PLoS ONE* **15**, e0233302 https://doi.org/10.1371/journal.pone.0233302
99 Kimura, S. and Kodama, Y. (2016) Actin-dependence of the chloroplast cold positioning response in the liverwort *Marchantia polymorpha* L. *PeerJ* **4**, e2513 https://doi.org/10.7717/peerj.2513
100 Yong, L.K. and Kodama, Y. (2023) Dark-induced chloroplast relocation depends on actin filaments in the liverwort *Apopellia endiviifolia* along with the light- and cold-induced relocations. *Plant Cell Environ.* **46**, 1822–1832 https://doi.org/10.1111/pce.14566
101 Tanaka, H., Sato, M., Ogasawara, Y., Hamashima, N., Buchner, O., Holzinger, A. et al. (2017) Chloroplast aggregation during the cold-positioning response in the liverwort *Marchantia polymorpha*. *J. Plant Res.* **130**, 1061–1070 https://doi.org/10.1007/s10265-017-0958-9
102 Ovsyannikov, A.Y. and Koteyeva, N.K. (2020) Seasonal movement of chloroplasts in mesophyll cells of two Picea species. *Protoplasma* **257**, 183–195 https://doi.org/10.1007/s00709-019-01427-6
103 Kumar, A.S., Park, E., Nedo, A., Alqarni, A., Ren, L., Hoban, K. et al. (2018) Stromule extension along microtubules coordinated with actin-mediated anchoring guides perinuclear chloroplast movement during innate immunity. *eLife* **7**, e23625 https://doi.org/10.7554/eLife.23625
104 Ding, X., Jimenez-Gongora, T., Krenz, B. and Lozano-Duran, R. (2019) Chloroplast clustering around the nucleus is a general response to pathogen perception in *Nicotiana benthamiana*. *Mol. Plant Pathol.* **20**, 1298–1306 https://doi.org/10.1111/mpp.12840
105 Park, E., Caplan, J.L. and Dinesh-Kumar, S.P. (2018) Dynamic coordination of plastid morphological change by cytoskeleton for chloroplast-nucleus communication during plant immune responses. *Plant Signal. Behav.* **13**, e1500064 https://doi.org/10.1080/15592324.2018.1500064
106 Caplan, J.L., Kumar, A.S., Park, E., Padmanabhan, M.S., Hoban, K., Modla, S. et al. (2015) Chloroplast stromules function during innate immunity. *Dev. Cell* **34**, 45–57 https://doi.org/10.1016/j.devcel.2015.05.011
107 Erickson, J.L., Kantek, M. and Schattat, M.H. (2017) Plastid-nucleus distance alters the behavior of stromules. *Front. Plant Sci.* **8**, 1135 https://doi.org/10.3389/fpls.2017.01135
108 Savage, Z., Duggan, C., Toufexi, A., Pandey, P., Liang, Y., Segretin, M.E. et al. (2021) Chloroplasts alter their morphology and accumulate at the pathogen interface during infection by *Phytophthora infestans*. *Plant J.* **107**, 1771–1787 https://doi.org/10.1111/tpj.15416
109 Irieda, H. and Takano, Y. (2021) Epidermal chloroplasts are defense-related motile organelles equipped with plant immune components. *Nat. Commun.* **12**, 2739 https://doi.org/10.1038/s41467-021-22977-5
110 Irieda, H. (2022) Emerging roles of motile epidermal chloroplasts in plant immunity. *Int. J. Mol. Sci.* **23**, 4043 https://doi.org/10.3390/ijms23074043
111 Hanson, M.R. and Conklin, P.L. (2020) Stromules, functional extensions of plastids within the plant cell. *Curr. Opin. Plant Biol.* **58**, 25–32 https://doi.org/10.1016/j.pbi.2020.10.005

112 Fujiwara, M.T., Yoshioka, Y., Hirano, T., Kazama, Y., Abe, T., Hayashi, K. et al. (2012) Visualization of plastid movement in the pollen tube of *Arabidopsis thaliana*. *Plant Signal. Behav.* **7**, 34–37 https://doi.org/10.4161/psb.7.1.18484
113 Fujiwara, M.T., Hashimoto, H., Kazama, Y., Hirano, T., Yoshioka, Y., Aoki, S. et al. (2010) Dynamic morphologies of pollen plastids visualised by vegetative-specific FtsZ1-GFP in *Arabidopsis thaliana*. *Protoplasma* **242**, 19–33 https://doi.org/10.1007/s00709-010-0119-7
114 Logan, D.C. and Leaver, C.J. (2000) Mitochondria-targeted GFP highlights the heterogeneity of mitochondrial shape, size and movement within living plant cells. *J. Exp. Bot.* **51**, 865–871 PMID:10948212
115 Zhang, Y., Sheng, X., Meng, X. and Li, Y. (2014) The circular F-actin bundles provide a track for turnaround and bidirectional movement of mitochondria in Arabidopsis root hair. *PLoS ONE* **9**, e91501 https://doi.org/10.1371/journal.pone.0091501
116 Zheng, M., Wang, Q., Teng, Y., Wang, X., Wang, F., Chen, T. et al. (2010) The speed of mitochondrial movement is regulated by the cytoskeleton and myosin in *Picea wilsonii* pollen tubes. *Planta* **231**, 779–791 https://doi.org/10.1007/s00425-009-1086-0
117 Van Gestel, K., Kohler, R.H. and Verbelen, J.P. (2002) Plant mitochondria move on F-actin, but their positioning in the cortical cytoplasm depends on both F-actin and microtubules. *J. Exp. Bot.* **53**, 659–667 https://doi.org/10.1093/jexbot/53.369.659
118 Lovy-Wheeler, A., Cardenas, L., Kunkel, J.G. and Hepler, P.K. (2007) Differential organelle movement on the actin cytoskeleton in lily pollen tubes. *Cell Motil. Cytoskeleton* **64**, 217–232 https://doi.org/10.1002/cm.20181
119 Colaco, R., Moreno, N. and Feijo, J.A. (2012) On the fast lane: mitochondria structure, dynamics and function in growing pollen tubes. *J. Microsc.* **247**, 106–118 https://doi.org/10.1111/j.1365-2818.2012.03628.x
120 Zhang, S., Wang, C., Xie, M., Liu, J., Kong, Z. and Su, H. (2018) Actin bundles in the pollen tube. *Int. J. Mol. Sci.* **19**, 3710 https://doi.org/10.3390/ijms19123710
121 Tian, X., Wang, X. and Li, Y. (2021) Myosin XI-B is involved in the transport of vesicles and organelles in pollen tubes of *Arabidopsis thaliana*. *Plant J.* **108**, 1145–1161 https://doi.org/10.1111/tpj.15505
122 Romagnoli, S., Cai, G., Faleri, C., Yokota, E., Shimmen, T. and Cresti, M. (2007) Microtubule- and actin filament-dependent motors are distributed on pollen tube mitochondria and contribute differently to their movement. *Plant Cell Physiol.* **48**, 345–361 https://doi.org/10.1093/pcp/pcm001
123 Ni, C.Z., Wang, H.Q., Xu, T., Qu, Z. and Liu, G.Q. (2005) AtKP1, a kinesin-like protein, mainly localizes to mitochondria in *Arabidopsis thaliana*. *Cell Res.* **15**, 725–733 https://doi.org/10.1038/sj.cr.7290342
124 Wang, X., Sheng, X., Tian, X., Zhang, Y. and Li, Y. (2020) Organelle movement and apical accumulation of secretory vesicles in pollen tubes of *Arabidopsis thaliana* depend on class XI myosins. *Plant J.* **104**, 1685–1697 https://doi.org/10.1111/tpj.15030
125 Zhang, L. and Xing, D. (2008) Methyl jasmonate induces production of reactive oxygen species and alterations in mitochondrial dynamics that precede photosynthetic dysfunction and subsequent cell death. *Plant Cell Physiol.* **49**, 1092–1111 https://doi.org/10.1093/pcp/pcn086
126 Zhang, L., Li, Y., Xing, D. and Gao, C. (2009) Characterization of mitochondrial dynamics and subcellular localization of ROS reveal that HsfA2 alleviates oxidative damage caused by heat stress in Arabidopsis. *J. Exp. Bot.* **60**, 2073–2091 https://doi.org/10.1093/jxb/erp078
127 Gao, C., Xing, D., Li, L. and Zhang, L. (2008) Implication of reactive oxygen species and mitochondrial dysfunction in the early stages of plant programmed cell death induced by ultraviolet-C overexposure. *Planta* **227**, 755–767 https://doi.org/10.1007/s00425-007-0654-4
128 Islam, M.S., Niwa, Y. and Takagi, S. (2009) Light-dependent intracellular positioning of mitochondria in *Arabidopsis thaliana* mesophyll cells. *Plant Cell Physiol.* **50**, 1032–1040 https://doi.org/10.1093/pcp/pcp054
129 Oikawa, K., Imai, T., Thagun, C., Toyooka, K., Yoshizumi, T., Ishikawa, K. et al. (2021) Mitochondrial movement during its association with chloroplasts in *Arabidopsis thaliana*. *Commun. Biol.* **4**, 292 https://doi.org/10.1038/s42003-021-01833-8
130 Rai, A.K., Chen, T. and Moroney, J.V. (2021) Mitochondrial carbonic anhydrases are needed for optimal photosynthesis at low CO_2 levels in *Chlamydomonas*. *Plant Physiol.* **187**, 1387–1398 https://doi.org/10.1093/plphys/kiab351
131 Fu, X. and Walker, B.J. (2023) Dynamic response of photorespiration in fluctuating light environments. *J. Exp. Bot.* **74**, 600–611 https://doi.org/10.1093/jxb/erac335
132 Hu, J. and Desai, M. (2008) Light control of peroxisome proliferation during Arabidopsis photomorphogenesis. *Plant Signal. Behav.* **3**, 801–803 https://doi.org/10.4161/psb.3.10.5876
133 Desai, M. and Hu, J. (2008) Light induces peroxisome proliferation in Arabidopsis seedlings through the photoreceptor phytochrome A, the transcription factor HY5 HOMOLOG, and the peroxisomal protein PEROXIN11b. *Plant Physiol.* **146**, 1117–1127 https://doi.org/10.1104/pp.107.113555
134 Desai, M., Pan, R. and Hu, J. (2017) Arabidopsis forkhead-associated domain protein 3 negatively regulates peroxisome division. *J. Integr. Plant Biol.* **59**, 454–458 https://doi.org/10.1111/jipb.12542
135 Piacentini, D., Corpas, F.J., D'Angeli, S., Altamura, M.M. and Falasca, G. (2020) Cadmium and arsenic-induced-stress differentially modulates arabidopsis root architecture, peroxisome distribution, enzymatic activities and their nitric oxide content. *Plant Physiol. Biochem.* **148**, 312–323 https://doi.org/10.1016/j.plaphy.2020.01.026
136 Rodriguez-Serrano, M., Romero-Puertas, M.C., Sanz-Fernandez, M., Hu, J. and Sandalio, L.M. (2016) Peroxisomes extend peroxules in a fast response to stress via a reactive oxygen species-mediated induction of the peroxin PEX11a. *Plant Physiol.* **171**, 1665–1674 https://doi.org/10.1104/pp.16.00648
137 Terron-Camero, L.C., Rodriguez-Serrano, M., Sandalio, L.M. and Romero-Puertas, M.C. (2020) Nitric oxide is essential for cadmium-induced peroxule formation and peroxisome proliferation. *Plant Cell Environ.* **43**, 2492–2507 https://doi.org/10.1111/pce.13855
138 Jedd, G. and Chua, N.H. (2002) Visualization of peroxisomes in living plant cells reveals acto-myosin-dependent cytoplasmic streaming and peroxisome budding. *Plant Cell Physiol.* **43**, 384–392 https://doi.org/10.1093/pcp/pcf045
139 Peremyslov, V.V., Prokhnevsky, A.I., Avisar, D. and Dolja, V.V. (2008) Two class XI myosins function in organelle trafficking and root hair development in Arabidopsis. *Plant Physiol.* **146**, 1109–1116 https://doi.org/10.1104/pp.107.113654
140 Barton, K., Mathur, N. and Mathur, J. (2013) Simultaneous live-imaging of peroxisomes and the ER in plant cells suggests contiguity but no luminal continuity between the two organelles. *Front. Physiol.* **4**, 196 https://doi.org/10.3389/fphys.2013.00196
141 Rodriguez-Serrano, M., Romero-Puertas, M.C., Sparkes, I., Hawes, C., del Rio, L.A. and Sandalio, L.M. (2009) Peroxisome dynamics in arabidopsis plants under oxidative stress induced by cadmium. *Free Radic. Biol. Med.* **47**, 1632–1639 https://doi.org/10.1016/j.freeradbiomed.2009.09.012
142 Gao, H., Metz, J., Teanby, N.A., Ward, A.D., Botchway, S.W., Coles, B. et al. (2016) *In vivo* quantification of peroxisome tethering to chloroplasts in tobacco epidermal cells using optical tweezers. *Plant Physiol.* **170**, 263–272 https://doi.org/10.1104/pp.15.01529