



**HAL**  
open science

# Methods to Visualize the Actin Cytoskeleton During Plant Cell Division

Marie-Cécile Caillaud

► **To cite this version:**

Marie-Cécile Caillaud. Methods to Visualize the Actin Cytoskeleton During Plant Cell Division. Marie-Cécile Caillaud. Plant Cell Division. Methods and Protocols, 2382, Springer US, pp.1-16, 2022, Methods in Molecular Biology, 978-1-0716-1746-5. 10.1007/978-1-0716-1744-1\_1 . hal-04075954

**HAL Id: hal-04075954**

**<https://hal.science/hal-04075954>**

Submitted on 20 Apr 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Methods to visualize the Actin Cytoskeleton During Plant Cell Division

Marie-Cécile Caillaud

Laboratoire Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA, F-69342 Lyon, France.

Corresponding author: [marie-cecile.caillaud@ens-lyon.fr](mailto:marie-cecile.caillaud@ens-lyon.fr)

## ii. Abstract

Mitosis in plants consists of separating the mother cell in two daughter cells by the centrifugal growth of a new wall. This process involves the reorganization of the structural elements of the cell, namely the microtubules and actin cytoskeleton which allow the coordination, the orientation and the progression of mitosis. In addition to its implication in those plant-specific structures, the actin cytoskeleton, in close association with the plasma membrane, abhors specific patterning at the cortex of the dividing cells, and might act as a signalling component. This review proposes an overview of the techniques available to visualize the actin cytoskeleton in fixed tissues or living cells during division, including electron, fluorescent and super resolution microscopy techniques.

## iii. Key words

actin, cell biology, plant, Fluorescent-tagged reporters, electron tomography, immunogold labeling, Phallotoxins, super-resolution microscopy

## 1. Introduction

The topology of the tissue in the green lineage relies deeply on a precise spatio-temporal control and coordination of division and growth. As plant cells cannot relocate by migration, the orientation of the cell division is brought about by a complex interplay between the cell-cycle machinery and other cellular structures, including the microtubule and actin cytoskeletons as well as the plasma membrane. When the cell cycle is progressing toward mitosis, most cellular microtubules together with the actin filaments (F-actin) relocate at the cell's cortex in close association with the plasma membrane forming the preprophase band at the cortical division zone (CDZ). In acentrosomal plant cells, the mitotic spindle allows the separation of the genetic material during metaphase/anaphase transition while the phragmoplast drives the formation of a new cell wall separating the daughter cells in telophase at the earlier defined cortical division site (CDS) (Livanos and Muller 2019). Microtubule and F-actin cytoskeletons are therefore closely associated and regulated to form and orient these three distinct structures. During this process, the actin cytoskeleton, in close association with the plasma membrane, adopts specific patterning at the cortex of the dividing cells, leading to the formation of F-actin enriched (actin twin peaks) and depleted zone (ADZ, Figure 1). This review proposes an overview of the techniques available to visualize the actin cytoskeleton in fixed tissues or living cells during division, including electron, fluorescent and super resolution microscopy techniques.

## 2. Electron tomography

Actin cytoskeleton and its interaction with microtubule cytoskeleton was visualized using chemical fixed plant cells (Seagull and Heath 1979; Hardham et al. 1980; Heath and Seagull 1982). However, chemical fixation and dehydration techniques, were later revealed to be inadequate for the preservation of cytoskeleton (Tiwari and Polito 1988; Lancelle et al. 1987). The efficacy of the technique of freeze-substitution for the preservation of cytoskeletal details is now widely recognized (Tiwari and Polito 1988; Tiwari et al. 1984; Lancelle et al. 1987). Using such approach, the actin cytoskeleton at the cortex of the cell was describe to be associated together with the plasma membrane, but also with the endoplasmic reticulum beneath the cortical microtubules (Tiwari and Polito 1988). More recently, the ultrastructure of the actin cytoskeleton in the PPB in onion cotyledon epidermal cells was achieved using high-pressure freezing/freeze substitution techniques. This approach allowed the collection of quantitative information on the number and length of F-actin inside the PPB. Using this

approach, F-actin cables ~170-nm-long were observed to bind and to cross-link microtubules (Takeuchi et al. 2016). Electron microscopic observations of glycerinated *Haemanthus* cells indicate the presence of actin-F in the kinetochore fibers (Forer and Jackson, 1979). But these data are still debated because the preparative techniques used might have induced changes in actin-F distribution (Schroeder, 1976).

### **3. Immunogold labeling of F-actin**

Using actin monoclonal antibodies, which gave the finest resolution with immunogold staining, it was shown that F-actin was often seen in the near vicinity of another bundle or microtubule, in an end to side or Y-shaped connection, closely parallel or juxtaposed to microtubules. Actin-F bundle length often reached 10-20  $\mu\text{m}$  (Schmit and Lambert 1988a). The distribution of F-actin cables was later visualized using an immunogold-silver-enhanced method and compared with the arrangement of immunogold-stained microtubules in the same cells (Molè-Bajer et al. 1988). In the dividing endosperm cells of the monocot *Haemanthus*, F-actin was observed on the spindle surface and those present within the spindle are arranged parallel to the long axis of the spindle. Using this approach, F-actin was found to not reorient outside the spindle, while in the polar region, F-actin appears to be compressed by the elongating spindle (Molè-Bajer et al. 1988). In anaphase, shorter F-actin orients in various directions accumulate at the equator, where some of them are incorporated into the phragmoplast (Molè-Bajer et al. 1988).

### **4. Phallotoxins**

The biologically active components of poisonous *Amanita* mushrooms such as phallotoxins are known to be specific for the F-actin (Dancker et al. 1975; Wieland et al. 1978; Wulf et al. 1979). Phallotoxins are low in molecular weight, and thus can penetrate plant cells without prior cell wall digestion, and can also be used with or without fixation before the staining step. The first attempt to use phalloxin to stain actin in the phragmoplast and cell cortex was unsuccessful (Clayton & Lloyd, 1985; Lloyd *et al.* 1985; Gunning & Wick, 1985). Later, staining the unfixed cells with rhodaminyl lysine phalloxin (rhodamine-phalloidin) in detergent extraction buffer in carrot suspension cells set up the protocol to visualize the actin cytoskeleton during cell division (Lloyd and Traas 1988; Traas et al. 1987). Using this approach, the organization of F-actin in the PPB, mitotic spindle and phragmoplast was

described in various plant species including maize, *Allium*, *Tradescantia* and BY2 cells (Panteris et al. 2009; Yasuda et al. 2005; Valster et al. 1997; Clayton and Lloyd 1985a). Using the BY-2 cell culture stably expressing a fluorescent reporter for microtubules, the behavior of the F-actin cytoskeleton was assessed by co-staining with rhodamine-phalloidin (Hoshino et al. 2003). Using this approach, the actin depleted zone (ADZ) which appear before the prophase was reported to appear first from one side of the dividing cell, possibly as a cause of polar cytokinesis, as is often observed in BY-2 cells (Hoshino et al. 2003) and as was reported in *Arabidopsis* cells (Cutler and Ehrhardt 2002). The ADZ finally extends to the other side of the dividing cell forming a ring at the division plane deprived of F-actin (Hoshino et al. 2003).

## 5. Immunolabeling in fixed tissues

Immunofluorescence labeling, using a monoclonal antibody developed against actin is often used to visualize F-actin in fixed tissues. Using this technique, the formation of an actin ring at the cortex of the cell in preprophase, the “actin PPB” was visualized (Palevitz 1987). Double labeling of both microtubules and F-actin was therefore possible with this method, permitting to correlate the F-actin organization in regards to the known organization of the microtubule rearrangement that occurs throughout division (McCurdy and Gunning 1990; Li et al. 2010). Colocalization of F-actin with rhodamine-phalloidin and microtubules with tubulin immunocytochemistry in *Triticum aestivum* L. cv. Kite showed that F-actin and microtubules colocalized at the PPB (Palevitz 1987). As the PPB narrows, the F-actin is also confined to an increasingly restricted zone usually wider than the PPB in *Allium* (Liu and Palevitz 1992). In synchronized tobacco BY2 cells, the triple staining of microtubules, F-actin and DNA was performed and allowed a better resolution in time and space on the relationship between the mitosis progression and formation of the PPB (Katsuta et al. 1990). In wheat root-tip cells, the immunolocalization of actin revealed the absence of F-actin at the cortical division zone (McCurdy et al. 1988). However, depending on the antibody, such a zone was more or less wide (McCurdy et al. 1988; Panteris 2008). Early work using immunolocalization of actin cytoskeleton in rye leaf tissue revealed the presence of a meshwork of actin that concentrated near the end walls that face the spindle poles (Cho and Wick 1991). Consistently, immunofluorescence labeling of F-actin revealed a more intense reactivity to the actin antibody in the cell pole facing the spindle than the longitudinal side walls (Figure 1, Baluska et al. 1997; Liu and Palevitz 1992; Li et al. 2010). Furthermore, the ADZ is short-lived from metaphase through the anaphase/telophase transition (Clayton and Lloyd 1985b; Kakimoto and Shibaoka 1987a). The presence of actin filaments before phragmoplast formation in endosperm cells

suggest that microfilaments could act as a substitute for cell-plate "memory" (Schmit and Lambert 1988a).

Co-visualization of F-actin and microtubules by dual immunolabelling in BY-2 showed that the length of phragmoplast F-actin is shorter than that of microtubules (Li et al. 2010; Yoneda et al. 2004). During telophase, the two zones of aligned cortical F-actin over the ends of the cell gradually disappear and are replaced by new interphase networks (Liu and Palevitz 1992). Despite the handiness of using phallotoxins and the option of removing possible damage to F-actin from the fixation procedure, it has been questioned whether unfixed cells stained with phallotoxins could provide a misleading picture of F-actin configurations in vivo (Kakimoto and Shibaoka 1987b; Sonobe and Shibaoka 1989). Moreover, labeling procedures without fixation cannot be used for double labeling and therefore reduce the possibility to study the dynamics of the F-actin together with the microtubule cytoskeleton, for instance.

F-actin staining and immunolabelling approaches proved useful when the study takes place in an organism that cannot be transformed, or in multiple mutants for which crosses could be difficult. In these cases, the staining techniques described above are still used today. In parallel, advances in live imaging have led to the use of fluorescent-tagged actin-binding domains (ABD) or proteins interacting with the actin cytoskeleton (Li et al. 2010). These methods allow us to visualize the dynamics of the F-actin cytoskeleton during plant mitosis in vivo using for example confocal microscopy, tracking system and super-resolution microscopy (Table 1).

## **6. Fluorescent-tagged actin reporters**

- The fluorescent-tagged C-terminal part of the mouse Talin (GFP-mTn<sup>2345-2541</sup>)

Talin is a cytoskeletal associated protein that localizes specifically in adherents-type junctions with the extracellular matrix (Burrige and Connell 1983) and is absent from the plant genome. The amino acids 2345–2541 in the C-terminus of the mouse Talin (mTalin<sup>2345-2541</sup>) are responsible for its actin-binding capacity (Kost et al. 1998). Unlike the full-length protein, mTalin<sup>2345-2541</sup> is unable to nucleate actin polymerization and was, therefore, use as a reporter to visualize F-actin in plants (Kost et al. 2000; Kost et al. 1998). In tobacco BY-2 suspension cells, GFP-mTalin<sup>2345-2541</sup> colocalizes with rhodamine-phalloidin (Kost et al. 2000). Stable and ubiquitous expression of GFP-mTalin<sup>2345-2541</sup> in *Arabidopsis* transgenic lines was used to visualize F-actin in all tissues with no apparent phenotypic effects (Kost et al. 2000). While using GFP-

mTalin<sup>2345–2541</sup> the F-actin behaviors in different cell types and organisms were described in interphasic cells and in pollen tubes of different species (Holweg et al. 2004; Hoffmann and Nebenführ 2004; Kost et al. 2000). To my knowledge, F-actin dynamics during cell division was not analyzed using this reporter. Later study revealed that while GFP-mTalin<sup>2345–2541</sup> does not affect actin polymerization, it inhibits its depolymerization by affecting the actin-depolymerizing factor (Ketelaar et al. 2004). These findings led to the dropping of this reporter to study F-actin in plants.

### The fluorescent-tagged Fimbrin

Fimbrin is a cytoskeletal protein associated with F-actin in various metazoan structures, such as in microvilli, microspikes, membrane ruffles, and cell-substrate attachment (Bretscher 1981). The first plant fimbrin-like gene, *AtFIM1*, was identified in Arabidopsis (McCurdy and Kim 1998) and since then has been extensively used as a reporter to visualize F-actin cytoskeleton inside plant cells (Kovar et al. 2001; Wang et al. 2008; Sheahan et al. 2004b; Wang et al. 2004; Yoneda et al. 2007). The Fimbrin 1 actin-binding domain 2 fused to GFP (GFP-fABD2) corresponds to the N-terminal fusion of GFP to the C-terminal half of AtFim1 (aa 325–687), which includes the second ABD and the C-terminal end of Arabidopsis Fimbrin 1 (McCurdy and Kim 1998; Ketelaar et al. 2004a). Compared with GFP fusions to full-length AtFim1, GFP-fABD2 is more visibly labeling the native F-actin organization in Arabidopsis (Sheahan et al. 2004a) and rapidly became a standard to visualize F-actin in planta (Voigt et al. 2005a).

In the tobacco BY-2 cell line stably transformed with a GFP-fABD2, the appearance of a broad F-actin band in the late G2 phase that later separates to form a structure corresponding to the so-called ADZ in mitosis was confirmed (Sano et al. 2005). Intriguingly, the GFP-fABD2 labeled structure at the cell cortex in mitosis appeared to form two bands rather than the ADZ. Measurements of fluorescent intensities indicated a GFP-fABD2 distribution that resembled two peaks, and we therefore named the structure actin 'twin peaks' (Figure 1, Sano et al. 2005). The cell plate was shown to form exactly in the middle of the actin 'twin peaks' suggesting that these structures act as a landmark for cytokinesis orientation (Sano et al. 2005). Live cell imaging in BY2 of the GFP-fABD2 distribution was next monitored together with the endocytic tracer, FM4-64, that labels the cell plate (Higaki et al. 2008). During cytokinesis, GFP-fABD2 accumulates at the phragmoplast and subsequently approaches the expanding cell plate edges (Higaki et al. 2008). In vivo visualization of F-actin in Arabidopsis plants stably expressing *35Spro::GFP-fABD2* showed that mitotic cells have an F-actin-depleted zone

around the equator, whereas F-actin accumulates at the plasma membrane which is facing the spindle poles (Voigt et al. 2005a). During cytokinesis, this overall actin polarity is still maintained (Voigt et al. 2005a). Using this reporter, the phragmoplast F-actin could be observed at different stages of cytokinesis (Voigt et al. 2005a).

In Arabidopsis, the expression of the *GFP-fABD2-GFP* reporter under the 35S promoter (Wang et al. 2008) has inhibitory effects on cell and organ growth (Dyachok et al. 2014). The use of the ubiquitin10 (UBQ10) promoter to drive expression of the GFP-fABD2-GFP reporter minimized some of the artifacts that researchers might encounter when using fABD2-based and other F-actin reporters for studying F-actin dynamics in living plants (Dyachok et al. 2014).

### Lifact reporter

An important development is the identification of a 17-amino-acid peptide (Lifact) derived from the N-terminus of yeast Abp140 protein that binds specifically to F-actin with a low affinity and appears to be a faithful reporter of actin dynamics without disrupting them (Riedl et al. 2008). While Lifact decorates F-actin with minimum perturbation of actin dynamics in plants, its expression needs to be optimized to reach a lower expression level than *fADB2* in plants (Era et al. 2009). Indeed, It was reported that Arabidopsis seedlings overexpressing at high level *Lifact-YFPv* reporter under the 35S promoter had less dense and more bundled F-actin in some cell types compared with fABD2-based reporter (Dyachok et al. 2014).

To test whether Lifact-psRFP affects the dynamics of actin-F in BY-2 stable line, the cells were challenged with Latrunculin B, a drug which eliminates actin-F depending on their innate turnover. The authors assumed that if the expression of Lifact-psRFP increases the stability of actin-F, the cells expressing Lifact-psRFP should be less sensitive to Latrunculin B. However, the Lifact-psRFP expressing cell line was more sensitive, which argues against reduced dynamics of actin-F (Durst et al. 2014).

Using Arabidopsis plants, stably expressing *Lifact* as a reporter, we tracked the behaviors of the F-actin cytoskeleton in time with an unprecedented precision (Lebecq et al., in prep for Plants 2021 special issue). To follow *in vivo* division in the root meristem overtime, manual adjustment is needed, otherwise the area under study will grow out of the field of view (the root tip grows out of the field of view in about 20 min). By developing an unsupervised approach to automatically track cells using spinning disk confocal microscopy (Doumane et al., 2017), we are now able to automatically track the region under study in real time during



acquisition so that root growth can be compensated. This keeps the region under study within frame for the duration of the experiment. By imaging *in vivo*, the fluorescent-tagged Lifeact under the control of UB10 and RPS5a promoters—both less strong than 35S promoter—we confirmed and quantified earlier reports describing an apical-basal accumulation of the actin filaments in the late stage of cell division (Figure 1, Cho and Wick 1991; Baluska et al. 1997; Liu and Palevitz 1992; Voigt et al. 2005, Sadot et al., 2019; Lebecq et al., in prep for Plants 2021 special issue). By visualizing fluorescent-tagged Lifeact together with cell division markers in the dividing root tip cells, we observed that the F-actin polarization occurs at the metaphase/anaphase transition and persists in time throughout cytokinesis (Lebecq et al., in prep for Plants 2021 special issue). In this study, we generated a set of fluorescent-tagged Lifeact markers in cyan, yellow, and red (Lebecq et al., in prep for Plants 2021 special issue), that should be helpful in performing colocalization analysis in *Arabidopsis*.

To validate the use of this reporter to visualize the F-actin in plant, BY-2 cells stably expressing Lifeact–psRFP were PFA-fixed, and stained using Alexa-Fluor®488 labeled phalloidin. This experiment showed a clear colocalization of Alexa-Fluor®488-phalloidin and Lifeact–psRFP for the perinuclear actin. However, in the cell cortex, only the green fluorescence of Alexa-Fluor®488-phalloidin could be detected, whereas the red Lifeact–psRFP signal was absent, indicating that there is a clear structural difference between these actin sub-populations (Durst et al. 2014).

While studies relying on fluorescent-based reporters multiplied, it is important to keep in mind that these biosensors might not label the entire population of F-actin filaments in cells due to competition with endogenous actin regulatory proteins. As such, accurate detection of F-actin by immunolocalization might be considered as a complementary approach in certain cell types (Sadot et al., 2019).

## **7. MT-actin interactions and how to image both at the same time**

Interaction between microtubules and F-actin has been reported in various plant systems, and proteins connecting these two cytoskeletal networks were identified, even if the molecular mechanism is still elusive (Takeuchi et al. 2017; Sampathkumar et al. 2011; SCHMIT and LAMBERT 1988b). Among them, Kinesins with a calponin homology domain (KCHs) have been identified recently as a plant-specific subgroup of the kinesin-14 family and are suspected to act as microtubule–actin filament cross-linkers. The Cotton kinesin GhKCH2, which also contains an actin-binding calponin homology (CH) domain in the N-terminus, decorates the

phragmoplast midzone in dividing root tip cells using immunolocalization assays (Xu et al. 2009). Similarly, NtKCH, a KCH homolog of tobacco BY-2 cells, accumulates in the PPB (Klotz and Nick 2012). Using the transient *Agrobacterium*-mediated transformation of BY-2 cells (TAMBY2), the *Arabidopsis* kinesin 14-type molecular motor (AtKinG) was found to strongly label the PPB (Buschmann et al. 2011). Fluorescence double-labeling in BY-2 cells showed that AtKinG localizes to both microtubules and F-actin (Buschmann et al. 2011). In BY-2 cells, the fluorescent-tagged kinesin-like AtKCA1 protein from *Arabidopsis* that interacts with the Cyclin-dependent kinases AtCDKA labels the plasma membrane in a non-homogenous manner (Vanstraelen et al. 2004; Vanstraelen et al. 2006). In a stable BY-2 line, GFP-AtKCA1 is excluded from the cortical division zone (CDZ) forming the 'KCA1 depleted zone' (KDZ) (Vanstraelen et al. 2006). This finding contrasts with what was observed for the fluorescent-tagged actin nucleator FORMIN 6 (AtFH6-GFP) which labeled the plasma membrane without showing regions of reduced fluorescence (Vanstraelen et al. 2006). To visualize F-actin together with the AtKCA1 fusion protein, fABD2 was fused N-terminally to RFP (fABD2-RFP) and stably transformed in BY-2 cells expressing GFP-AtKCA1 (Vanstraelen et al. 2006). Dual live-cell imaging showed that the ADZ and the KDZ colocalize (Vanstraelen et al. 2006). However, the localization of AtKCA1 in its native system was not yet reported and therefore the existence of the KDZ in a dividing plant tissue still needs to be confirmed.

The Myosins which constitute a large superfamily of proteins that share a common domain of interaction with F-actin, were also reported to colocalize with F-actin and microtubules. The most studied of them, the moss Myo8, was shown to interact with both F-actin and microtubules. In *Physcomitrella*, Myo8 localizes to the division site and the phragmoplast during cell division and may promote phragmoplast guidance via F-actin to the division site (Wu and Bezanilla 2014).

While microtubule-associated proteins are rarely reported to also binds to F-actin, a 190 KDa Microtubules Associated Protein (NtMAP-190) isolated from mini-protoplasts of Tobacco cultured cells was shown to bind to both F-actin and microtubules and to localized at the mitotic spindle (Igarashi et al. 2000).

The Formins are involved in the polymerization of actin and associate with the fast-growing end of the F-actin. In moss, the fluorescent-tagged class II Formin 2A (For2A) strongly labels the center of the expanding phragmoplast, as it was also reported for the type I *Arabidopsis* Formins AtFH5 and AtFH8 (Ingouff et al. 2005b; Xue et al. 2011a; Wu and Bezanilla 2014). These observations suggest that the fast-growing ends of F-actin are anchored at its midzone and the pointed ends are in the cytoplasm (Wu and Bezanilla 2014). By contrast, the type II *Arabidopsis* Formin AtFH14 that, like For2A, acquires an N-terminal

PTEN-like domain (van Gisbergen and Bezanilla 2013), colocalizes with  $\alpha$ -tubulin at the entire PPB, spindle and phragmoplast when expressed in BY-2 and Arabidopsis suspension cells (Li et al. 2010). These finding highlights specificity among the Formins in their interaction with the cytoskeleton and anionic lipids. Indeed, the type I Arabidopsis Formin AtFH4 is able to bind microtubules in vitro and in vivo (Deeks et al. 2010a), suggesting that specific Formins could have an impact on both the F-actin and microtubule cytoskeleton during cell division.

While these fluorescent-tagged actin-binding proteins are available (Table1), the fact that they often localize to both actin and microtubules can make the interpretation of labeling experiments difficult. However, peculiar staining patterns such as the one observed for Myo8 during cytokinesis will be key to study in detail in time and space the events occurring for example at the cortex of the cell in late telophase.

Name	Localization	remarks	orga nism	Vector/ Resistance	reference
35Spro:GFP-Talin	all arrays	prevent F-actin depolymerization	Col-0	pCAMBIA-1390 (hygro & kana)	(Wang et al. 2004; Wang et al. 2008)
35Spro:DsRed2-Talin	all arrays	prevent F-actin depolymerization	Col-0	pCAMBIA-1390 (hygro & kana)	(Dyachok et al. 2014)
35Spro:DsRed2-Talin	all arrays		BY-2	pGWB2 (basta)	(Buschmann et al. 2011)
35Spro:CFP-fABD2-CFP	all arrays	morphological defects	Col-0	pCAMBIA-1390 (hygro & kana)	(Dyachok et al. 2014)
35Spro:GFP-fABD2-GFP	all arrays	morphological defects	Col-0	pCAMBIA-1390 (hygro & kana)	(Wang et al. 2004; Wang et al. 2008)
35Spro:GFP-fABD2	all arrays		Col-0	pCAMBIA-1300 (hygro & kana)	(Xue et al. 2011b)
UBQ10pro:GFP-fABD2-GFP	all arrays		Col-0	pCAMBIA-1390 (hygro & kana)	(Dyachok et al. 2014)

					(Voigt et al. 2005a)
35Spro:GFP-Fim1	all arrays		Col-0	pCB302 (hygro)	
					(Voigt et al. 2005a)
35Spro:GFP-fABD2	all arrays		Col-0	pCB302 (hygro)	
35Spro:YFP-fABD2-YFP	all arrays	morphological defects	Col-0	pCAMBIA-1390 (hygro & kana)	(Dyachok et al. 2014)
35Spro:mOrange-fABD2-mOrange	all arrays	morphological defects	Col-0	pCAMBIA-1390 (hygro & kana)	(Dyachok et al. 2014)
35Spro:tdTom-fABD2	all arrays		BY-2	pMDC100 (kana)	(Kojo et al. 2014; Kojo et al. 2013)
35Spro:mCh-fABD2-mCherry	all arrays	morphological defects	Col-0	pCAMBIA-1390 (hygro & kana)	(Dyachok et al. 2014)
35Spro:RFP-fABD2	all arrays		BY-2	pH7WGR2 (hygro)	(Klotz and Nick 2012)
35Spro:YFP-TUB-tdTomato-ABD2	MTs and F-actin		BY-2	pMDC101 (kana)	(Kojo et al. 2014; Kojo et al. 2013)
UBQ10pro:mCh-ABD2-mCh	all arrays		Col-0	pCAMBIA-1390 (hygro & kana)	(Dyachok et al. 2014)
Ub10pro:Lifeact-2mTU2	all arrays		Col-0	pK7m34GW (kana)	Lebecq et al., Plants in prep
			Col-0;		(Dyachok et al. 2014; Era et al. 2009)
			Marc hanti		
35Spro:Lifeact-YFPv	all arrays	morphological defects	a	pGWB2 (basta)	
Ub10pro:Lifeact-YFPv	all arrays		Col-0	pB7m34GW (basta)	(Doumane et al. 2020)
RPS5apro:Lifeact-tdTom	all arrays		Col-0		Lebecq et al., Plants in prep

UB10pro:Lifeact-mCh	all arrays		moss	pTHUbi-gate (hygro)	(van Gisbergen et al. 2012)
35Spro:Lifeact-psRFP	all arrays		BY-2	pH7WG2 (hygro)	(Durst et al. 2014)
35Spro:AtKinG-GFP	PPB and phragmoplast	Minus-end directed kinesin	BY-2	pGFP-N-Bin	(Buschmann et al. 2011)
35Spro:GFP-NtKCH	PPB and phragmoplast	Associate with both MTS and AFs	Toba cco	pK7WGF2 (kana)	(Klotz and Nick 2012)
35Spro:Myo8A-3XmEGFP	Spindle, center of phragmoplast & CDS	Actin-based molecular motors	BY-2		(Wu and Bezanilla 2014)
PAtFH8:AtFH8-GFP	newly formed cell wall	too weak to be detected	At	pCAMBIA1300 template (kana)	(Xue et al. 2011b)
XVEpro-AtFH8-GFP	newly formed cell wall		At	pER8 (hygro)	(Xue et al. 2011b)
XVEpro:AtFH14-GFP	PPB, spindle and phragmoplast	Bundle MTs and AFs and cross-link them	BY-2	pER8 (hygro)	(Li et al. 2010)
UB10pro:For2A-3XmEGFP	center of the growing phragmoplast		moss	pTHUbi-gate (hygro)	(Wu and Bezanilla 2014)
35Spro:AtFH5-eGFP	center of the growing phragmoplast		At	pK7WGF2 (kana)	(Ingouff et al. 2005a)
35Spro:AtFH4-GFP		Associate with both MTS and AFs	BY-2	pMDC43 (kana)	(Deeks et al. 2010b)
UB10pro:Myo8A-3XmEGF	Spindle, center of phragmoplast & CDS	Actin-based molecular motors	moss	pTKUbi-gate (kana)	(Wu and Bezanilla 2014)

**Table 1. Examples of Fluorescent marker available to visualize F-actin in vivo**

## **8. Visualization of F-actin in vivo by super-resolution microscopy**

As electron microscopy did in the past, super-resolution microscopy provides the ability to see details of cellular and even macromolecular structures that were not possible to see before. Photo-Activated Localization Microscopy (PALM) has attracted considerable attention because they allow structures below the Abbe limit to be resolved (Hedde and Nienhaus 2014). In recent years, a growing interest to use this method in plant research is obvious even though only a few laboratories are equipped with a super-resolution microscope which allows the detection of the emission from individual fluorophores. Importantly, these emitters must be photoactivatable or photoconvertible by irradiation with visible light. By activating them sparsely and stochastically, each CCD camera frame contains only a few spots of the size of the point-spread function, which allows each molecule to be precisely localized.

Using BY-2 cells stably transformed with photoactivatable fluorescent proteins fused to the actin-binding probe Lifeact, the dynamic reorganization of the actin cytoskeleton during the cell cycle was recently assessed by PALM with optical sectioning (Durst et al. 2014). While the Lifeact–monomeric variant fused to IrisFP was found to indiscriminately label central and cortical actin filaments, the tetrameric Lifeact–photoswitchable red fluorescent protein (Lifeact–psRFP) labeled only a specific perinuclear sub-population of actin called the “nuclear basket” (Durst et al. 2014). During mitosis, Lifeact–psRFP was exclusively localizing at the nuclear basket, arranged in a fine mesh-like structure until the end of metaphase and excluded from the spindle. At the beginning of the anaphase, Lifeact–psRFP signal in the nuclear basket was reduced. In telophase, while the new nuclear envelope was formed, a clear gradient of the Lifeact–psRFP was observed toward the cell poles (Durst et al. 2014).

## **9. Conclusion**

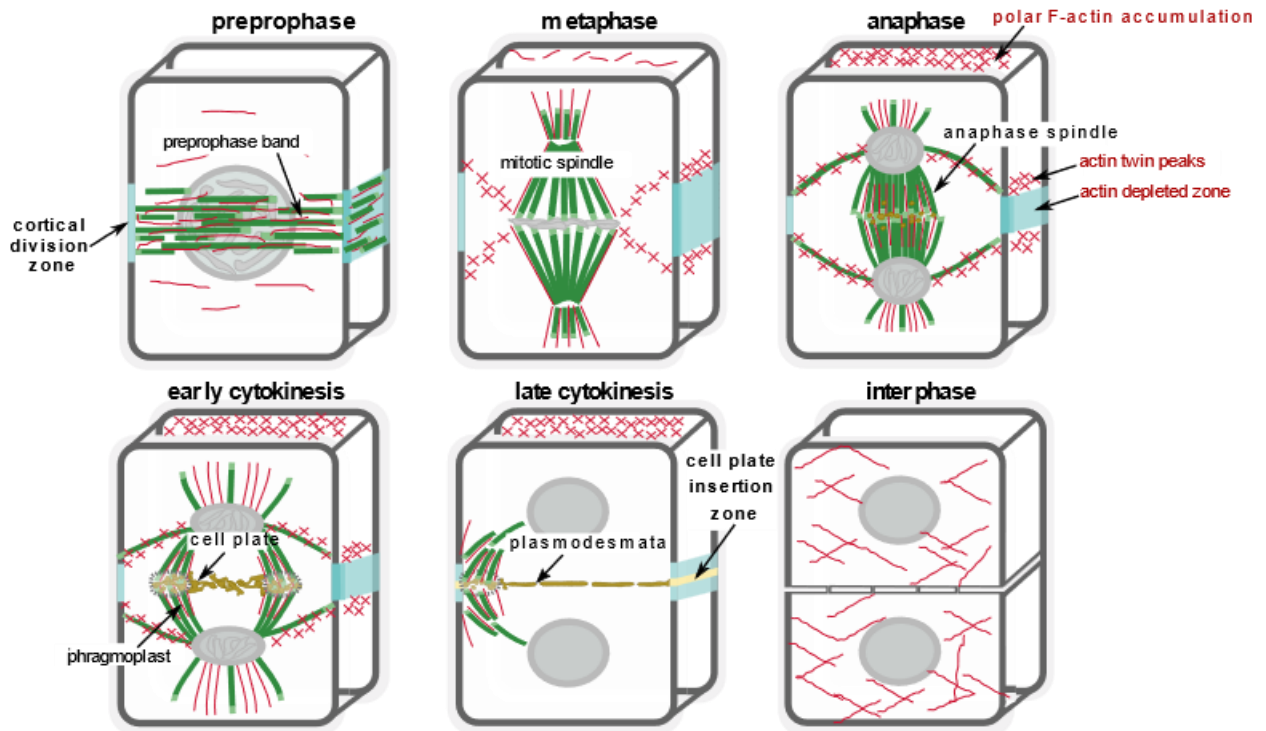
In the past few years, the increasing interest in the visualization of the actin cytoskeleton is illustrated by the number of different techniques and fluorescent reporter lines generated. Yet, so far, the behaviors of the actin cytoskeleton during mitosis have been mostly studied in a single cell context such as the BY-2 cell line. The dynamics of the actin cytoskeleton in growing tissues remain partial, even if recent studies started to address it. To date, there are still difficulties to visualize dividing cells in the meristematic part of the plant at super-resolution

because of the topology of the tissues or the presence of the root cap. Many other technical challenges are still blocking progress in the field, to address for example whether or not the ADZ is also observed in a dividing cell in its physiological context. In a tissue context, the fluorescence observed in the surrounding interphase cells, might mask potential domains at the cortex of the dividing cell such as the ADZ. The development of an inducible promoter or artificial system to visualize the dividing cell at better resolution might be the next task. Furthermore, while many zonation in the cortex of the dividing cells (CDZ, ADZ, actin twin peaks, KDZ for example) were described in different systems, the temporal and spatial relationship between them is still cryptic. Integration of the localization of the F-actin cytoskeleton in a referential together with the key player of the cell division seems to be the only way to assemble the pieces of the puzzle during this crucial process. In animals, actin mutants have strong phenotypes. It's more difficult to understand the role of F-actin in plants, simply because the phenotypes are weak. It would be therefore helpful to obtain more quantitative data about the localization of the actin during cell division and to develop systems to modify the actin cytoskeleton in order to build up a model in the light of what was done for the microtubules cytoskeleton in plants.

## **Acknowledgement**

We are grateful to the SiCE group (RDP, Lyon, France) and to Oliver Hamant (RDP, Lyon, France) and Jan Traas (RDP, Lyon, France) for comments and discussions. This work was supported by ANRJC/JC INTERPLAY (ANR-16-CE13-0021) and ANR PRC PlantScape and a SEED FUND ENS LYON-2020.

## **10. Figure Legend**



**Figure 1.** Schematic representation of the different steps of the plant cell division, with a focus on the actin cytoskeleton (red).

## 11. References

- Baluska F, Vitha S, Barlow PW, Volkmann D (1997) Rearrangements of F-actin arrays in growing cells of intact maize root apex tissues: a major developmental switch occurs in the postmitotic transition region. *European journal of cell biology* 72 (2):113-121
- Bretscher A (1981) Fimbrin is a cytoskeletal protein that crosslinks F-actin in vitro. *Proceedings of the National Academy of Sciences* 78 (11):6849-6853
- Burridge K, Connell L (1983) Talin: A cytoskeletal component concentrated in adhesion plaques and other sites of actin-membrane interaction. *Cell motility* 3 (5):405-417
- Buschmann H, Green P, Sambade A, Doonan J, Lloyd C (2011) Cytoskeletal dynamics in interphase, mitosis and cytokinesis analysed through *Agrobacterium*-mediated transient transformation of tobacco BY-2 cells. *New Phytologist* 190 (1):258-267
- Cho SO, Wick SM (1991) Actin in the developing stomatal complex of winter rye: A comparison of actin antibodies and Rh-phalloidin labeling of control and CB-treated tissues. *Cell motility and the cytoskeleton* 19 (1):25-36



- Clayton L, Lloyd CW (1985) Actin organization during the cell cycle in meristematic plant cells. Actin is present in the cytokinetic phragmoplast. *Exp Cell Res* 156 (1):231-238. doi:10.1016/0014-4827(85)90277-0
- Cutler SR, Ehrhardt DW (2002) Polarized cytokinesis in vacuolate cells of *Arabidopsis*. *Proceedings of the National Academy of Sciences* 99 (5):2812-2817
- Dancker P, Löw I, Hasselbach W, Wieland T (1975) Interaction of actin with phalloidin:: Polymerization and stabilization of F-actin. *Biochimica et Biophysica Acta (BBA)-Protein Structure* 400 (2):407-414
- Deeks MJ, Fendrych M, Smertenko A, Bell KS, Oparka K, Cvrckova F, Zarsky V, Hussey PJ (2010) The plant formin AtFH4 interacts with both actin and microtubules, and contains a newly identified microtubule-binding domain. *J Cell Sci* 123 (Pt 8):1209-1215. doi:10.1242/jcs.065557
- Ding B, Turgeon R, Parthasarathy M (1991) Microfilaments in the preprophase band of freeze substituted tobacco root cells. *Protoplasma* 165 (1-3):209-211
- Doumane M, Colin L, Lebecq A, Fangain A, Bareille J, Hamant O, Belkhadir Y, Jaillais Y, Caillaud M-C (2020) iDePP: a genetically encoded system for the inducible depletion of PI(4,5)P<sub>2</sub> in *Arabidopsis thaliana*. bioRxiv:2020.2005.2013.091470. doi:10.1101/2020.05.13.091470
- Doumane M, Lionnet C, Bayle V, Jaillais Y, Caillaud MC (2017) Automated Tracking of Root for Confocal Time-lapse Imaging of Cellular Processes. *Bio Protoc* 7 (8). doi:10.21769/BioProtoc.2245
- Durst S, Hedde PN, Brochhausen L, Nick P, Nienhaus GU, Maisch J (2014) Organization of perinuclear actin in live tobacco cells observed by PALM with optical sectioning. *J Plant Physiol* 171 (2):97-108. doi:10.1016/j.jplph.2013.10.007
- Dyachok J, Sparks JA, Liao F, Wang YS, Blancaflor EB (2014) Fluorescent protein-based reporters of the actin cytoskeleton in living plant cells: fluorophore variant, actin binding domain, and promoter considerations. *Cytoskeleton* 71 (5):311-327
- Era A, Tominaga M, Ebine K, Awai C, Saito C, Ishizaki K, Yamato KT, Kohchi T, Nakano A, Ueda T (2009) Application of Lifeact reveals F-actin dynamics in *Arabidopsis thaliana* and the liverwort, *Marchantia polymorpha*. *Plant and Cell Physiology* 50 (6):1041-1048
- Hardham AR, Green PB, Lang JM (1980) Reorganization of cortical microtubules and cellulose deposition during leaf formation in *Graptopetalum paraguayense*. *Planta* 149 (2):181-195
- Heath I, Seagull R (1982) Oriented cellulose fibrils and the cytoskeleton: a critical comparison of models. *The cytoskeleton in plant growth and development*:163-182
- Hedde PN, Nienhaus GU (2014) Super-resolution localization microscopy with photoactivatable fluorescent marker proteins. *Protoplasma* 251 (2):349-362

- Higaki T, Kutsuna N, Sano T, Hasezawa S (2008) Quantitative analysis of changes in actin microfilament contribution to cell plate development in plant cytokinesis. *BMC Plant Biol* 8:80. doi:10.1186/1471-2229-8-80
- Hoffmann A, Nebenführ A (2004) Dynamic rearrangements of transvacuolar strands in BY-2 cells imply a role of myosin in remodeling the plant actin cytoskeleton. *Protoplasma* 224 (3-4):201-210
- Holweg C, Süßlin C, Nick P (2004) Capturing in vivo dynamics of the actin cytoskeleton stimulated by auxin or light. *Plant and Cell Physiology* 45 (7):855-863
- Hoshino H, Yoneda A, Kumagai F, Hasezawa S (2003) Roles of actin-depleted zone and preprophase band in determining the division site of higher-plant cells, a tobacco BY-2 cell line expressing GFP-tubulin. *Protoplasma* 222 (3-4):157-165. doi:10.1007/s00709-003-0012-8
- Igarashi H, Orii H, Mori H, Shimmen T, Sonobe S (2000) Isolation of a novel 190 kDa protein from tobacco BY-2 cells: possible involvement in the interaction between actin filaments and microtubules. *Plant and Cell Physiology* 41 (8):920-931
- Ingouff M, Fitz Gerald JN, Guerin C, Robert H, Sorensen MB, Van Damme D, Geelen D, Blanchoin L, Berger F (2005) Plant formin AtFH5 is an evolutionarily conserved actin nucleator involved in cytokinesis. *Nature cell biology* 7 (4):374-380. doi:10.1038/ncb1238
- Kakimoto T, Shibaoka H (1987) A new method for preservation of actin filaments in higher plant cells. *Plant and cell physiology* 28 (8):1581-1585
- KATSUTA J, HASHIGUCHI Y, SHIBAOKA H (1990) The role of the cytoskeleton in positioning of the nucleus in premitotic tobacco BY-2 cells. *Journal of Cell Science* 95 (3):413-422
- Ketelaar T, Allwood EG, Anthony R, Voigt B, Menzel D, Hussey PJ (2004) The actin-interacting protein AIP1 is essential for actin organization and plant development. *Current Biology* 14 (2):145-149
- Klotz J, Nick P (2012) A novel actin-microtubule cross-linking kinesin, NtKCH, functions in cell expansion and division. *New Phytol* 193 (3):576-589. doi:10.1111/j.1469-8137.2011.03944.x
- Kojo KH, Yasuhara H, Hasezawa S (2014) Time-sequential observation of spindle and phragmoplast orientation in BY-2 cells with altered cortical actin microfilament patterning. *Plant Signal Behav* 9
- Kojo KH, Yasuhara H, Kutsuna N, Hasezawa S (2013) Dual Observation of Microtubules and Actin Microfilaments during Cell Cycle Progression in BY-YTRF1 Cells. *CYTOLOGIA* 78 (3):203-204
- Kost B, Spielhofer P, Chua NH (1998) A GFP-mouse talin fusion protein labels plant actin filaments in vivo and visualizes the actin cytoskeleton in growing pollen tubes. *The Plant Journal* 16 (3):393-401

- Kost B, Spielhofer P, Mathur J, Dong C-H, Chua N-H (2000) Non-invasive F-actin visualization in living plant cells using a GFP-mouse talin fusion protein. In: Actin: a dynamic framework for multiple plant cell functions. Springer, pp 637-659
- Kovar DR, Gibbon BC, McCurdy DW, Staiger CJ (2001) Fluorescently-labeled fimbrin decorates a dynamic actin filament network in live plant cells. *Planta* 213 (3):390-395
- Lancelle S, Cresti M, Hepler P (1987) Ultrastructure of the cytoskeleton in freeze-substituted pollen tubes of *Nicotiana glauca*. *Protoplasma* 140 (2-3):141-150
- Li Y, Shen Y, Cai C, Zhong C, Zhu L, Yuan M, Ren H (2010) The type II Arabidopsis formin14 interacts with microtubules and microfilaments to regulate cell division. *The Plant Cell* 22 (8):2710-2726
- Liu B, Palevitz BA (1992) Organization of cortical microfilaments in dividing root cells. *Cell motility and the cytoskeleton* 23 (4):252-264
- Livanos P, Muller S (2019) Division Plane Establishment and Cytokinesis. *Annual review of plant biology* 70:239-267. doi:10.1146/annurev-arplant-050718-100444
- Lloyd CW, Traas J (1988) The role of F-actin in determining the division plane of carrot suspension cells. *Development* 102 (1):211-221
- McCurdy DW, Gunning BE (1990) Reorganization of cortical actin microfilaments and microtubules at preprophase and mitosis in wheat root-tip cells: a double label immunofluorescence study. *Cell motility and the cytoskeleton* 15 (2):76-87
- McCurdy DW, Kim M (1998) Molecular cloning of a novel fimbrin-like cDNA from *Arabidopsis thaliana*. *Plant molecular biology* 36 (1):23-31
- McCurdy DW, Sammut M, Gunning BES (1988) Immunofluorescent visualization of arrays of transverse cortical actin microfilaments in wheat root-tip cells. *Protoplasma* 147 (2):204-206. doi:10.1007/BF01403349
- Molè-Bajer J, Bajer AS, Inoué S (1988) Three-dimensional localization and redistribution of F-actin in higher plant mitosis and cell plate formation. *Cell Motility and the Cytoskeleton* 10 (1-2):217-228
- Murata T, Karahara I, Kozuka T, Giddings Jr TH, Staehelin LA, Mineyuki Y (2002) Improved method for visualizing coated pits, microfilaments, and microtubules in cryofixed and freeze-substituted plant cells. *Journal of electron microscopy* 51 (2):133-136
- Palevitz BA (1987) Actin in the preprophase band of *Allium cepa*. *The Journal of cell biology* 104 (6):1515-1519
- Panteris E (2008) Cortical actin filaments at the division site of mitotic plant cells: a reconsideration of the 'actin-depleted zone'. *New Phytologist* 179 (2):334-341
- Panteris E, Adamakis I-DS, Tzioutziou NA (2009) Abundance of actin filaments in the preprophase band and mitotic spindle of brick1 *Zea mays* mutant. *Protoplasma* 236 (1-4):103-106

- Riedl J, Crevenna AH, Kessenbrock K, Yu JH, Neukirchen D, Bista M, Bradke F, Jenne D, Holak TA, Werb Z (2008) Lifeact: a versatile marker to visualize F-actin. *Nature methods* 5 (7):605-607
- Sadot, E. and E. B. Blancaflor (2019). The Actomyosin System in Plant Cell Division: Lessons Learned from Microscopy and Pharmacology. *The Cytoskeleton*, Springer: 85-100.
- Sampathkumar A, Lindeboom JJ, Debolt S, Gutierrez R, Ehrhardt DW, Ketelaar T, Persson S (2011) Live cell imaging reveals structural associations between the actin and microtubule cytoskeleton in Arabidopsis. *Plant Cell* 23 (6):2302-2313. doi:10.1105/tpc.111.087940
- Sano T, Higaki T, Oda Y, Hayashi T, Hasezawa S (2005) Appearance of actin microfilament 'twin peaks' in mitosis and their function in cell plate formation, as visualized in tobacco BY-2 cells expressing GFP-fimbrin. *Plant J* 44 (4):595-605. doi:10.1111/j.1365-313X.2005.02558.x
- Schmit AC, Lambert AM (1988) Plant actin filament and microtubule interactions during anaphase--telophase transition: effects of antagonist drugs. *Biol Cell* 64 (3):309-319. doi:10.1016/0248-4900(88)90005-6
- Seagull R, Heath I (1979) The effects of tannic acid on the in vivo preservation of microfilaments. *European journal of cell biology* 20 (2):184-188
- Sheahan MB, Staiger CJ, Rose RJ, McCurdy DW (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 physiology* 136 (4):3968-3978
- Sonobe S, Shibaoka H (1989) Cortical fine actin filaments in higher plant cells visualized by rhodamine-phalloidin after pretreatment with m-maleimidobenzoyl N-hydroxysuccinimide ester. *Protoplasma* 148 (2-3):80-86
- Takeuchi M, Staehelin A, Mineyuki Y (2017) Actin-microtubule interaction in plants. *Cytoskeleton-Structure, Dynamics, Function and Disease InTechOpen*:33-54
- Takeuchi M, Karahara I, Kajimura N, Takaoka A, Murata K, Misaki K, Yonemura S, Staehelin LA, Mineyuki Y (2016) Single microfilaments mediate the early steps of microtubule bundling during preprophase band formation in onion cotyledon epidermal cells. *Mol Biol Cell* 27 (11):1809-1820. doi:10.1091/mbc.E15-12-0820
- Tiwari SC, Polito VS (1988) Organization of the cytoskeleton in pollen tubes of *Pyrus communis*: a study employing conventional and freeze-substitution electron microscopy, immunofluorescence, and rhodamine-phalloidin. *Protoplasma* 147 (2-3):100-112

- Tiwari SC, Wick SM, Williamson RE, Gunning B (1984) Cytoskeleton and integration of cellular function in cells of higher plants. *The Journal of cell biology* 99 (1 Pt 2):63s
- Traas JA, Doonan JH, Rawlins DJ, Shaw PJ, Watts J, Lloyd CW (1987) An actin network is present in the cytoplasm throughout the cell cycle of carrot cells and associates with the dividing nucleus. *The Journal of cell biology* 105 (1):387-395
- Valster AH, Pierson ES, Valenta R, Hepler PK, Emons A (1997) Probing the Plant Actin Cytoskeleton during Cytokinesis and Interphase by Profilin Microinjection. *Plant Cell* 9 (10):1815-1824. doi:10.1105/tpc.9.10.1815
- van Gisbergen PA, Bezanilla M (2013) Plant formins: membrane anchors for actin polymerization. *Trends in cell biology* 23 (5):227-233
- van Gisbergen PA, Li M, Wu S-Z, Bezanilla M (2012) Class II formin targeting to the cell cortex by binding PI (3, 5) P2 is essential for polarized growth. *Journal of Cell Biology* 198 (2):235-250
- Vanstraelen M, Acosta JAT, De Veylder L, Inzé D, Geelen D (2004) A plant-specific subclass of C-terminal kinesins contains a conserved a-type cyclin-dependent kinase site implicated in folding and dimerization. *Plant physiology* 135 (3):1417-1429
- Vanstraelen M, Van Damme D, De Rycke R, Mylle E, Inze D, Geelen D (2006) Cell cycle-dependent targeting of a kinesin at the plasma membrane demarcates the division site in plant cells. *Current biology : CB* 16 (3):308-314. doi:10.1016/j.cub.2005.12.035
- Voigt B, Timmers AC, Samaj J, Muller J, Baluska F, Menzel D (2005) GFP-FABD2 fusion construct allows in vivo visualization of the dynamic actin cytoskeleton in all cells of *Arabidopsis* seedlings. *European journal of cell biology* 84 (6):595-608. doi:10.1016/j.ejcb.2004.11.011
- Wang YS, Motes CM, Mohamalawari DR, Blancaflor EB (2004) Green fluorescent protein fusions to *Arabidopsis* fimbrin 1 for spatio-temporal imaging of F-actin dynamics in roots. *Cell motility and the cytoskeleton* 59 (2):79-93
- Wang YS, Yoo CM, Blancaflor EB (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 Phytologist* 177 (2):525-536
- Wieland T, Faulstich H, Fiume L (1978) Amatoxins, phallotoxins, phallolysin, and antamanide: the biologically active components of poisonous *Amanita* mushroom. *CRC critical reviews in biochemistry* 5 (3):185-260
- Wu SZ, Bezanilla M (2014) Myosin VIII associates with microtubule ends and together with actin plays a role in guiding plant cell division. *Elife* 3. doi:10.7554/eLife.03498

- Wulf E, Deboen A, Bautz F, Faulstich H, Wieland T (1979) Fluorescent phalloxin, a tool for the visualization of cellular actin. *Proceedings of the national academy of sciences* 76 (9):4498-4502
- Xu T, Qu Z, Yang X, Qin X, Xiong J, Wang Y, Ren D, Liu G (2009) A cotton kinesin GhKCH2 interacts with both microtubules and microfilaments. *Biochemical Journal* 421 (2):171-180
- Xue X-H, Guo C-Q, Du F, Lu Q-L, Zhang C-M, Ren H-Y (2011) AtFH8 is involved in root development under effect of low-dose latrunculin B in dividing cells. *Molecular plant* 4 (2):264-278
- Yasuda H, Kanda K, Koiwa H, Suenaga K, Kidou S, Ejiri S (2005) Localization of actin filaments on mitotic apparatus in tobacco BY-2 cells. *Planta* 222 (1):118-129. doi:10.1007/s00425-005-1522-8
- Yoneda A, Akatsuka M, Kumagai F, Hasezawa S (2004) Disruption of actin microfilaments causes cortical microtubule disorganization and extra-phragmoplast formation at M/G1 interface in synchronized tobacco cells. *Plant Cell Physiol* 45 (6):761-769. doi:10.1093/pcp/pch091
- Yoneda A, Kutsuna N, Higaki T, Oda Y, Sano T, Hasezawa S (2007) Recent progress in living cell imaging of plant cytoskeleton and vacuole using fluorescent-protein transgenic lines and three-dimensional imaging. *Protoplasma* 230 (3-4):129-139