MPMI Vol. 36, No. 4, 2023, pp. 256–259, https://doi.org/10.1094/MPMI-01-22-0023-A

A Medicago truncatula Cell Biology Resource: Transgenic Lines Expressing Fluorescent Protein–Based Markers of Membranes, Organelles, and Subcellular Compartments

Funding

Financial support was provided by the United States National Science Foundation (NSF) Plant Genome Program grant numbers IOS-1733470 and IOS-2139351. The confocal microscope in the BTI Plant Cell Imaging Center was purchased with a United States NSF Instrumentation grant, number DBI-0618969.

Keywords

actin, apoplast, endoplasmic reticulum, Golgi bodies, mCherry, microtubules, mitochondria plasmodesmata, nucleus, peroxisomes, phosphoinositides, plasma membrane, plastid, tonoplast, trans-Golgi network

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Medicago truncatula Cell Biology Resource

Plant lines expressing genetically encoded fluorescent markers of organelles, membranes, and subcellular compartments have had a major impact on plant biology research and are particularly useful for co-localization and cell biology studies. There are many *Arabidopsis* transgenic lines in which membrane compartments and organelles are labeled or in which reporters enable alterations in ions, hormones, or lipids to be visualized (Desvoyes et al. 2020; Geldner et al. 2009; Jones et al. 2014; Nelson et al. 2007; Rizza et al. 2017; Simon et al. 2014). Large-scale fluorescent marker resources have also been established in maize (Wu et al. 2013) and rice (Chen et al. 2019). *Medicago truncatula* is used widely for studies of two major plant-microbe symbioses, the N_2-fixing association with rhizobia and the endosymbiosis with arbuscular mycorrhizal fungi (Bravo et al. 2016; Canas and Beltran 2018; Chen et al. 2018; Jones et al. 2007; Young et al. 2011), for studies of legume interactions with pathogens and pests, as well as legume biology in general (Costa et al. 2021; Gavrin and Schornack 2019; Gou et al. 2018; Kamphuis et al. 2013; Saqib et al. 2011). Here, we report 18 transgenic *M. truncatula* lines that constitutively express fluorescent protein markers of membranes, organelles, cytoskeletal elements, and two phosphoinositides, namely, phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) and phosphatidylinositol 4-phosphate (PI4P), in cells throughout the plant. This resource will enhance research of *M. truncatula*–microbe interactions and *M. truncatula* cell biology in general.

M. truncatula transgenic lines expressing mCherry-based markers under the control of the constitutive *AtUBQ10* promoter were generated by *Agrobacterium tumefaciens*–mediated transformation of *M. truncatula* R108. The mCherry-fusion constructs are identical to those described previously (Ivanov and Harrison 2014) but the vector backbone differs, as the *bar* gene is expressed from the *CaMV35S* promoter rather than the *Nos* promoter. Transgenic lines expressing markers that label the nucleus, endoplasmic reticulum, *trans*-Golgi network, plasma membrane, apoplast, late endosomes, transient late endosome/tonoplasts, tonoplast, mitochondria, plastids, peroxisomes, plasmodesmata, microtubules, and actin filaments were generated (Table 1; Supplementary

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***e*-Xtra:** Supplementary material is available online.

The author(s) declare no conflict of interest.

Accepted for publication 31 August 2022.

Fig. 1. Fluorescent protein–based markers of organelles, membranes, and elements of the cytoskeleton in *Medicago truncatula* transgenic lines. Subcellular localization of markers in guard cells (arrowheads) and pavement cells (arrows) of the cotyledon epidermis. Markers of **A,** nucleus (NLS-mCherry), **B,** endoplasmic reticulum (mCherry-HDEL), **C and D,** *trans*-Golgi network (mCherry-MtSYP61), **E,** plasma membrane (AtPIP2a-mCherry), **F,** apoplast (MtBCPsp-mCherry), **G and H,** late endosome/pre-vacuolar compartment (mCherry-MtRAB5A2), **I and J,** transitory late endosome (TLE)/tonoplast (mCherry-MtRAB7A2), **K,** tonoplast (AtTIP1-1-mCherry), **L,** mitochondria (ScCOX4sp-mCherry), **M,** plastids (MtRubisco1sp-mCherry), **N,** peroxisomes (mCherry-SKL), **O and P,** plasmodesmata (AtPDLP1-mCherry), **Q and R,** microtubule (mCherry-MAP4-MBD), **S and T,** actin (AtFim1-ABD2), **U and V,** phosphatidylinositol 4-phosphate (GFP-PH$_{FAPP1}$), and **W and X,** phosphatidylinositol 4,5-bisphosphate (*GFP-PH$_{PLC\delta1}$*) were expressed from the constitutive *AtUBQ10* promoter. Images presented in B, Q, R, S, and T are projections of 10 optical sections on the *z* axis taken at 0.5-μm intervals. Scale bars = 10 μm. The markers show the correct subcellular locations with the caveat that stomatal guard cells, which show very high expression, may display nonspecific fluorescence in the vacuole.

Table 1. Expression cassettes used to create stable transgenic lines of the fluorescent protein-based markers in *Medicago truncatula*

Cell compartment	**Expression cassette**[a]
Nucleus	*AtUBQ10p::NLS-mCherry-GUS-tAtUBQ10*
Endoplasmic reticulum	*AtUBQ10p::AtWAK2-mCherry-HDEL-tAtUBQ10*
trans-Golgi network/early endosome	*AtUBQ10p::mCherry-MtSYP61-t35S*
	AtUBQ10p::mCherry-MtSYP41-t35S
Plasma membrane	*AtUBQ10p::AtPIP2a-mCherry-tAtUBQ10*
Apoplast	*AtUBQ10p::MtBCP1sp-mCherry-t35S*
Late endosome/pre-vacuolar compartment	*AtUBQ10p::mCherry-MtRAB5A2-t35S*
Transitory late endosome/tonoplast	*AtUBQ10p::mCherry-MtRAB7A2-t35S*
Tonoplast	*AtUBQ10p::AtTIP1-1-mCherry-tAtUBQ10*
Actin microfilaments	*AtUBQ10p::AtFim1-ABD2-mCherry- tAtUBQ10*
Microtubules	*AtUBQ10p::mCherry-MAP4-MBD-t35S*
Mitochondria	*AtUBQ10p::ScCOX4sp-mCherry-tAtUBQ10*
Plastids	*AtUBQ10p::MtRubisco1sp-mCherry-tAtUBQ10*
Peroxisomes	*AtUBQ10p::mCherry-SKL(PTS1)-tAtUBQ10*
Plasmodesmata	*AtUBQ10p::AtPDLP1-mCherry-tAtUBQ10*
Autophagosomes	*AtUBQ10p::mCherry-MtLC3-t35S*
PI4P[b]	*AtUBQ10p::GFP-PH$_{FAPP1}$-t35S*
PI(4,5)P_2	*AtUBQ10p::GFP-PH$_{PLC\delta1}$-t35S*

[a] Each transferDNA also contains the cauliflower mosaic virus (CaMV) 35Sp::bar-t35S. The bar gene originates from *Streptomyces hygroscopicus*. The constructs were generated in pCAMBIA 3301.

[b] PI4P = phosphatidylinositol 4-phosphate and PI(4,5)P_2 = phosphatidylinositol 4,5-bisphosphate.

Information). We were unable to regenerate transgenic lines expressing GmMAN49-mCherry or LifeAct-mCherry, which are markers for Golgi bodies and actin filaments, respectively. Additionally, we generated *M. truncatula* transgenic lines expressing green fluorescent protein (GFP) fusions that report the presence of phosphoinositides PI(4,5)P_2 and PI4P, on the cytoplasmic face of the membrane bilayer (Table 1; Supplementary Information). These constructs are the same as those described by Ivanov and Harrison (2019).

Previously, we evaluated the subcellular localization of these fluorescent markers in *M. truncatula* roots, in which they showed the expected locations (Ivanov and Harrison 2014, 2019). Here, we verified marker expression and location in the epidermal cells of the cotyledons in the stable transgenic lines in the T1 and T2 generations. All markers showed the correct subcellular location in the pavement cells (Fig. 1; Table 1). In guard cells, the fluorescence intensity was much higher and, in some instances, this high expression negatively affected the localization of the markers and resulted in a strong fluorescent signal in the lumen of the vacuoles. A comparison of the two cell types, located adjacent to each other, provides a useful, internally controlled example of the effects of high expression of the markers (Fig. 1; Table 1). High expression of the *AtUBQ10* promoter in guard cells has been reported previously in *Arabidopsis* (Grefen et al. 2010; Schlucking et al. 2013), thus indicating that this is not species-specific but rather a feature of this promoter in guard cells.

Data Availability

Seed of these transgenic lines can be obtained upon request.

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