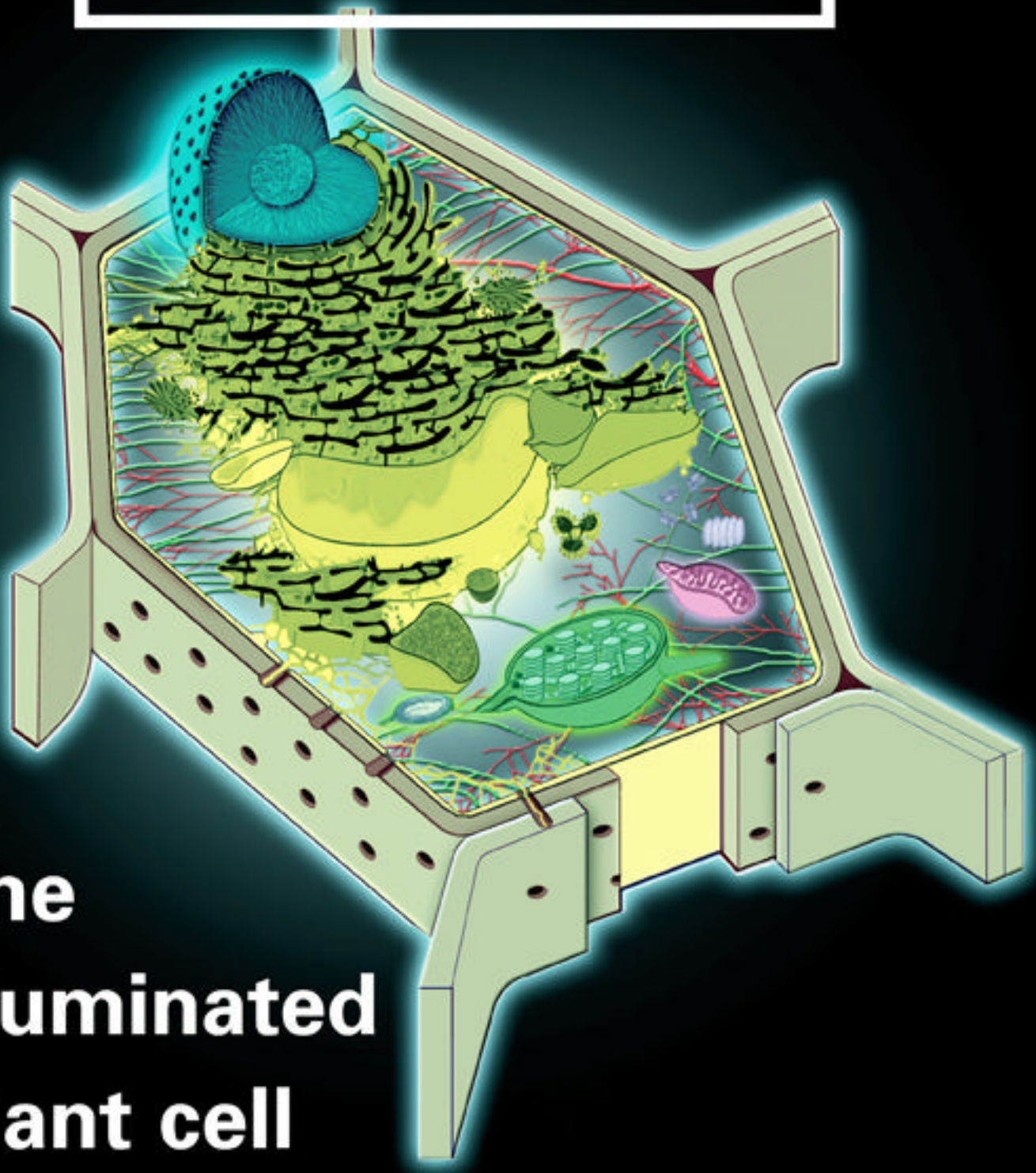
ELSEVIER

TRENDS in Plant Science



The illuminated plant cell

Trends Plant Sci. November 2007 Vol. 12 No. 11, pp. 483 – 527 ISSN 1360-1385

Review TRENDS in Plant Science Vol.12 No.11

The illuminated plant cell

Jaideep Mathur

Laboratory of Plant Development and Interactions, Department of Molecular and Cellular Biology, College of Biological Science, University of Guelph, 588 Gordon Street, Guelph, Ontario, N1G 2W1, Canada

The past decade has provided biologists with a palette of genetically encoded, multicolored fluorescent proteins. The living plant cell turned into a 'coloring book' and today, nearly every text-book organelle has been highlighted in scintillating fluorescent colors. This review provides a concise listing of the earliest representative fluorescent-protein probes used to highlight various targets within the plant cell, and introduces the idea of using the numerous multicolor, subcellular probes for the development of an early intracellular response profile of plants.

Visualizing the plant-cell interior

The wall encasing the plant cell has been the largest barrier to the visualization and understanding of subcellular processes in living plants. Traditionally, plant scientists have relied on squashing, maceration, sectioning or enzyme-mediated degradation of the cell wall to gain access to the inner compartments of the plant cell. Although detailed observations of the plant cell constitute the foundations of plant biology, the fact remains that many of the descriptions are extrapolations of observations made on fixed, dead plant tissue. By comparison, the analysis of live-cell phenomena, such as cytoplasmic streaming and organelle interactions, has been rather limited ([1] and references cited therein) because only those plant tissues that allow light to be transmitted through are amenable to non-invasive visualization techniques involving time-lapse video recordings. With the advent of fluorescence microscopy and the availability of cell permeant dyes, such as the nucleotide-binding SYTO stains, the endomembrane-staining DIOC6 and FM4–64 dyes and the mitochondria-specific mitotracker dyes [2], plant biologists have obtained a short time window for live-cell imaging before toxicity-related concerns become pertinent. Also, microinjection of specific stains and fluorescent-protein analogs into living plant cells has become a powerful tool for the observation of subcellular processes [3]. Unfortunately, microinjection procedures do require skilled researchers, are often labor intensive and are limited in terms of useful cell types, observable cell numbers and experimental reproducibility. Thus, only a handful of researchers could explore their potential and, for plant biologists, they never reached the status of a routine technique.

Here, I provide a brief overview of how our ability to look inside the plant cell received a tremendous boost in the early 1990s, with the cloning and rapid availability of a 27-kDa green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* [4].

Fluorescent proteins light up the plant cell interior

The genetically encoded GFP swept away many of the cell wall-imposed limitations on live imaging of the plant cell interior because, in stark contrast to the cumbersome external loading of stains and dyes, GFP and its derivative fluorescent proteins (FPs) are produced by the cells themselves and do not require exogenous substrates, cofactors or chemical treatments for their activity [5,6]. Through their fusion to specific nucleotide sequences, FPs can be targeted to literally any compartment or component of the cell. Once introduced into a plant cell, either for transient or for stable transgene expression after integration in the plant genome, FPs follow the general rules governing subcellular protein dynamics, localization and interactions. The fusion proteins are thus able to respond to both cell-intrinsic and external environmental cues. Through concomitant advancements in non-invasive, CCD-based epifluorescent and confocal laser scanning microscopy, FPs can be readily visualized in living plant cells [6].

Whereas, many modern laboratories using FPs in their research trace their initial acquisition of GFP clones to Douglas Prasher and Martin Chalfie [4], Roger Tsien [7] or to the commercial source Clontech (http://www.clontech.com), a large portion of the credit for modifying GFP for optimal expression in plants and popularizing its use among fellow plant scientists through its unconditional sharing goes to Jim Haseloff and his research team [8]. By late 1997, many plant research laboratories had introduced the cDNAs for mGFP5 and mGFP5-ER (targeted to the endoplasmic reticulum) into their plant dissection strategies involving transcriptional and translational fusion proteins.

The rapid acceptance of GFP as a live reporter protein provided a strong motivation for the creation of newer versions of FPs with altered spectral characteristics [9]. Today, multicolored FPs spanning the visible spectrum have been obtained from a variety of organisms [9,10]. In plants, two complementary FP-based investigative strategies have been adopted:

(i) those creating chimeric translational and transcriptional constructs using FPs to gain spatiotemporal information about gene activity in the plant developmental context;

(ii) those that specifically target FPs to organelles and vesicles or to the cell boundary components to understand subcellular dynamics and interactions (Table 1).

Corresponding author: Mathur, J. (jmathur@uoguelph.ca).

 1360-1385/$ – see front matter doi:10.1016/j.tplants.2007.08.017

Table 1. A non-comprehensive list of different targeted Fluorescent Protein probes available for plants

Target compartment	Fusion Protein[a] – Brief description	Refs
Apoplastic space[b]	secGFP – Secretory GFP created by fusing a chitinase signal peptide to GFP; transits through the ER lumen	[29]
Cell wall[b]	CFP::PRP2 – A cyan fluorescent protein fused to *A. thaliana* full-length proline-rich protein 2A	[41]
Chloroplast	RBCS1A::GFP – *A. thaliana* ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit 1A N-terminal transit peptide (amino acid residues 1–55) fused to GFP	[66]
	RECA::GFP – *A. thaliana* recombinase-A N-terminal transit peptide (amino acid residues 1–15) fused to GFP	[16]
Plastid nucleoids	PEND::GFP – N-terminal region from plastid envelope DNA-binding protein fused to GFP	[67]
Amyloplast	TP::YFP – Transit peptide from wheat granule bound starch synthase fused to YFP and placed under a rice actin promoter	[68]
Cytosol	GFP/smGFP – Soluble, modified GFP. Non-targeted GFP accumulates in the cytosol	[8,69]
Endoplasmic reticulum (ER)	ss-GFP::KDEL – GFP fused to a potato patatin storage protein N-terminal signal sequence (amino acid residues 1–23) and *Catharanthus roseus* heat-shock protein 90 C-terminal KDEL ER retrieval sequence (amino acid residues 814–817)	[70]
ER-membrane	spGFP5::CX – GFP5 fused at the N-terminal end to a sporamin signal peptide calnexin	[71]
ER-body	ss-GFP::HDEL – GFP fused to an *A. thaliana* basic chitinase N-terminal signal sequence (amino acid residues 1–21) and a C-terminal HDEL ER retrieval sequence	[8,36]
Early-type endosome	ARA7::GFP – *A. thaliana* full-length Rab isoform 7 fused to GFP	
Late-type[b] endosome	ARA6::GFP – *Arabidopsis* full-length Rab isoform 6 fused to GFP	[31]
Golgi body	ERD2::GFP – *Arabidopsis* full-length ER retention-defective-2 protein (HDEL-ER retrieval signal receptor protein) fused to GFP	[23]
cis Golgi	*Gm*MAN1::GFP – α(1,2)-mannosidase I, a resident Golgi protein from *Glycine max* fused to GFP	[72]
medial Golgi	XylT36::GFP – First 36 amino acids of β(1,2)-xylosyltransferase from *A. thaliana* fused to GFP	[73]
trans Golgi	Sttmd::GFP – Rat α(2,6)-sialyltransferase N-terminus (amino acid residues 1–52 including the single transmembrane domain) fused to GFP	[23]
Microfilaments (F-actin)	GFP::mTalin – GFP fused to the F-actin-binding domain (amino acid residues 2345–2541) from mouse *Talin* gene	[17]
	GFP::ABD2 – GFP fused to the actin-binding domain-2 of *A. thaliana FIMBRIN1* gene	[39]
Microtubules	GFP::MAP4 – GFP fused to the microtubule-binding domain (amino acid residues 935–1084) from the mouse microtubule-associated protein 4 fused to GFP	[18]
Microtubule+ end	GFP::EB1 – GFP fused to the full length cDNA of microtubule plus end-binding protein (EB1a/EB1b) from *A. thaliana*	[19,27]
Mitochondrion	CoxIV::GFP – Cytochrome oxidase subunit IV N-terminal presequence (amino acid residues 1–29) from *Saccharomyces cerevisiae* fused to GFP	[74]
	GGPS6::GFP – *A. thaliana* geranylgeranyl pyrophosphate synthase isoform 6 N-terminal (amino acids residues 1–42) fused to GFP	[75]
Nucleus	SM40::GFP – Mammalian simian virus 40 large T-antigen nuclear-localization signal (amino acid residues 126–132) fused to GFP	[66]
	C2NLS::GFP – Tobacco etch virus polypeptide c2 nuclear-localization signal (amino acid residues 1810–1854) fused to GFP	[65]
Nuclear envelope	LBR::GFP – The first 238 amino acids of the human lamin B-Receptor fused to GFP	[71]
Nuclear pore	MOS3::GFP – Full-length cDNA for *A. thaliana MOS3* (*MOFIDIER OF SNC1*) fused to GFP	[76]
Nucleolus	AtFbr1::smGFP – Full-length cDNA for *A. thaliana FIBRILLARIN1* gene fused to GFP	[77]
Chromatin	H2B::YFP – *A. thaliana* cDNa for histone 2B fused to YFP	[78]
Oil body	Oleosin::GFP – *A. thaliana* full-length oleosin isoform S3 fused to GFP	[79]
Peroxisome	GFP::PTS1 – GFP fused to pumpkin hydroxypyruvate reductase isoform 1 C-terminus (amino acid residues 377–386 of peroxisomal targeting signal type 1)	[80]
Plasma membrane	GFP::ROP6 – GFP fused to *A. thaliana* full-length Rho of plants isoform 6	[81]
Plasmodesmata	MP::GFP – full-length tobacco mosaic tobamovirus movement protein fused to GFP	[15]
Precursor-accumulating vesicle	SP::GFP::PV72C – Pumpkin 2S albumin signal peptide/sequence (residues 1–22) fused to GFP with the C-terminus (amino acid residues 557–624) of pumpkin precursor-accumulating vesicle 72-kDa protein	[82]
Proteasome	PAF::GFP – Tobacco full-length proteasome α 6(F) subunit of 20S proteasome fused to GFP	[64]
Ribosomes	THI1::GFP – The N-terminus of *A. thaliana THI1* cDNA (nucleotides1–315) fused to GFP	[63]
Lytic-type vacuole	Aleurain::GFP – N-terminus of barley aleurain thiol protease precursor (amino acid residues 1–143 including an ER-targeting signal sequence and vacuolar targeting propeptide) fused to GFP	[83]
Storage-type vacuole	ss-GFP::VSS – N-terminal signal sequence (amino acid residues 1–23) of tobacco chitinase A fused to GFP and C-terminal vacuolar sorting signal (amino acid residues 318–324) fusion construct	[84]

[a]The late-type endosome is also referred to as a prevacuolar compartment or multivesicular body [31].
[b]The apoplastic space and the wall are not considered subcellular components but are intimately related to the plant cell.

Whereas this review focuses largely on subcellular markers for use in plants, the visualization of FPs within a tissue/organ has been pivotal in educating us about various important aspects of plant development including those related to signaling [11,12] and patterning [13,14]. Many of the stable transgenic lines of *Arabidopsis thaliana* exhibiting tissue- or cell-type-specific expression patterns, such as the GAL4–GFP enhancer trap lines from Jim Haseloff and from Scott Poethig, have been created using the mGFP5-ER construct and are now available through public domain seed stock centers, such as NASC (http://*arabidopsis*.info/). In addition to their uses as marker lines in research and in helping to elucidate gene function within the developmental context, *Arabidopsis* lines exhibiting tissue-specific GFP expression are turning out to be excellent teaching material.

Understanding subcellular dynamics and interactions through targeted FPs

The ss-GFP–HDEL fusion construct, one of the first subcellular targeted probes to be created, fluorescently

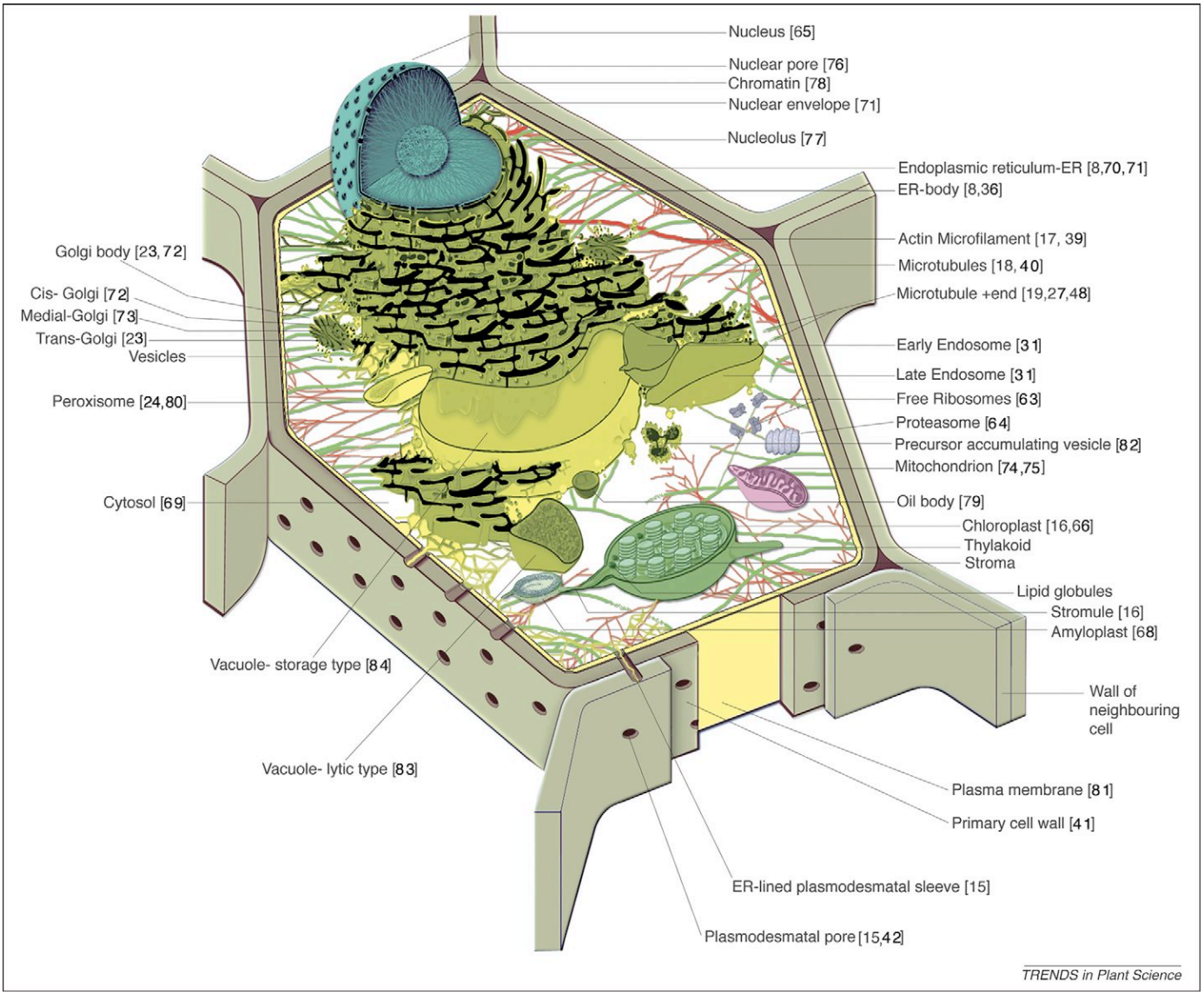


Figure 1. Diagrammatic representation of a generalized plant cell providing the reference locations of subcellular components and compartments to which the fluorescent proteins are targeted, along with the pertinent citations. The diagram is not drawn to scale and does not accurately reflect the relative numbers of subcellular components. Ribosomes [63] and proteasomes [64] have nuclear as well as free cytoplasmic locations. The figure is based on Ref. [62].

highlights the endoplasmic reticulum (ER) [8] and has been used by many laboratories to obtain their first glimpse of green fluorescence in plant cells. Subsequently, this probe has been used as a useful control when targeting FPs to other organelles (Figure 1). Although every discovery relating to the inner workings of the plant cell should be considered important and find a mention in this overview, a listing of the myriad of discoveries and the numerous probes created during the past decade has to be curtailed owing to space limitations. However, in recognition of the rapid growth of the field and the necessity of keeping it frequently updated, a new online resource devoted to 'the Illuminated Plant Cell' (http://www.illuminatedcell.com/) is being created. This website aims to provide a comprehensive listing of probes and related information to the community. Nevertheless, certain findings, such as the elucidation of mechanisms of plasmodesmatal functioning and viral movement [15], the rediscovery of plastid stromules [16], the visualization of the intricate cytoskeletal organization [17,18] and unraveling of its functioning [19–22], the recognition of novel actin-based mechanisms for organelle motility [23–25] and subcellular interactions [26–28], the recognition of exocytosis- [29] and endocytosis-mediated [30,31] mechanisms in plant development, the visualization of cellulase synthase (CESA6) organization and *in situ* activity [32], and the use of targeted GFP in microscope-based mutant screens [33–35], have resulted directly from the use of targeted fluorescent proteins.

One of the benefits of using FPS for live imaging is the observation of transient subcellular phenomena, such as the conditional highlighting of spindle-shaped ER bodies [8] in response to defense-inducing conditions (e.g. herbivory [36]) and the quick evaluation of organelle behavior in response to stress and apoptotic signals. Conditional dual targeting of certain probes, such as the ERD2–GFP, which can accumulate specifically in Golgi bodies (Figure 1) or be localized in both the ER and Golgi stacks [23], has also

been possible using targeted FPs. Furthermore, although (based on similar localization patterns) a large number of proteins are known to highlight peroxisomes and mitochondria [37,38], the need for multiple probes has been felt for the labeling of F-actin [17,39] and microtubules [18,40] following concerns that a single probe might not label the entire gamut of arrays displayed by these ubiquitous cytoskeletal elements. Thus, the number of targeted probes created sometimes out of dire necessity but often through routine experiments designed to localize a gene product, continues to grow steadily. An approach that has been particularly useful in generating probes has been the creation of random cDNA::FP fusions to identify new subcellular structures in plant cells [37,41–43]. The increased diversity in probes targeted towards the same subcellular structure provide the researcher with a range of protein tools to suit specific experimental requirements, act as the much-needed independent probes for controls and confirmation of observations, and are leading to more detailed dissection of suborganellar properties [21,22,25,44]. Because the creation, merits, demerits and numerous uses of probes targeted to specific organelles have been reviewed in detail [44–47], Table 1 and Figure 1 do not provide a comprehensive listing of every targeted fusion protein created to-date. Rather, they serve to emphasize the fact that a vast majority of subcellular compartments and components of the plant cell have become fluorescently highlighted.

The following sections briefly explore the long-term implication of the availability of multiple subcellular probes for achieving a better understanding of the plant cell.

A target within reach: the multicolored plant cell

Most early organelle-targeted probes used different versions of GFP. These probes allowed the clear visualization of the targeted organelle (Table 1) and the dissection of interactions occurring between similar organelles, such as chloroplast–chloroplast interactions through stromules [16] or the 'kiss and run' transient interaction between mitochondria [45]. However, they did not allow the visualization of interactions occurring between two or more different organelles. Single FP-based observations thus reveal only a small part of the dynamic subcellular world. The vital subcellular cooperation and coordination of interactions can best be pursued when more than one FP tags are used for targeting different structures [24,27,48]. Therefore, there is a growing trend to replace GFP with other compatible, colored FPs to achieve the simultaneous, multicolor visualization of multiple organelles and their interactions. For example, the GFP–MAP4 (MBD) probe, which has served as a very useful label for cortical microtubule arrays [18], is now also available in cyan (CFP–MAP4) [48], yellow (YFP–MAP4) [27] and red (DsRed–MAP4) [49] versions. The availability of these different FP-tagged versions of MAP4 has allowed the fine analysis of microtubule dynamics and led to numerous new insights [19,20,26,27,48,49]. The multicolor visualization approach is also resulting in a slow reversal of the tendency to exclude chlorophyll autofluorescence in green tissues by creating specific narrow band filters for GFP. In fact, in many live visualization strategies, not only does the orange-red chlorophyll provide a bright counter-fluorescence to GFP but it also allows the simultaneous visualization of chloroplasts with other FP-labeled organelles (Figure 2a,h). Most importantly, the photobleaching of chloroplasts can be used as an internal control for photodamage-induced artifacts (Box 1). Figure 2 provides a few examples of multicolor probes that are being used for the simultaneous visualization of different organelles in plant cells. In the simplest strategy for creating a multicolor line, two stable transgenic lines carrying dissimilar FP-probes targeted to separate organelles can be crossed. By including chloroplasts as autofluorescent structures, this strategy easily allows the visualization of three different organelles within the same cell (Figure 2a,h,i). Although subject to limitations imposed by gene-silencing mechanisms, the two-color-FP-containing plant can be further transformed and selected for inclusion of an additional FP-marker (Figure 2b–e). Although the expression of four FPs [e.g. ECFP (emission max. ca. 475 nm), EGFP (emission max. 509 nm), EYFP (emission max. ca. 527 nm) and RFP (monomeric DsRed/mRFP, emission max. ca. 607 nm) [9,10,50]] can be achieved, the actual visualization and convincing separation of more than four colors within a cell is still a technically challenging task. Theoretically, very fine spectral separation can be achieved by confining data collection to peak emission wavelengths for different

Box 1. Knowing the 'darker side' of FP-based technology

The FP-based method for studying plants does require a cautionary note. Because each FP has a specific size and characteristic folding properties, the addition of an FP-tag to determine the subcellular localization and behavioral properties of another protein of interest must be mindful of issues related to alterations of protein mobility, turnover and stability, in addition to the possible alterations in subcellular localization patterns. The size of the protein being fused to the FP, the information related to the folding of the fusion protein, the shielding of a signal sequence or the inadvertent snipping off of a portion of the C-terminal sequence, the introduction of a 'hinge' between the protein of interest and the FP, the unintentional introduction of a mutation in a PCR-based cloning approach, are all considerations that should be matched by adequate controls. Artifacts might also result from using multimeric versus monomeric versions of a given FP or by relying overly on transient overexpression data versus stable expression (or vice versa) for a particular fusion-protein probe. Although transient expression experiments, including those resulting in a sudden subcellular flooding of a FP-probe through the use of inducible promoters, can result in major misinterpretations of protein behavior and localization, even stable transgenic lines should be carefully screened for the range of protein expression, plasmid insertion related effects and the possible developmental consequences before putting forward an opinion on gene function. The most common misinterpretations result from faulty imaging methods and conditions, especially where broadband filters that allow a bleed-through of native autofluorescence are used. This specific imaging artifact is a major concern in conclusions based on FP-colocalization or FRET interactions. Great caution must be exercised when observing motile organelles over time, because even transient changes in high-intensity laser-induced photobleaching as well as membrane damage can greatly skew motility data. Again, data overextraction through the use of non-transparent algorithms and data extrapolation software are concerns associated with FP-technology. More detailed discussions on the advantages and disadvantages of FP-based probes and the limitations of light microscopy are available [5–7,9,10,47,50,60].

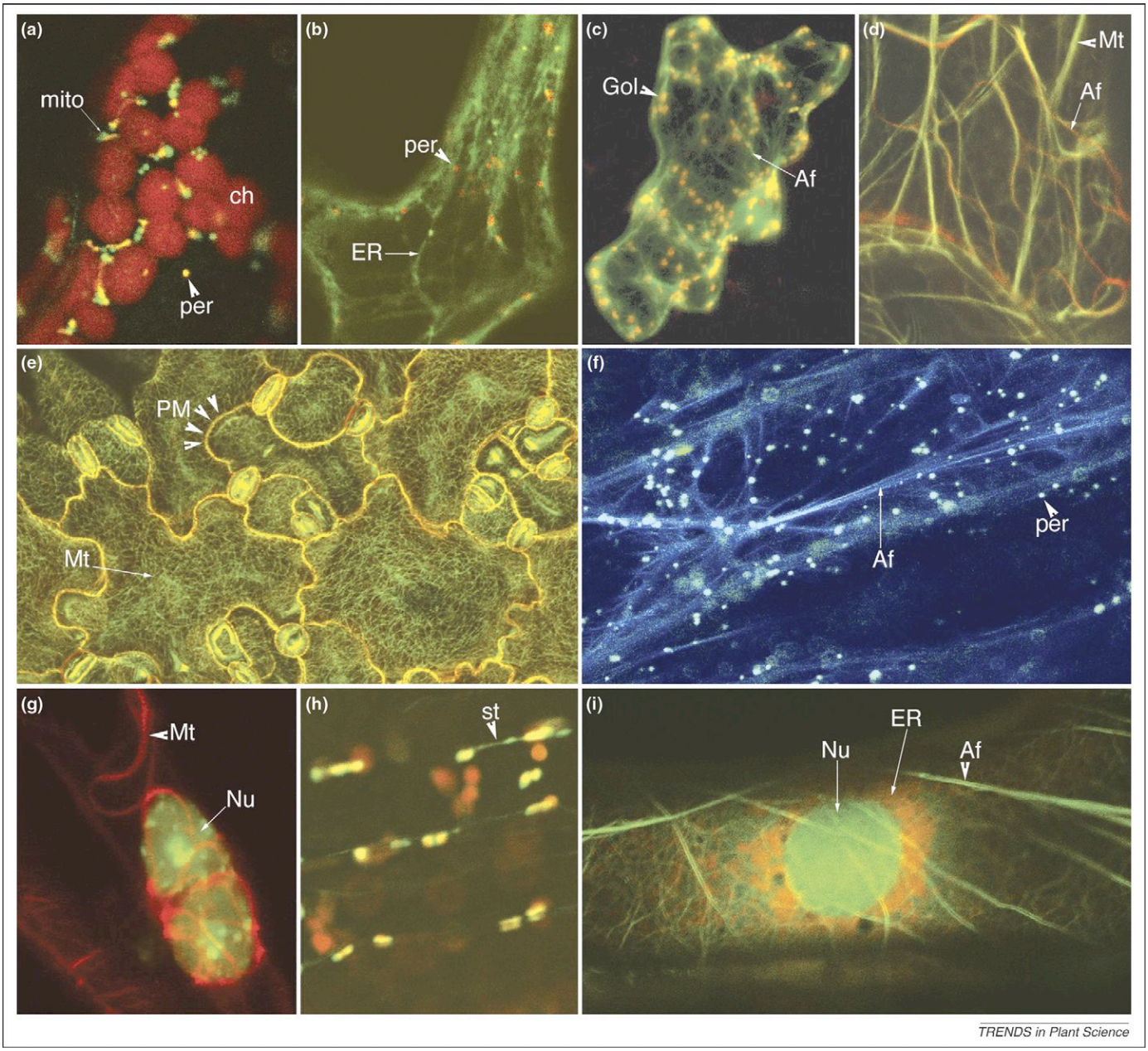


Figure 2. Transgenic lines in *Arabidopsis thaliana* carrying combinations of probes targeted to different organelles and compartments are being created for simultaneous multicolor visualization of the living plant cell. (**a**) Chloroplasts (ch; red autofluorescence), mitochondria (mito; green fluorescent) and peroxisomes (per; yellow fluorescence [24]) visualized simultaneously. (**b**) Peroxisomes (per; false allocated red color for YFP–SKL target [24]) and endoplasmic reticulum (GFP targeted to the ER [8]) visualized in a single confocal section. (**c**) Golgi bodies (Gol; false allocated red color for ERD2–GFP target [23]) and actin microfilaments (Af; false allocated green color for YFP–mTalin target [17]) visualized in a pavement cell. (**d**) Simultaneous visualization of the two major cytoskeletal elements in plants through GFP labeled F-actin (Af; targeted through GFP–mTalin [17]) and DsRed2–MAP4(MBD)-labeled microtubules (Mt; red color). (**e**) Visualization of YFP-labeled plasma membrane [37] and GFP-labeled microtubules [18]. (**f**) Peroxisomes (per; YFP–SKL target [24]) and cyan colored F-actin (Af; CFP–mTalin) covisualized using dual band CFP–YFP filter (Chroma technology-filter set 59017). (**g**) Nucleus (Nu) highlighted using GFP–NLS [65] within a microtubule cage (Mt; red color; MBD). (**h**) Multicolor visualization can offer clues about the relative amounts of proteins, viz, GFP (green) and Chlorophyll (red autofluorescence) as well as their coincident localization (yellow) in different chloroplasts (ch) within the same area of the cell. Also shown are stromules (st) connecting chloroplasts. (**i**) The simultaneous visualization strategy can be greatly augmented by using morphological criterion in addition to multicolor FP targeting as shown in this single confocal scan. The round GFP–NLS targeted [65] nucleus (Nu), is clearly distinguished from the GFP–mTalin [17] targeted F-actin (Af) and the net-like endoplasmic reticulum (ER; targeted using a HKDEL [8] fusion to DsRed2).

probes. Whereas this is possible for brightly fluorescent cells where the different FPs display roughly similar levels of fluorescence intensities, the large overlaps in excitation and emission spectra for most commonly used FPs, combined with the subcellular motility of organelles, frequently create confusing color-overlaps. Approaches aimed at increasing wavelength resolution for multicolor imaging range from spectrophotometric separation or/and algorithm-based FP-specific spectral profiling for protein discrimination. Alternatively, FPs possessing more stringent spectral characteristics are available for use [9,10]. Recently, the creation of several inducible promoters has introduced another exciting range of possibilities whereby the FP remains unexpressed until the chimeric gene is triggered by the exogenous application of an inducing chemical or change of temperature regime [21,51,52]. In

addition, several photo-inducible FPs have become available. These include PA-GFP (photoactivable-GFP), which becomes activated by 405 nm-light to produce a many-fold increase in fluorescence [53]. PA-GFP has been targeted to the ER to understand protein dynamics within this compartment [28]. Similarly EosFP [54] and Kaede [55], two FPs that rapidly change color from green to red upon activation by near-UV light (ca. 390 nm) have been used for the visualization of endocytosis events at the plasma membrane [30] and to improve our understanding of mitochondrial fusion and division [56], respectively. FPs that accumulate in the cytosol and respond to specific activator molecules, such as reactive oxygen species [57] or to changes in the status of various ions, including H^+, Ca^{2+}, Cl^- and NO_3^- (reviewed in Ref. [58]), and destabilized versions of FP [59] have been developed and are valuable additions to the fluorescent protein tool kit.

Although not discussed here, advanced imaging techniques, such as bimolecular fluorescence complementation (BiFC [60]), biluminescence resonance energy transfer (BRET), Forster or fluorescence resonance energy transfer (FRET), fluorescence lifetime imaging (FLIM) and fluorescence recovery after photobleaching (FRAP [61]) (reviewed in Refs [5–7,9,10,50]), are all off-shoots of FP-based technology whose application in plant research is gathering momentum.

Targeted FPs and EIRPing of plants: an emerging concept

As discussed earlier, FPs have provided us with the ability to look inside living plant cells and have revealed that, within their rigid walls, plant cells actually display rapid subcellular dynamics. To ensure survival, a rooted plant needs to respond very quickly to diverse environmental cues. Interestingly, our understanding of a plant's response to a given stimulus comes from observations that are usually made long after the causal event has occurred. For example, although we know that, like all other living organisms, plants suffer from stress, we do not know the earliest subcellular indications of stress shown by a plant cell. Similarly, we recognize that plants are susceptible to pests and diseases but have only a hazy idea about the earliest responses of a plant cell to the invasion of its epidermal surface.

The fluorescently illuminated plant cell thus has a lot of new information to offer through its rapid response to environmental cues. In the long term, this information can be judiciously combined with molecular-genetic strategies to devise better strategies for plant improvement and management. These thoughts and the availability of numerous targeted fluorescent protein probes and transgenic lines have led to the idea of generating an early intracellular response profile for plants (EIRPP; http://www.uoguelph.ca/~jmathur/research/EIRP.html). Proof of concept studies for EIRPP using the model plant *Arabidopsis thaliana* are already underway.

Acknowledgements

An overview on FP-based contributions to understanding plants merits the inclusion of many more references than has been possible here. I hope that the comprehensive online resource being created will serve as a suitable atonement for the omissions. I thank Alison Sinclair and Preetinder Dhanoa for help in compiling the initial list of FP probes that appeared in a similar mini-review [62], Anshudeep Mathur for the creation of the Interactive flash animation and the design of the webpage, David Logan, Sean Cutler, Geoff Wasteneys, Rob Mullen and Hugo Zhang for their critical inputs. The Natural Sciences and Engineering Research Council of Canada (NSERC) fund the EIRPP program initiated by my laboratory.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2007.08.017.

References

1 Grolig, F. and Pierson, E.S. (2000) Cytoplasmic streaming: From flow to track. In *Actin: A dynamic framework for multiple plant cell functions* (Staiger, C. *et al.*, eds), pp. 165–190, Kluwer Academic Publishers
2 Fricker, M. *et al.* (2001) Fluorescent probes for living plant cells. In *Plant cell biology* (2nd edn) (Hawes, C. and Satiat-Jeunemaitre, B., eds), pp. 35–84, Oxford University Press
3 Zhang, D. *et al.* (1990) Microtubule dynamics in living dividing plant cells: confocal imaging of microinjected fluorescent brain tubulin. *Proc. Natl. Acad. Sci. U. S. A.* 87, 8820–8824
4 Chalfie, M. *et al.* (1994) Green fluorescent protein as a marker for gene expression. *Science* 263, 802–805
5 Fricker, M. *et al.* (2006) Quantitative fluorescence microscopy: From Art to Science. *Annu. Rev. Plant Biol.* 57, 79–107
6 Haseloff, J. and Siemering, K.R. (2006) The uses of green fluorescent protein in plants. *Methods Biochem. Anal.* 47, 259–284
7 Tsien, R.Y. (2003) Imagining imaging's future. *Nat. Rev. Mol. Cell Biol.* 4, SS16–SS21
8 Haseloff, J. *et al.* (1997) Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. *Proc. Natl. Acad. Sci. U. S. A.* 94, 2122–2127
9 Shaner, N.C. *et al.* (2005) A guide to choosing fluorescent proteins. *Nat. Methods* 2, 905–909
10 Giepmans, B.N. *et al.* (2006) The fluorescent toolbox for assessing protein location and function. *Science* 312, 217–224
11 Benkova, E. *et al.* (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell* 115, 591–602
12 Fu, Y. *et al.* (2005) *Arabidopsis* interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis. *Cell* 120, 687–700
13 Berger, F. *et al.* (1998) Stomata patterning on the hypocotyl of *Arabidopsis thaliana* is controlled by genes involved in the control of root epidermis patterning. *Dev. Biol.* 194, 226–234
14 Kurup, S. *et al.* (2005) Marking cell lineages in living tissues. *Plant J.* 42, 444–453
15 Heinlein, M. *et al.* (1995) Interaction of tobamovirus movement proteins with the plant cytoskeleton. *Science* 270, 1983–1985
16 Kohler, R.H. *et al.* (1997) Exchange of protein molecules through connections between higher plant plastids. *Science* 276, 2039–2042
17 Kost, B. *et al.* (1998) A GFP-mouse talin fusion protein labels plant actin filaments *in vivo* and visualizes the actin cytoskeleton in growing pollen tubes. *Plant J.* 16, 393–401
18 Marc, J. *et al.* (1998) A GFP-MAP4 reporter gene for visualizing cortical microtubule rearrangements in living epidermal cells. *Plant Cell* 10, 1927–1939
19 Chan, J. *et al.* (2003) EB1 reveals mobile microtubule nucleation sites in *Arabidopsis*. *Nat. Cell Biol.* 5, 967–971
20 Shaw, S.L. *et al.* (2003) Sustained microtubule treadmilling in *Arabidopsis* cortical arrays. *Science* 300, 1715–1718
21 Ketelaar, T. *et al.* (2004) Green fluorescent protein-mTalin causes defects in actin organization and cell expansion in *Arabidopsis* and inhibits actin depolymerizing factor's actin depolymerizing activity *in vitro*. *Plant Physiol.* 136, 3990–3998
22 Mathur, J. *et al.* (2003) *Arabidopsis CROOKED* encodes for the smallest subunit of the ARP2/3 complex and controls cell shape by region specific fine F-actin formation. *Development* 130, 3137–3144
23 Boevink, P. *et al.* (1998) Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. *Plant J.* 15, 441–447

24 Mathur, J. *et al.* (2002) Simultaneous visualization of peroxisomes and cytoskeletal elements reveals actin and not microtubule-based peroxisome motility in plants. *Plant Physiol.* 128, 1031–1045
25 Kim, H. *et al.* (2005) Actin filaments play a critical role in vacuolar trafficking at the Golgi complex in plant cells. *Plant Cell* 17, 888–890
26 Dhonukshe, P. *et al.* (2005) Microtubule plus-ends reveal essential links between intracellular polarization and localized modulation of endocytosis during division-plane establishment in plant cells. *BMC Biol.* 3, 11
27 Mathur, J. *et al.* (2003) A novel localization pattern for an EB1-like protein links microtubule dynamics to endo-membrane organization. *Curr. Biol.* 13, 1991–1998
28 Runions, J. *et al.* (2006) Photoactivation of GFP reveals protein dynamics within the endoplasmic reticulum membrane. *J. Exp. Bot.* 57, 43–45
29 Batoko, H. *et al.* (2000) A rab1 GTPase is required for transport between the endoplasmic reticulum and Golgi apparatus and for normal Golgi movement in plants. *Plant Cell* 12, 2201–2217
30 Dhonukshe, P. *et al.* (2007) Clathrin-mediated constitutive endocytosis of PIN auxin efflux carriers in *Arabidopsis*. *Curr. Biol.* 17, 520–527
31 Ueda, T. *et al.* (2004) Functional differentiation of endosomes in *Arabidopsis* cells. *Plant J.* 40, 783–789
32 Paredez, A.R. *et al.* (2006) Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* 312, 1491–1495
33 Whittington, A.T. *et al.* (2001) MOR1 is essential for organizing cortical microtubules in plants. *Nature* 411, 610–613
34 Logan, D.C. *et al.* (2003) The genetic control of plant mitochondrial morphology and dynamics. *Plant J.* 36, 500–509
35 Mano, S. *et al.* (2004) An *Arabidopsis* dynamin-related protein, DRP3A, controls both peroxisomal and mitochondrial division. *Plant J.* 38, 487–498
36 Matsushima, R. *et al.* (2003) The ER body, a novel endoplasmic reticulum-derived structure in *Arabidopsis*. *Plant Cell Physiol.* 44, 661–666
37 Cutler, S.R. *et al.* (2000) Random GFP:cDNA fusions enable visualization of subcellular structures in cells of *Arabidopsis* at a high frequency. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3718–3723
38 Li, S. *et al.* (2006) Systematic analysis of *Arabidopsis* organelles and a protein localization database for facilitating fluorescent tagging of full-length *Arabidopsis* proteins. *Plant Physiol.* 141, 527–539
39 Sheahan, M.B. *et al.* (2004) A green fluorescent protein fusion to actin-binding domain 2 of *Arabidopsis* fimbrin highlights new features of a dynamic actin cytoskeleton in live plant cells. *Plant Physiol.* 136, 3968–3978
40 Ueda, K. *et al.* (1999) Visualization of microtubules in living cells of transgenic *Arabidopsis thaliana*. *Protoplasma* 206, 201–206
41 Tian, G.W. *et al.* (2004) High-throughput fluorescent tagging of *Arabidopsis* full-length gene products *in planta*. *Plant Physiol.* 135, 25–38
42 Escobar, N.M. *et al.* (2003) High-throughput viral expression of cDNA-green fluorescent protein fusions reveals novel sub-cellular addresses and identifies unique proteins that interact with plasmodesmata. *Plant Cell* 15, 1507–1523
43 Koroleva, O.A. *et al.* (2005) High-throughput protein localization in *Arabidopsis* using *Agrobacterium*-mediated transient expression of GFP-ORF fusions. *Plant J.* 41, 162–174
44 Matheson, L.A. *et al.* (2006) Traffic between the plant endoplasmic reticulum and Golgi apparatus: to the Golgi and beyond. *Curr. Opin. Plant Biol.* 9, 601–609
45 Logan, D.C. (2007) The mitochondrial compartment. *J. Exp. Bot.* 58, 1225–1243
46 Weber, A.P. and Fischer, K. (2007) Making the connections - The crucial role of metabolite transporters at the interface between chloroplast and cytosol. *FEBS Lett.* 581, 2215–2222
47 Wasteneys, G.O. and Yang, Z. (2004) New views on the plant cytoskeleton. *Plant Physiol.* 136, 3884–3891
48 Dhonukshe, P. and Gadella, T.W., Jr (2003) Alteration of microtubule dynamic instability during preprophase band formation revealed by yellow fluorescent protein-CLIP170 microtubule plus-end labeling. *Plant Cell* 15, 597–611
49 Dixit, R. and Cyr, R. (2004) Encounters between dynamic cortical microtubules promote ordering of the cortical array through angle-dependent modifications of microtubule behavior. *Plant Cell* 16, 3274–3284
50 Dixit, R. *et al.* (2006) Using intrinsically fluorescent proteins for plant cell imaging. *Plant J.* 45, 599–615
51 Zuo, J. *et al.* (2006) Applications of chemical-inducible expression systems in functional genomics and biotechnology. *Methods Mol. Biol.* 323, 329–334
52 Young, L.W. *et al.* (2005) A high- and low-temperature inducible *Arabidopsis thaliana* HSP101 promoter located in a nonautonomous Mutator-like element. *Genome* 48, 547–555
53 Patterson, G.H. and Lippincott-Schwartz, J. (2004) Selective photolabeling of proteins using photoactivatable GFP. *Methods* 32, 445–450
54 Wiedenmann, J. and Nienhaus, G.U. (2006) Live-cell imaging with EosFP and other photoactivatable marker proteins of the GFP family. *Expert Rev. Proteomics* 3, 361–374
55 Gurskaya, N.G. *et al.* (2006) Engineering of a monomeric green-to-red photoactivatable fluorescent protein induced by blue light. *Nat. Biotechnol.* 24, 461–465
56 Arimura, S. *et al.* (2004) Frequent fusion and fission of plant mitochondria with unequal nucleoid distribution. *Proc. Natl. Acad. Sci. U. S. A.* 101, 7805–7808
57 Bulina, M.E. *et al.* (2006) A genetically encoded photosensitizer. *Nat. Biotechnol.* 24, 95–99
58 Truong, K. *et al.* (2007) Calcium indicators based on calmodulin-fluorescent protein fusions. *Methods Mol. Biol.* 352, 71–82
59 Triccas, J.A. *et al.* (2002) Destabilized green fluorescent protein for monitoring transient changes in mycobacterial gene expression. *Res. Microbiol.* 153, 379–383
60 Walter, M. *et al.* (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. *Plant J.* 40, 428–438
61 Martens, H.J. *et al.* (2006) Quantification of plasmodesmatal endoplasmic reticulum coupling between sieve elements and companion cells using fluorescence redistribution after photobleaching. *Plant Physiol.* 142, 471–480
62 Dhanoa, P.K. *et al.* (2006) Illuminating subcellular structures and dynamics in plants: a fluorescent protein toolbox. *Can. J. Bot.* 84, 515–522
63 Chabregas, S.M. *et al.* (2003) Differential usage of two in-frame translational start codons regulates subcellular localization of *Arabidopsis thaliana* THI1. *J. Cell Sci.* 116, 285–291
64 Kim, M. *et al.* (2003) Molecular characterization of NbPAF encoding the alpha6 subunit of the 20S proteasome in *Nicotiana benthamiana*. *Mol. Cells* 15, 127–132
65 Grebenok, R.J. *et al.* (1997) Green-fluorescent protein fusions for efficient characterization of nuclear targeting. *Plant J.* 11, 573–586
66 Chiu, W. *et al.* (1996) Engineered GFP as a vital reporter in plants. *Curr. Biol.* 6, 325–330
67 Terasawa, K. and Sato, N. (2005) Visualization of plastid nucleoids *in situ* using the PEND–GFP fusion protein. *Plant Cell Physiol.* 46, 649–660
68 Langeveld, S.M. *et al.* (2000) B-type granule containing protrusions and interconnections between amyloplasts in developing wheat endosperm revealed by transmission electron microscopy and GFP expression. *J. Exp. Bot.* 51, 1357–1361
69 Davis, S.J. and Vierstra, R.D. (1998) Soluble, highly fluorescent variants of green fluorescent protein (GFP) for use in higher plants. *Plant Mol. Biol.* 36, 521–528
70 Boevink, P. (1996) Virus-mediated delivery of the green fluorescent protein to the endoplasmic reticulum of plant cells. *Plant J.* 10, 935–941
71 Irons, S.L. *et al.* (2003) The first 238 amino acids of the human lamin B receptor are targeted to the nuclear envelope in plants. *J. Exp. Bot.* 54, 943–950
72 Nebenfuhr, A. *et al.* (1999) Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system. *Plant Physiol.* 121, 1127–1142
73 Follet-Gueye, M.L. *et al.* (2003) An improved chemical fixation method suitable for immunogold localization of green fluorescent protein in the Golgi apparatus of tobacco Bright Yellow (BY-2) cells. *J. Histochem. Cytochem.* 51, 931–940
74 Kohler, R.H. *et al.* (1997) The green fluorescent protein as a marker to visualize plant mitochondria *in vivo*. *Plant J.* 11, 613–621

75 Zhu, X.F. *et al.* (1997) Geranylgeranyl pyrophosphate synthase encoded by the newly isolated gene *GGPS6* from *Arabidopsis thaliana* is localized in mitochondria. *Plant Mol. Biol.* 35, 331–341
76 Zhang, Y. and Li, X. (2005) A putative nucleoporin 96 is required for both basal defense and constitutive resistance responses mediated by suppressor of npr1-1,constitutive 1. *Plant Cell* 17, 1306–1316
77 Pih, K.T. *et al.* (2000) Molecular cloning and targeting of a fibrillarin homolog from *Arabidopsis*. *Plant Physiol.* 123, 51–58
78 Boisnard-Lorig, C. *et al.* (2001) Dynamic analyses of the expression of the HISTONE:YFP fusion protein in *Arabidopsis* show that syncytial endosperm is divided in mitotic domains. *Plant Cell* 13, 495–509
79 Wahlroos, T. *et al.* (2003) Oleosin expression and trafficking during oil body biogenesis in tobacco leaf cells. *Genesis* 35, 125–132
80 Mano, S. *et al.* (1999) Light regulates alternative splicing of hydroxypyruvate reductase in pumpkin. *Plant J.* 17, 309–320
81 Bischoff, F. *et al.* (2000) Localization of AtROP4 and AtROP6 and interaction with the guanine nucleotide dissociation inhibitor AtRhoGDI1 from *Arabidopsis*. *Plant Mol. Biol.* 42, 515–530
82 Shimada, T. *et al.* (2002) A vacuolar sorting PV72 on the membrane of vesicles that accumulate precursors of seed storage proteins (PAC Vesicles). *Plant Cell Physiol.* 43, 1086–1095
83 Di Sansebastiano, G.P. *et al.* (2001) Regeneration of a lytic central vacuole and of neutral peripheral vacuoles can be visualized by green fluorescent proteins targeted to either type of vacuoles. *Plant Physiol.* 126, 78–86
84 Di Sansebastiano, G.P. *et al.* (1998) Specific accumulation of GFP in a non-acidic vacuolar compartment via a C-terminal propeptide-mediated sorting pathway. *Plant J.* 15, 449–457