

Illuminating subcellular structures and dynamics in plants: a fluorescent protein toolbox¹

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Abstract: The discovery and development of multicoloured fluorescent proteins has led to the exciting possibility of observing a remarkable array of subcellular structures and dynamics in living cells. This minireview highlights a number of the more common fluorescent protein probes in plants and is a testimonial to the fact that the plant cell has not lagged behind during the live-imaging revolution and is ready for even more in-depth exploration.

Key words: fluorescent protein, live imaging, microscopy, organelle, targeting signal.

Résumé : La découverte et le développement de protéines fluorescentes multicolores ouvrent d'intéressantes possibilités pour l'observation de remarquables arrangements de structures et de dynamiques sub-cellulaires dans les cellules vivantes. Cette mini revue considère un certain nombre des sondes protéiniques fluorescentes les plus communes et témoigne du fait que la cellule végétale n'est pas en retard dans la révolution de l'imagerie in vivo et est prête pour des explorations encore plus approfondies.

Mots clés : protéine fluorescente, imagerie vitale, microscopie, organelle, signal de ciblage.

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Introduction

Cell biology is beginning to rely increasingly on the ability to visualize, track, and quantify gene activity to fully comprehend gene function in living cells. Toward this end, genetically encoded fluorescent proteins, such as the green fluorescent protein (GFP) and its numerous colour variants (Shaner et al. 2005) that span the visible spectrum and do not require exogenous substrates, cofactors, or chemical treatments, are now considered standard tools in almost all live-imaging techniques (Chalfie et al. 1994; Haraguchi et al. 2002; Tsien 2003). Indeed, the creation of fluorescent protein fusions to a full-length or truncated gene product of choice is now a routine task in most molecular biology laboratories. Further, new innovations in live imaging are continuing to be developed at a remarkable pace as additional novel fluorescent proteins are discovered, as computer-assisted image acquisition and data management capabilities become stronger, and as researchers delve deeper into nanometric dimensions for elucidating the intricacies of interorganelle and protein-protein interactions in

cells (Zhang et al. 2002; Tsien 2003; Wada and Suetsugu 2004; Chapman et al. 2005).

Fluorescent fusion proteins are useful probes for studying different subcellular compartments and structures in plant cells

With the genomic sequences of *Arabidopsis thaliana* and *Oryza sativa* readily available (Tyagi et al. 2004; Bevan and Walsh 2005), fluorescent protein aided live-imaging technologies have become arguably the most powerful tools for plant scientists interested in placing any given gene product back into its intracellular context. Consequently, many different strategies (reviewed in Leffel et al. 1997; Haseloff et al. 1999; Hanson and Kohler 2001; Hawes et al. 2001; Pimpl and Denecke 2002; Ehrhardt 2003; Brandizzi et al. 2004; Kurup et al. 2005; Haseloff and Siemering 2006) have converted the plant cell into a metaphorical "colouring book" and resulted in the targeting of different multicoloured fluorescent protein probes to specific organelles and subcellular compartments. Some of these probes, such as the ss-GFP-HDEL serving as a marker for the lumen of the endoplasmic reticulum (Boevink et al. 1996; Haseloff et al. 1997) (see Table 1), are already considered to be "classic" organelle marker proteins and have been used for the generation of numerous stable transgenic lines that are now available through public domain seed-stock centers (see <http://www.plantsci.cam.ac.uk/Haseloff/geneControl/catalogFrame.html>). The short, noncomprehensive list of other various classic fluorescent protein probes in Table 1 represents primarily the first report(s) that allow for the vis-

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Table 1. Various fluorescent fusion proteins used as probes for different organelles or other subcellular compartments/structures in plant cells.

Compartment	Fusion protein ^a	Reference(s)
Cell wall	sec-GFP: <i>Arabidopsis</i> basic chitinase N-terminal signal sequence (amino acid residues 1–21) fused to GFP	Batoko et al. 2000
Chloroplast	CFP-PRP2: <i>Arabidopsis</i> full-length proline-rich protein 2A fused to CFP	Tian et al. 2004
	RBCS1A-GFP: <i>Arabidopsis</i> ribulose 1,5 bisphosphate carboxylase/oxygenase small subunit 1A N-terminal transit peptide (amino acid residues 1–55) fused to the GFP	Chiu et al. 1996
Endoplasmic reticulum (ER)	RecA-GFP: <i>Arabidopsis</i> recombinase-A N-terminal transit peptide (amino acid residues 1–15) fused to GFP	Kohler et al. 1997a
	ss-GFP-KDEL: potato patatin storage protein N-terminal signal sequence (amino acid residues 1–23) and <i>Catharanthus roseus</i> heat shock protein 90 C-terminal KDEL ER retrieval sequence (amino acid residues 814–817) fused to GFP	Boevink et al. 1996
ER body	ss-GFP-HDEL: <i>Arabidopsis</i> basic chitinase N-terminal signal sequence (amino acid residues 1–21) and a C-terminal HDEL ER retrieval sequence fused to GFP	Haseloff et al. 1997
	ss-GFP-HDEL: <i>Arabidopsis</i> basic chitinase N-terminal signal sequence (amino acid residues 1–21) and a C-terminal HDEL ER retrieval sequence fused to GFP	Gunning 1998; Haseloff and Siemerling 1998
Endosome early-type	Ara7-GFP ^b : <i>Arabidopsis</i> full-length RabF2b GTPase (isoform 7) fused to GFP	Ueda et al. 2001
	YFP-AtRabF2b ^b : <i>Arabidopsis thaliana</i> full-length Rab GTPase isoform F2b (Ara7) fused to YFP	Kotzer et al. 2004
	GFP-AtVAMP27 ^b : <i>Arabidopsis thaliana</i> full-length vesicle-associated membrane protein 727 (refer to Uemura et al. (2004) for information on VAMP27 and other members in the R class of the <i>Arabidopsis</i> SNARE gene family) fused to GFP	Uemura et al. 2004
Endosome late-type ^c	Ara6-GFP: <i>Arabidopsis</i> full-length Rab isoform 6 fused to GFP	Ueda et al. 2001
	GmMan1-GFP: <i>Glycine max</i> full-length α -1,2-mannosidase 1 (minus the protein's C-terminal 11 amino acid residues) fused to GFP	Nebenfuhr et al. 1999
Golgi cis	XylT36-GFP: <i>Arabidopsis</i> β 1–2-xylosyltransferase N terminus (amino acid residues 1–36 including the protein's single transmembrane domain) fused to GFP	Pagny et al. 2003
Golgi medial	STtmd-GFP: rat 2,6-sialyl transferase N terminus (amino acid residues 1–52 including the protein's single transmembrane domain) fused to GFP	Boevink et al. 1998
	GnTI-GFP: tobacco β 1,2- <i>N</i> -acetylglucosaminyl-transferase I N terminus (amino acid residues 1–77) fused to GFP	Essl et al. 1999
Microfilaments	GFP-mTalin: mouse talin f-actin-binding domain (amino acid residues 2345–2541) fused to GFP	Kost et al. 1998
Microtubules	GFP-MAP4: mouse microtubule-associated protein 4 microtubule-binding domain (amino acid residues 935–1084) fused to GFP	Marc et al. 1998
	GFP-EB1b: <i>Arabidopsis</i> full-length microtubule plus end-binding protein 1b fused to GFP	Mathur et al. 2003
Mitochondrion	CoxIV-GFP: <i>Saccharomyces cerevisiae</i> cytochrome oxidase subunit IV N-terminal presequence (amino acid residues 1–29) fused to GFP	Kohler et al. 1997b
Nuclear envelope	GGPS6-GFP: <i>Arabidopsis</i> geranylgeranyl pyrophosphate synthase isoform 6 N-terminal presequence (amino acids residues 1–42) fused to GFP	Zhu et al. 1997
	MFP1-GFP ^d : Tomato MAR (matrix attachment region binding protein) binding filament-like protein 1 fused to GFP	Gindullis and Meier 1999; Samaniego et al. 2005
Nucleus	LBR-GFP ^{5d} : human lamin B receptor N terminus (amino acid residues 1–238) with codon usage optimized for in planta expression fused to GFP ₅ (GFP ₅ is a modified version of GFP that lacks an aberrant cryptic intron (Haseloff et al. 1997))	Irons et al. 2003
	SM40-GFP: mammalian simian virus 40 large T-antigen nuclear localization signal (amino acid residues 126–132) fused to GFP	Chiu et al. 1996
Nucleus	C2NLS-GFP: tobacco etch virus polypeptide c2 nuclear localization signal (amino acid residues 1810–1854) fused to GFP	Grebenok et al. 1997
	Dof1-GFP: maize DNA binding with one finger isoform 1 N terminus (amino acid residues 1–147) fused to GFP	Yanagisawa and Sheen 1998

Table 1 (concluded).

Compartment	Fusion protein ^a	Reference(s)
Oil body	Oleosin-GFP: <i>Arabidopsis</i> full-length oleosin isoform S3 (refer to Kim et al. (2002) for information on the oleosin isoform S3 and other members of the <i>Arabidopsis</i> oleosin gene family) fused to GFP	Wahlroos et al. 2003
Peroxisome	GFP-PTS1: pumpkin hydroxypyruvate reductase isoform 1 C terminus (amino acid residues 377–386 including the C-terminal peroxisomal targeting signal type 1) fused to GFP	Mano et al. 1999
Plasma membrane	GFP-ROP6: <i>Arabidopsis</i> full-length Rho of plants isoform 6 fused to GFP	Bischoff et al. 2000
Plasmodesmata	MP-GFP: tobacco full-length mosaic tobamovirus movement protein fused to GFP	Heinlein et al. 1995
Precursor accumulating vesicle	CKL6-GFP: <i>Arabidopsis</i> full-length casein kinase-like isoform 6 fused to GFP	Lee et al. 2005
	SP-GFP-PV72C: pumpkin 2S albumin signal peptide/sequence (amino acid residues 1–22) and pumpkin precursor accumulating vesicle 72 kDa protein C-terminus (amino acid residues 557–624) fused to GFP	Shimada et al. 2002
Proteasome	PAF-GFP: tobacco full-length proteasome α 6(F) subunit of 20S proteasome fused to GFP	Kim et al. 2003
Vacuole lytic-type	Aleurain-GFP: barley aleurain thiol protease precursor N terminus (amino acid residues 1–143 including the protein's ER targeting signal sequence and vacuolar targeting propeptide) fused to GFP	Di Sansebastiano et al. 2001
Vacuole storage-type	ss-GFP-VSS: tobacco chitinase A N-terminal signal sequence (amino acid residues 1–23) and C-terminal vacuolar sorting signal (amino acid residues 318–324) fused to GFP	Di Sansebastiano et al. 1998

Note: This table does not represent a comprehensive list of all the fluorescent fusion proteins currently available as probes for different organelles or other subcellular compartments/structures in plant cells. Instead, it represents, primarily, the first report(s) that allowed for the visualization of a particular organelle, compartment, or structure in live plants.

^aGenBank accession numbers for proteins (or protein portions) fused to GFP are as follows: Ara6, NP_567008; Ara7, NP_193699; *Arabidopsis* basic chitinase, NP_566426; barley aleurain thiol protease, CAA28804; C2NLS, NP_062908; *Catharanthus roseus* heat shock protein 90, AAA16785; CKL6, AAY24535; CoxIV, NP_011328; Dof1, CAA46875; EB1b, BAB11500; GGPS6, NP_175376; GmMan1, AAF16414; GnTI, CAC80702; LBR, NP_002287; MAP4, NP_032659; MFP1, CAA69181; MP, NP_597748; mTalin, NP_035732; *Arabidopsis* oleosin S3, CAA44225; PAF, BAB10635; potato patatin storage protein, CAA81735; PRP2, NP_179710; pumpkin 2S albumin, BAA03993; pumpkin hydroxypyruvate reductase, BAA08410; pumpkin precursor accumulating vesicle 72 kDa protein, BAA25079; RBCS1A, NP_176880; recA, NP_565198; ROP6, NP_195228; SM40, NP_043127; STmd, NP_00102682; tobacco chitinase A, CAA01263; TUA6, NP_849388; VAMP27, NP_190998; XylIT, AAF77064.

^bWhile the fluorescent fusion protein Ara7-GFP was reported by Ueda et al. (2001) as a marker for early-type endosomes in tobacco BY-2 suspension cultured cells, more recent observations suggest that another version of this fluorescent fusion protein (i.e., YFP-AtRabF2b) localizes in tobacco leaf cells to late-type endosomes and, to a lesser extent, Golgi bodies (Kotzer et al. 2004). Possible reasons for the reported differences in the localization of these two related fluorescent fusion proteins include the different expression systems employed (i.e., suspension cells versus leaf cells) (Kotzer et al. 2004) and the relative position (i.e., N or C terminal) of the appended fluorescent proteins. Given these seemingly contradictory findings for Ara7 (RabF2b) as a marker for early-type endosomes, the reader may want to consider other fluorescent fusion proteins as probes for this compartment, e.g., GFP-AtVAMP727 encoding the *Arabidopsis* vesicle-associated membrane protein (also referred to as a R-SNARE) 727 fused to GFP (Uemura et al. 2004).

^cThe late-type endosome is also referred to as a prevacuolar compartment or multivesicular body (Ueda et al. 2004; Uemura et al. 2004; Tse et al. 2004).

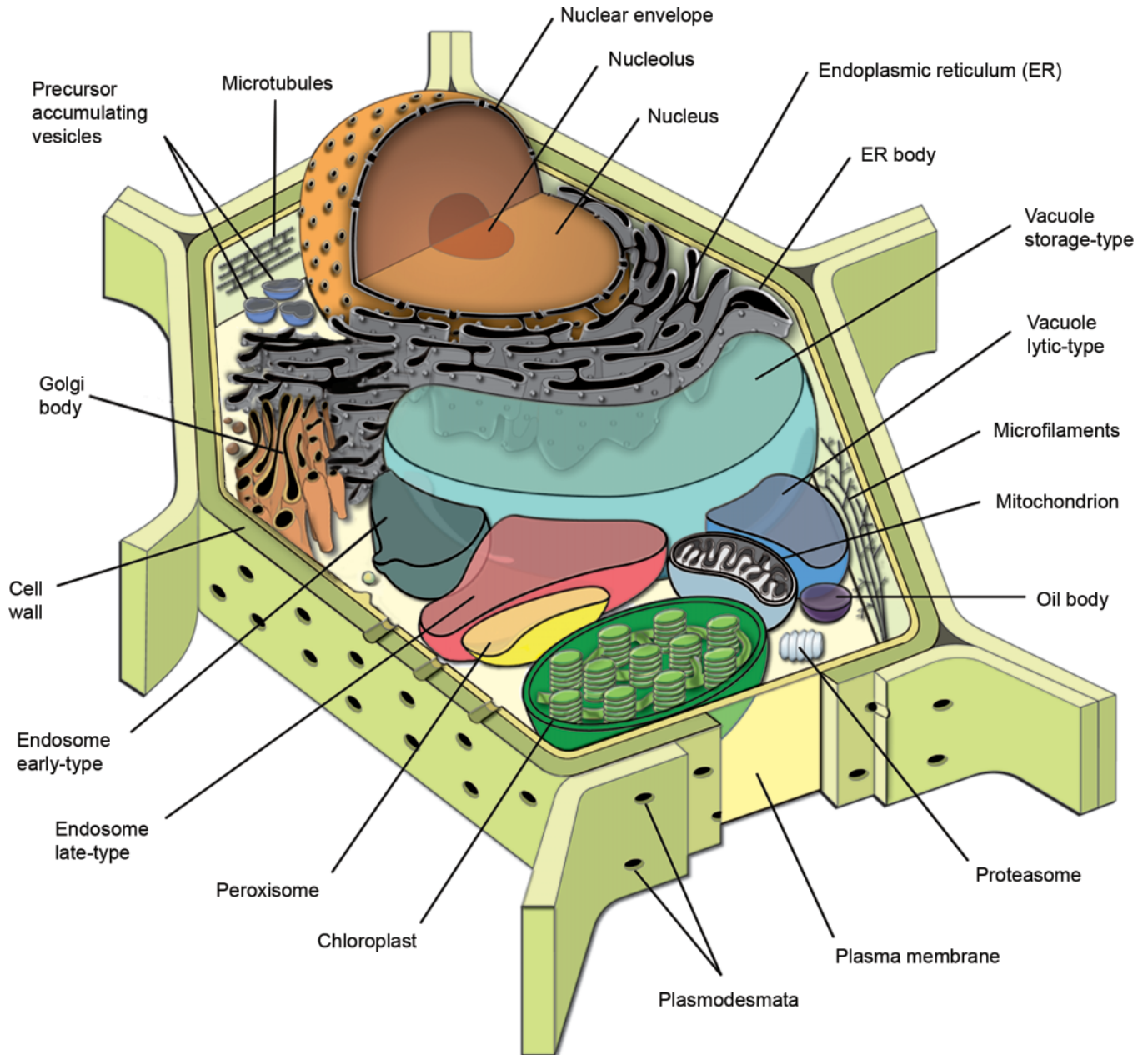
^dAlthough the fluorescent fusion protein MFP1-GFP was originally reported to be localized to the nuclear matrix (Gindullis and Meier 1999), subsequent studies revealed that this protein is localized also to the stromal side of thylakoids (Samaniego et al. 2005). Given this dual localization for MFP1, the reader may want to consider other fluorescent fusion proteins as probes for the nuclear envelope, e.g., LBR-GFP₅ encoding the N-terminal 238 amino acid residues of the human lamin B receptor fused to GFP₅ (Irons et al. 2003).

ualization of a particular organelle, compartment, or structure in live plants.

In many cases, the initial use of the fluorescent proteins listed in Table 1 was associated with important cell biological discoveries and advances such as the mechanisms of virus transport and plasmodesmatal functioning (Heinlein et al. 1995), the characterization of plastid stromules (Kohler et al. 1997a), and the intricacies of cytoskeleton organization (Kost et al. 1998; Marc et al. 1998). Nearly all of these early probes used a GFP. However, more recently, the GFP has been replaced with a variety of different-coloured fluorescent proteins. For example, GFP-MAP4, consisting of GFP linked to the microtubule-binding domain of the mouse microtubule-associated protein 4 and serving as a marker protein for microtubules (Marc et al. 1998) (Table 1), is now available in cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and red fluorescent protein (DsRed) versions (Dhonukshe and Gadella 2003; Mathur et al. 2003; Dixit and Cyr 2003). In addition, the number of fluorescent protein based probes for plants has continued to grow steadily

over the years as researchers have created random cDNA::fluorescent protein fusions in their attempts to learn more about the plant cell (Cutler et al. 2000; Escobar et al. 2003; Tian et al. 2004; Koroleva et al. 2005) or as they strive to resolve the function(s) of a specific gene during plant growth and development through its fusion to a fluorescent protein. As a result, for most organelles, there are at least several different fluorescent protein probes available now, e.g., GFP-ABD2 (GFP fused to the actin-binding domain 2 of *Arabidopsis* fimbrin1; Sheahan et al. 2004), which, like the GFP-mTalin created earlier (Kost et al. 1998) (Table 1), labels filamentous actin and TUA6-GFP (*Arabidopsis* full-length tubulin alpha 6 fused to GFP; Ueda et al. 1999), which, like GFP-MAP4 (Marc et al. 1998), labels microtubule arrays. Such a diversity of probes targeted towards the same subcellular structure are a welcome development, as not only do they provide the researcher with a range of novel tools to suit specific experimental requirements but they also act as the much-needed independent probes whose combined use should be promoted to mini-

Fig. 1. Diagrammatic representation of a generalized plant cell depicting the principal organelles, compartments, and structures described in Table 1. Note that this diagram does not reflect the actual relative sizes and numbers of the different organelles or other subcellular compartments/structures shown in it. Adapted from Campbell and Reece (2002) with permission from Pearson Education Inc., publishing as Benjamin Cummings.



mize interpretational errors. With the numerous options available, Table 1 should be considered only as a useful starting point in the search for a fluorescent protein probe of choice. Further, while tabulating the different fluorescent protein probes shown in Table 1, it also became apparent that most standard textbooks present an oversimplified version of the plant cell whereby at least some of the organelles, compartments, and structures that are described in the table are not depicted. The diagrammatic representation of a generalized plant cell in Fig. 1 is aimed at overcoming this discrepancy.

Important considerations when using fluorescent fusion protein-based probes

An overview of fluorescent fusion proteins used as probes for live plant cells would not be complete without a cautionary note revealing the “darker side” of this technology. Knowing the caveats of fluorescent fusion proteins is essential for avoiding potential pitfalls and can only serve to make the technology stronger. For example, the use of an additional protein tag such as the approximately 27 kDa GFP to determine the subcellular localization and behaviour-

ral properties of a protein of interest raises issues of altered protein turnover and stability (Marcus et al. 2001) as well as possible changes in localization patterns (Sedbrook 2004). These and other GFP-linked artefacts are being detected with increasing frequency and in certain cases have been shown to have direct, unintended consequences for normal plant cell growth and development (Marcus et al. 2001; Dixit and Cyr 2003; Ketelaar et al. 2004). Similarly, artefacts that may result from using multimeric versus monomeric versions of a given fluorescent protein, transient overexpression versus stable expression (or vice versa) of a fluorescent fusion protein probe (e.g., Ara7/RabF2b; Kotzer et al. 2004) (refer also to Table 1), choice of protein probe that serves as an effector/regulator and that might disrupt normal functioning of a cellular system, misinterpretations that might be produced due to imaging methods and conditions, as well as data overextraction that may occur through the use of sophisticated extrapolation software based on nontransparent algorithms are all growing concerns associated with this technology. The reader is directed to several recent reviews including Wasteney and Yang (2004), Chapman et al. (2005), Shaner et al. (2005), and Dixit et al. (2006) for more detailed discussions on the advantages and disadvantages of GFP-based probes.

Future directions

The hitherto relatively dark, walled microworld of the plant cell may now be considered well illuminated in fluorescent colours. The biogenesis of different organelles, their intracellular motility and mutual interactions, as well as organelle responses to diverse biotic and abiotic stimuli are now open to real-time microscopic dissection. Furthermore, the use of novel cell biological techniques like bimolecular fluorescence complementation (BiFC) (Hu and Kerppola 2003; Walter et al. 2004), bioluminescence resonance energy transfer (BRET) (De and Gambhir 2005), Förster or fluorescence resonance energy transfer (FRET) (Eccleston et al. 2005; Wallrabe and Periasamy 2005), fluorescence lifetime imaging (FLIM) (Bastiaens and Squire 1999), fluorescence recovery after photobleaching (FRAP) (Bunt and Wouters 2004), proximity imaging microscopy (PRIM) (De Angelis et al. 1998; Dumas et al. 2004), and the incorporation of transgenic lines that carry several targeted probes and allow for simultaneous multicolour visualization (Mathur et al. 2002; Dhonukshe and Gadella 2003; Wada and Suetsugu 2004; Voigt et al. 2005), combined with the four-dimensional mapping of intracellular events (Dixit and Cyr 2004; Heisler et al. 2005), promises for even more exciting journeys into the living plant cell.

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