

Charles University, Faculty of Science  
Department of Experimental Plant Biology



**Mgr. Petra Cifrová**

**STORY OF THE TWO PLANT ACTIN NUCLEATORS:  
INTERACTION OF ARP2/3 COMPLEX AND FORMINS**

**Příběh dvou aktinových nukleátorů: interakce ARP2/3 komplexu a forminů**

**Doctoral thesis**

supervisor: RNDr. Kateřina Schwarzerová, Ph.D.

consultant: prof. RNDr. Fatima Cvrčková, Dr.

Prague, 2024

## STATEMENT

I hereby declare that I have prepared this dissertation independently under the supervision of the supervisor mentioned above. The thesis has not been submitted to any other university and the data given has not been used to obtain any other academic degree.

All literature sources used have been properly cited. The thesis identifies my personal contribution to the results of publications and manuscripts. Colleagues and co-authors of the draft have agreed to their inclusion in the dissertation. Artificial intelligence technologies were used solely to improve readability and language, not for data interpretation or drawing scientific conclusions.

In Prague on ..... Petra Cifrová

.....

## PROHLÁŠENÍ

Tímto prohlašuji, že jsem tuto disertační práci vypracovala samostatně pod vedením výše uvedeného školitele. Práce nebyla předložena na žádné jiné vysoké škole a uvedené údaje nebyly použity k získání jiného akademického titulu.

Všechny použité literární zdroje byly řádně citovány. V práci je uveden můj osobní podíl na výsledcích přiložených publikací a manuskriptu. Kolegové a spoluautoři manuskriptu souhlasili s jejich zařazením do disertační práce. Technologie umělé inteligence byly použity výhradně pro zlepšení čitelnosti a anglického jazyka, nikoliv pro vyvozování vědeckých závěrů nebo interpretaci dat.

V Praze dne ..... Petra Cifrová

.....

# TABLE OF CONTENT

<b>TABLE OF CONTENT</b>	<b>2</b>
<b>ACKNOWLEDGEMENTS</b>	<b>3</b>
<b>ABSTRAKT</b>	<b>5</b>
<b>ABSTRACT</b>	<b>6</b>
<b>INTRODUCTION</b>	<b>7</b>
Actin cytoskeleton in the plant cells	7
Actin-Binding Proteins (ABPs)	7
Actin nucleators: ARP2/3 complex and formins family	11
ARP2/3 Complex	12
Formins	15
Actin nucleators and pavement cell morphology	17
Actin nucleators and pollen tube tip growth	19
Actin nucleators and trichome shape	20
Actin nucleators and their connection with the cell wall and cell to cell adhesion	22
Actin nucleators and exocytosis and endocytosis	23
<b>DISSERTATION AIMS</b>	<b>25</b>
<b>PUBLICATIONS AND MANUSCRIPT ATTACHED TO THIS THESIS</b>	<b>26</b>
1. <i>Arabidopsis</i> FH1 Formin Affects Cotyledon Pavement Cell Shape by Modulating Cytoskeleton Dynamics	27
2. <i>Arabidopsis thaliana</i> plants lacking the ARP2/3 complex show defects in cell wall assembly and auxin distribution	28
3. <i>Arabidopsis</i> Class I Formin FH1 Relocates Between Membrane Compartments During Root Cell Ontogeny And Associates With Plasmodesmata	29
4. Division of Labor Between Two Actin Nucleators—the Formin FH1 and the ARP2/3 Complex—in <i>Arabidopsis</i> Epidermal Cell Morphogenesis	30
5. CRISPR-Cas9 <i>Arabidopsis</i> mutants of genes for ARPC1 and ARPC3 subunits of ARP2/3 complex reveal differential roles of complex subunits	31
6. ARP2/3 complex associates with peroxisomes to participate in pexophagy in plants	32
7. Pollen tube cell wall modification employs ARP2/3 complex-dependent endocytosis	33
<b>DISCUSSION</b>	<b>34</b>
The differences and similarities in the functions of plant actin nucleators	34
ARP2/3 complex and formins are vital for animal and yeast cells, but not for plant cells	40
Actin nucleators cooperate during pathogen-plant interactions	42
What could be behind the morphological and adhesion phenotypes of ARP2/3 complex mutant plants?	43
Future questions	48
<b>CONCLUSION</b>	<b>49</b>
<b>REFERENCES</b>	<b>50</b>
Used software	51
Theses and publications cited	51

# ACKNOWLEDGEMENTS

If human life is possible to compare with an actin filament, its complexity, and multifaceted roles would mirror the dynamic functions of the actin cytoskeleton within a cell. Just as an actin filament is essential for various cellular processes, each individual in society contributes uniquely to the intricate tapestry of life. Actin filaments provide dynamic movements and changes inside the cells, facilitating cell shape establishment. Similarly, the relationships and roles we assume - daughter, sister, wife, mother, friend, colleague - create the framework for our lives, helping us navigate through challenges and changes. Just as actin filaments interact with a network of proteins to maintain cell life and growth, we interact with those around us, forming bonds and networks that sustain and support us. The constant remodelling and dynamic nature of actin filaments mirror our own adaptability and growth as we encounter new experiences, and challenges, offering guidance and support to ensure that each of us reaches our potential destinations. Here I would like to express my deep gratitude to all those who helped me with this work to achieve my PhD shaped goal.

Most special thanks belong to my lab partner Jan Martinek. Without him, my scientific career and my passion for science would not have been possible. Thank you very much. I will cherish all our confocal-Mrtvá Ryba sessions and all the sunsets forever.

I would like to thank my supervisor Kateřina Schwarzerová for all her patience, support, and energy during the long years of my study. I would also like to thank my colleagues in the laboratory, especially Bára, Anička, Marie, Jana, Standa, Lenka, Erica, Honza, and all the others I have not mentioned, for the friendly atmosphere and creative working environment, for their time, advice and help. Furthermore, the formin family has been enthusiastically studied for decades in the lab next door by my colleagues Denisa, Amparo, and Eva. Our cool collaboration with Fatima Cvrčková's team has raised the exciting topic of ARP2/3 complex and formin interplay. Thank you for all the help, energy, and ideas during my studies. Finally, I would like to express my sincere gratitude to Roman Pleskot and his laboratory for welcoming me into my new scientific family, which helped me to complete and write my thesis. Please, accept my undying gratitude, Dan and Samy, for showing me that it is possible to use my own brain to do science. I am so grateful to be surrounded by such amazing people throughout my scientific journey.

The fundamental and loving support of my mother and sister and their love, laughter and understanding helped me through all my studies. Fn Fn Thank you!

Finally, I would like to thank my beloved family from the bottom of my heart: my husband Michal and my children Helena and Jakub for their love, support, understanding, time and great patience with my work. I could not finish without you by my side.

The ARP2/3 complex is the most studied actin nucleator, with hundreds of papers published in the last 25 years. I would like to acknowledge here the ARP2/3 complex centred theses of my laboratory team colleagues Jindřiška Fišerová (Fišerová, 2007), Lenka Havelková (Havelková, 2010), Kateřina Šlajcherová (Schiebertová, 2013), Pavlína Miklánková (Miklánková, 2015),

Jan Martinek (Martinek, 2015), Matěj Semerák (Semerák, 2016), Kamila Dubenecká (Dubenecká, 2018), Barbora Němcová (Němcová, 2020), Barbora Jelínková (Jelínková, 2021) and Maria Voloshina (Voloshina, 2023). As a team, we have collectively advanced our knowledge of actin nucleation a few steps further. Good work!

# ABSTRAKT

Cytoskelet rostlin, provázaná síť aktinových a mikrotubulových vláken, je zásadní pro mnoho buněčných procesů, které jsou nezbytné pro růst, vývoj a reprodukci rostlin. Důležitou součástí tohoto dynamického systému je aktinový cytoskelet, který je regulován skupinou aktin-vázajících proteinů, včetně dvou aktinových nukleátorů, ARP2/3 komplexu a rodinou forminů. Cílem mého doktorského výzkumu bylo objasnit rozdíly ve funkcích ARP2/3 komplexu a forminů a odhalit mechanismy, které stojí za morfologickými fenotypy rostlin s nefunkčním komplexem ARP2/3. Zatímco vliv ARP2/3 komplexu a forminů na morfogenezi buněk *Arabidopsis thaliana* byl popsán, interakce mezi těmito dvěma nukleátory zůstaly neprozkoumány. V rámci mé práce jsem porovnávala rostliny s mutovanými podjednotkami ARP2/3 komplexu a mutanty v nejzastoupenějším forminu v rostlinách (FORMIN1) společně s jejich dvojitými mutanty a zjistila jsem, že tvar trichomů a epidermálních buněk děložních lístků se u dvojitých mutantů podobá mutantům v podjednotkách ARP2/3 komplexu. Nicméně, hlubší analýza odhalila složitější, neantagonistický vztah mezi těmito dvěma nukleárními systémy. Následující studie aktinového a mikrotubulového cytoskeletu v rostlinách s mutantními aktinovými nukleátory, spolu s jejich odlišnou lokalizací v rostlinných buňkách, dále upřesnila naše porozumění jejich rolím. Navíc jsme v rámci našeho výzkumu přiloženého k této disertační práci studovali jak difúzně rostoucí buňky, tak apikálně rostoucí rostlinné buňky – pylové láčky. Zatímco role forminů jako aktinových nukleátorů v apikálně rostoucích pylových láčkách je dobře známá, role komplexu ARP2/3 v růstu pylových láček ještě nebyla zkoumána. Náš výzkum ukázal, že mutace v komplexu ARP2/3 ovlivňují růst pylových láček a následně produkci semen. Dále jsme zjistili, že podjednotka komplexu ARP2/3 – podjednotka ARPC3 – je důležitou součástí ARP2/3 komplexu specificky pro růst pylových láček. Mutace ARPC3 podjednotky neovlivňuje cirkularitu pokožkových buněk děložních lístků a tvar trichomů, ale ovlivňuje prodlužování pylových láček. Naše následná práce poté odhalila, že morfologické změny a problémy s adhezí pokožkových buněk popsané při nefunkčnosti ARP2/3 komplexu jsou spojeny s lokálně změněnými stavy pektinu v buněčné stěně. Moje disertační práce zkoumá různé role aktinových nukleátorů, komplexu ARP2/3 a forminů, v rostlinných buňkách, se zaměřením na jejich vliv na cytoskelet a následně na morfologii buněk.

# ABSTRACT

The plant cytoskeleton, a complex network of actin and microtubule filaments, is crucial for numerous processes essential to plant growth, development, and reproduction. An important part of this dynamic system is the actin cytoskeleton, which is regulated by various actin-binding proteins, including only two actin nucleators in plants, the ARP2/3 complex, and the family of formins. My doctoral research aims to elucidate the differences in function between ARP2/3 complex and formins as well as to uncover the mechanisms underlying the morphological phenotypes observed in ARP2/3 complex mutant plants. While the influence of the ARP2/3 complex and formins on *Arabidopsis thaliana* cell morphogenesis has been described, the interactions of the two nucleators have remained unexplored. By comparing plants with mutant subunits of the ARP2/3 complex and mutants of the most abundant formin in plants (FORMIN1), together with their double mutants, we found that the shape of trichomes and epidermal cells of cotyledons of the double mutants resembles those of the ARP2/3 complex mutants. However, a deeper analysis revealed a more complex, non-antagonistic relationship between the two nucleation systems. A following study of the actin and microtubule cytoskeleton in plants with mutant actin nucleators, along with their distinct localisation in plant cells, further refined our understanding of their roles. Furthermore, in our research work attached to this dissertation thesis, we studied both diffusely growing cells and tip growing plant cells - pollen tubes. While the role of formins as actin nucleators in the apical growing pollen tubes is well known, the role of the ARP2/3 complex in pollen tube growth has yet to be examined. Our research has shown that mutations in the ARP2/3 complex affect pollen tube growth and, subsequently, seed production. In addition, we found that a subunit of the ARP2/3 complex - ARPC3 subunit - is an important component of the ARP2/3 complex specifically for pollen tube growth, as its mutation does not affect the circularity of cotyledon epidermal cells and trichome shape but affect pollen tube elongation. Our subsequent work then revealed that morphological changes associated with the ARP2/3 complex are linked to locally altered pectin states in the cell wall, which stood behind affected cell-to-cell adhesion and cell shape of ARP2/3 complex mutant plants. My dissertation thesis explores the distinct roles of the actin nucleators, the ARP2/3 complex, and formins, in plant cells, with a focus on their effects on the cytoskeleton and, subsequently, on cell morphology.

# INTRODUCTION

## Actin cytoskeleton in the plant cells

The plant cytoskeleton consists of two types of filaments: microfilaments, known as the actin cytoskeleton, and microtubules. These filaments are interconnected and work together to form a dynamic network that allows plant cells to respond to a variety of stimuli and provides a scaffold crucial for essential intracellular processes such as cell growth, division, intracellular trafficking, and signalling (reviewed in X. Wang & Mao, 2019 and Yuan et al., 2023). Actin filaments are 7 nm thick, polarised, linear, right-handed, double-stranded helical filamentous protein polymers composed of the abundant cytoplasmic monomeric protein, actin. Actin is a 42kDa globular, monomeric protein, also called G-actin. When G-actin assembles into filaments, it is referred to as F-actin. These actin filaments are polarised, with a plus (barbed end) and a minus (pointed) end (Pollard & Borisy, 2003). Actin filaments are formed by diverse actin isoforms. Plants possess more isoforms of G-actin compared to mammals, which have only 6 isoforms (Perrin & Ervasti, 2010). *Arabidopsis thaliana* has genes encoding 8 actin isoforms, which are categorised into two groups based on their expression patterns - generative and vegetative actin isoforms. Generative actin isoforms are expressed in reproductive organs (ACT1, ACT3, ACT4, ACT11, ACT12), and vegetative isoforms are expressed in leaves, stems, and roots (ACT2, ACT7, ACT8) (McDowell et al., 1996) (Meagher et al., 1999) (Kijima et al., 2018). The remaining isoforms ACT5 and ACT9 did not show expression in any tested tissues and were initially considered pseudogenes. However, according to a database search, they are expressed in *Arabidopsis* endosperm (An and Meagher 2010) (Slajcherova et al., 2012). The functional importance of two distinct actin isoforms was demonstrated by Kandasamy and colleagues (2002) through the ectopic expression of the reproductive actin isoform ACT1 in vegetative tissues, which unexpectedly affected most plant organs and caused dwarfism (Kandasamy et al., 2002). Furthermore, mutation of reproductive actin isoform ACT11 is shown to delay pollen germination and enhance pollen tube growth (Chang & Huang, 2015). The actin network is highly dynamic, undergoing constant remodelling through processes such as filament growth, shrinkage, bundling, branching, bending, curling, and disintegration. This dynamic rearrangement of the actin network, known as actin polymerization and depolymerization, is mainly regulated by the timing and location of new filament nucleation, elongation, crosslinking, and disassembly. These processes are controlled by Actin-Binding Proteins (ABPs), which will be described in the next chapter.

## Actin-Binding Proteins (ABPs)

*Arabidopsis* actin-binding proteins are encoded by large, differentially expressed families whose primary role is the fine, precise, and rapid tuning of the actin network's dynamics and structure (see Figure 1). It is important to realise that actin-binding proteins can have one or multiple roles, each influencing and shaping the actin cytoskeleton network (see, e.g., Lian et

al., 2021 and García-González & van Gelderen, 2021 or Yuan et al., 2023). Key types of plant ABPs include:

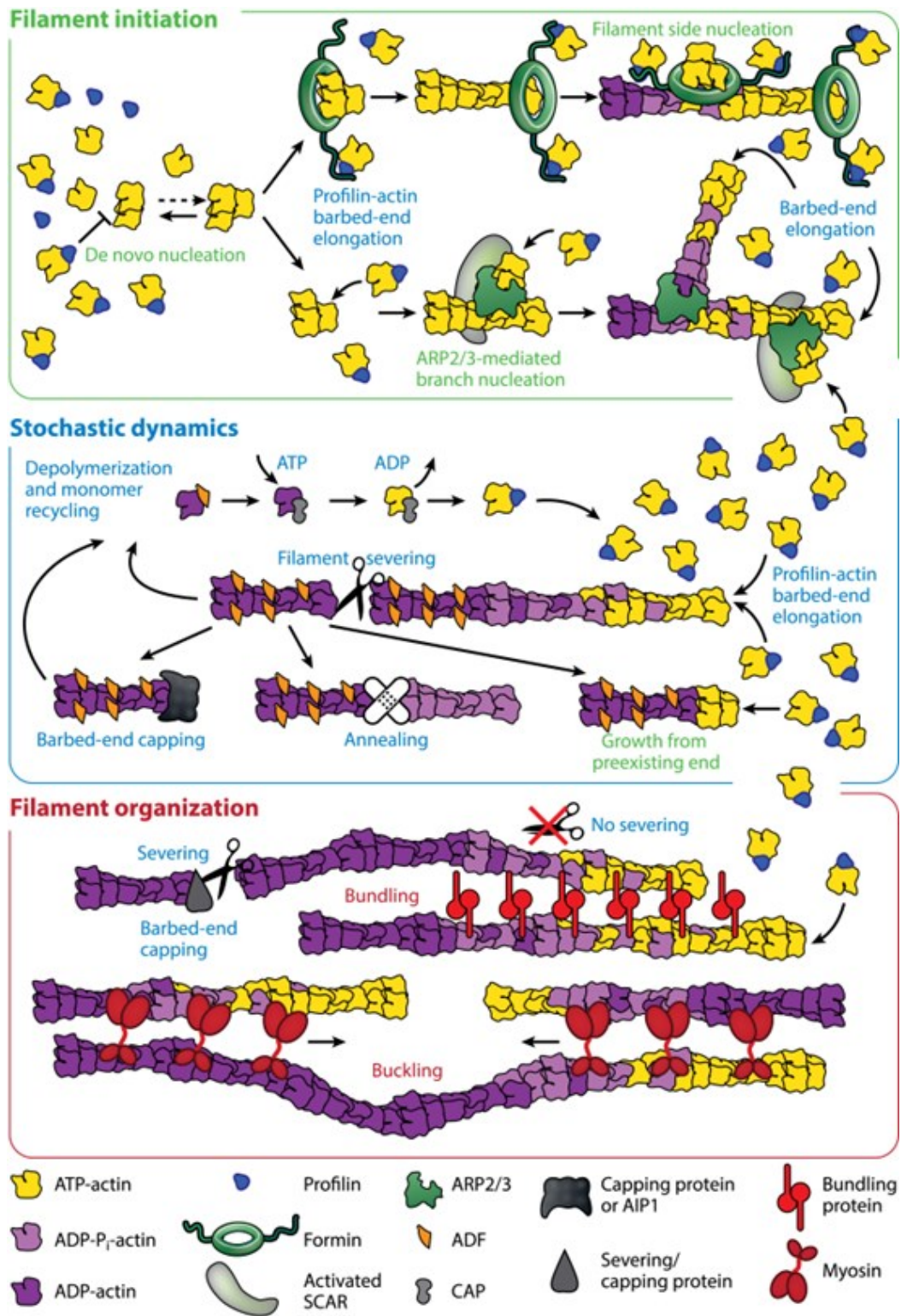
1. **Profilins:** Small proteins binding actin monomers (G-actin), preventing spontaneous nucleation and promoting polymerization when actin is needed. These proteins are crucial for formin-mediated actin filament nucleation.
2. **Actin-Depolymerizing Factors (ADF) and Severing proteins:** ADF proteins play a key role in regulating actin dynamics by binding to actin filaments (F-actin) and promoting their disassembly, which contributes to the rapid turnover of the actin cytoskeleton. Severing proteins such as villin, and some formins shorten filaments by creating breaks in the backbone and generating new ends for assembly and disassembly. These proteins are essential for actin filament reorganisation, with severing activity being the predominant mechanism for filament disassembly.
5. **Crosslinking and bundling proteins:** Actin filaments are often arrayed into higher-order structures like actin cables and bundles. The villins, fimbrins and some formins proteins crosslink actin filaments into bundles, which are vital for maintaining nucleus migration, organelles movement, and cytoplasmic streaming. They also have the capacity to sever and cap actin filaments.
6. **Capping proteins and villin:** These proteins bind to the barbed ends of actin filaments, preventing further addition of actin monomers, thus regulating filament length and dynamics.
7. **NETWORKED (NET) proteins:** This group of proteins associate actin filaments primarily with membranes, NET1 with plasma membrane and plasmodesmata, NET2A with the plasma membrane, NET3B with the endoplasmic reticulum and NET4 with tonoplast system.
8. **Myosin motors:** Myosins are actin-based motor proteins belonging to the myosin VIII and XI families. The *Arabidopsis* genome encodes thirteen members of the class XI myosins and four members of the class VIII motor proteins. They are essential for cytoplasmic streaming, as well as the movement and transport of organelles and vesicles. Plant myosins typically move from the minus to the plus end of the actin filament.
9. **Actin nucleators:** Actin nucleators, such as ARP2/3 complex and formins are integral to maintaining the dynamic nature of the actin cytoskeleton in plants. Generally, formins are believed to nucleate long actin filaments and actin bundles, while the ARP2/3 complex is responsible for generating fine, branched actin meshworks.

Actin-binding proteins can bind to G-actin or F-actin, or both. Actin nucleators, such as the ARP2/3 complex and family of formins generate starts for filament assembly from either the G-actin subunit monomer pool or profilin–G-actin complexes (Henty-Ridilla et al., 2013) (Li

et al., 2015). Most of the actin in plant cells exists in the monomer pool (G-actin), with only about 5–10% estimated to be in filamentous form (F-actin) (Henty-Ridilla et al., 2013). This monomer pool is buffered by an equally abundant amount of profilin, which forms a 1:1 complex with G-actin. In *in vitro* experiments, ATP-bound G-actin monomers are likely attached to the fast-growing barbed end of the actin filament. Conversely, ADP-bound G-actin monomers are disassembled from the slower-growing pointed end of the actin filament. Shortly after the ATP-loaded G-actin monomers are added to the fast-growing barbed end, nucleotide hydrolysis occurs, converting them to ADP-P<sub>i</sub>-loaded subunits. Subsequently, (inorganic phosphate) P<sub>i</sub> is released, leaving the ADP-loaded G-actin subunits. This results in older regions of the filament containing ADP-loaded G-actin, which can signal different types of actin-binding proteins, as some ABPs interact specifically with ADP-actin (Blanchoin et al., 2014).

Actin filament networks have been known to support and facilitate cytoplasmic streaming, organelle movements, and secretory pathways in plant cells (Yuan et al., 2023). As such, the actin cytoskeleton is recognised as a key player in establishing cellular morphogenesis, growth polarity, and cell wall assembly. The cortical actin cytoskeleton typically consists of two types of filaments: actin bundles and individual actin filaments. Actin bundles, labelled fluorescently, appear brighter, usually straighter, and have a longer lifespan than individual actin filaments. Individual actin filaments, which are very hard to observe are characterised by their lower fluorescent intensity (Staiger et al., 2009). These single actin filaments are highly dynamic, undergoing nucleation, depolymerization, polymerization, rearrangement, and fusion into bundles within tens of seconds (Vidali et al., 2010). This dynamic process is called the “treadmilling model”. It describes the process where G-actin assembles at the barbed end while disassembling at the pointed end, creating a flow of subunits through the filament over time (Staiger et al., 2009) (Carlier and Shekhar, 2017). Another model called “stochastic dynamic turnover model” describes the dynamic behavior of single filaments in the cortical array (Staiger et al., 2009) (Henty-Ridilla et al., 2013). To demonstrate the importance of actin-binding proteins in the regulation of the actin cytoskeleton network, a model is provided that illustrates the regulation of actin organisation and its stochastic dynamics (Figure 1).

The dynamic nature of actin filaments can be measured in *in vitro* experiments. It has been observed that the new filaments grow from one end at rates ranging from 1.6 to 3.4  $\mu\text{m/s}$  and can reach average maximum lengths of 10–15  $\mu\text{m}$  before starting to disassemble. It is estimated that 1  $\mu\text{m}$  long actin filament consists of approximately 370 actin subunits (Pollard et al., 2000). This rapid elongation is balanced by disassembly through severing activity, resulting in filaments that can attain lengths of about 15  $\mu\text{m}$  but remain short-lived (Michelot et al., 2007) (Staiger et al., 2009).



**Figure 1.: Model of the regulation of actin organisation and stochastic dynamics:** The model describes three overlapping levels of control: filament initiation, stochastic dynamics, and filament organisation (adapted from Li et al., 2015).

## **Actin nucleators: ARP2/3 complex and formins family**

It is essential to regulate both the timing and location of actin network polymerization in plant cells (Hussey et al. 2006). Important actin-binding proteins that initiate actin filament nucleation are actin nucleators. The only two actin nucleators identified so far in plant cells are the ARP2/3 complex and formins (Xu et al. 2023). The ARP2/3 complex and formins use different nucleation mechanisms and activation modes, and nucleate actin filaments at different speeds. The ARP2/3 complex is highly conserved, and its subunits do not have many isoforms in the genome. In contrast, the formin family is extensive and formins exhibit a diverse range of functions in plants beyond actin nucleation.

In animal cells, a variety of actin nucleators, beyond those found in plants, play crucial roles in cytoskeleton regulation. The ARP2/3 complex and formins are the most important but not the only actin nucleators. The ARP2/3 complex is a primary nucleator of branched actin networks. Formins are responsible for the nucleation and elongation of unbranched actin filaments. Another actin nucleator Spire nucleates actin filaments by binding to actin monomers and facilitating their assembly into short filaments. The Cordon-bleu (Cobl) nucleator creates long unbranched filaments and is involved in shaping cell morphology and neuron development. Leiomodin (Lmod) proteins promote the nucleation of actin filaments, particularly in muscle cells. JMY (Junction-mediating and regulatory protein) actin nucleator is involved in cell motility, DNA damage response, and transcriptional regulation. WHAMM (WASP homolog associated with actin, membranes, and microtubules) can nucleate actin filaments and is involved in vesicle transport and autophagy. Finally, Tandem Monomer-Binding Nucleators facilitate actin nucleation by stabilising actin monomers and promoting filament assembly and are involved in cell migration and signal transduction. The distinct and complementary roles of the actin nucleators in animal cells allow for dynamic and precise regulation of the actin cytoskeleton (see, e.g., Firat-Karalar & Welch, 2011).

Plant cells have only two actin nucleators, which makes them an ideal model for studying actin nucleation. In addition to actin nucleator mutants, cytoskeletal drugs, and actin nucleator inhibitors are commonly used to study actin cytoskeleton polymerization (Rosero et al., 2016). Cytoskeletal drugs are small molecules produced by various organisms, including fungi, sea sponges, and some plants. Actin depolymerizing drugs, such as Latrunculin B and Cytochalasin D, bind to globular actin, its nucleation, and polymerization, which leads to complete loss of actin filament structure. In contrast, phalloidin and jasplakinolide bind to F-actin and stabilise the filaments (Holzinger and Blaas, 2016). For microtubules, Oryzalin inhibits polymerization by binding to tubulin, while Taxol stabilises microtubules by binding to tubulin, preventing their depolymerization. Furthermore, targeted acute disruption of actin nucleation activity can be achieved with the use of actin nucleator inhibitors such as CK-666 and SMIFH2. CK-666 acts as an ARP2/3 complex inhibitor (Nolen et al. 2009), and SMIFH2 (small molecule inhibitor of FH2) (Cao et al. 2016) serves as a broad-spectrum formin inhibitor. Actin nucleation inhibitors have mainly been developed and tested in animal cells, but some initial studies have also explored their activity in plant cells (Ali et al. 2020) (Liu et al. 2020) (Xu et al., 2024).

## ARP2/3 Complex

The ARP2/3 complex consists of seven highly conserved subunits: two actin-related proteins (ARP2 and ARP3) and five additional subunits ARPC1-ARPC5, where the letter C denotes ARP2/3 complex Component. Actin-related proteins share partial sequence similarity with conventional actin monomers, with sequence homology ranging from 15% to 70% (Schroer et al., 1994) (Oma & Harata, 2011). The other five ARP2/3 complex Component subunits, ARPC1-ARPC5, mediate interactions with mother actin filaments, enabling the complex's branching function. ARPC2 and ARPC4 form the core of the complex, with ARPC4 being essential for structural integrity (Kotchoni et al., 2009) as illustrated in Figure 2.

The ARP2/3 complex subunits are expressed throughout the entire *Arabidopsis thaliana* plant (Li et al., 2003). Subunits ARPC1, ARPC2, and ARPC5 are encoded by two isoforms each in the *Arabidopsis* genome. The ARPC1 subunit has two isoforms, ARPC1A and ARPC1B, with almost identical sequences (Mergner et al., 2020). ARPC2 subunit is also coded by two isoforms, ARPC2A and ARPC2B, in the *Arabidopsis* genome, and it is a core subunit necessary for the integrity of the whole complex (El-Assal et al., 2004). The ARPC2B is not able to rescue the mutation in ARPC2A isoform, although it is widely expressed in plant tissues (El-Assal et al., 2004) (Mergner et al., 2020). This subunit is also important for the interaction of the ARP2/3 complex with microtubules (Havelková et al., 2015). Furthermore, ARPC3 is a peripheral subunit. Interestingly, it is not crucial for the integrity and function of the whole ARP2/3 complex, as the complex seems to be at least partially functional in vegetative tissues even without the ARPC3 subunit (Bellinvia et al., 2022). ARPC3 subunit is mainly expressed in pollen tubes (Li et al., 2003) (Mergner et al., 2020). The ARPC5 subunit has two isoforms in the *Arabidopsis thaliana* genome: ARPC5A and ARPC5B. Both isoforms are highly conserved. However, ARPC5B is not expressed throughout plant tissues and does not compensate for the ARPC5A mutant, suggesting that it is probably a pseudogene (Mergner et al., 2020). Notably, ARPC5 is the only subunit of the ARP2/3 complex that does not directly interact with either the mother or daughter actin filament. We can hypothesise that its role can be ARP2/3 complex stabilisation or can be involved in signalization and regulation of the ARP2/3 complex - visualised in Figure 2.

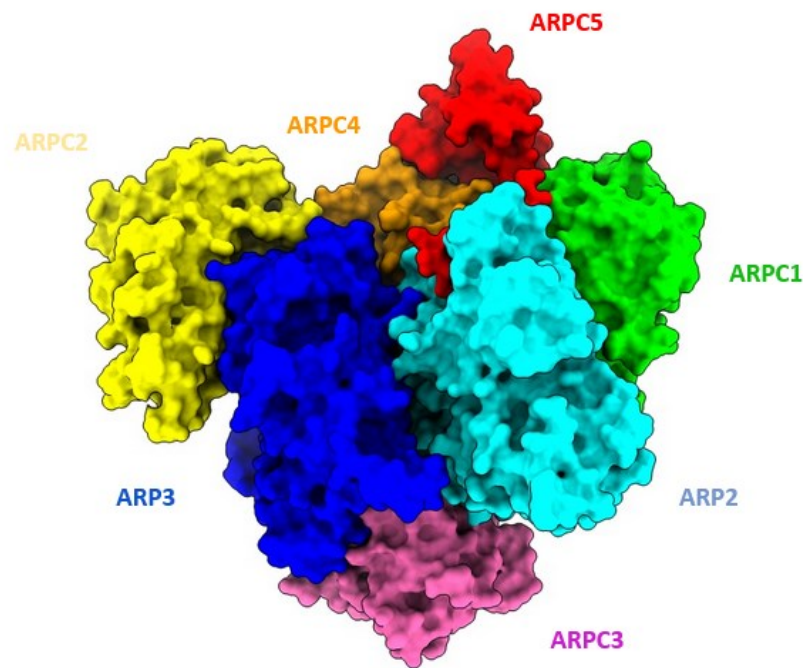
The ARP2/3 complex is essential for branching existing actin filaments by attaching to pre-existing (mother) filaments and nucleating new (daughter) filaments at approximately 70° angle, thereby forming a fine actin meshwork (Volkman et al., 2001). To nucleate actin filaments, the ARP2/3 complex must first be activated by nucleation-promoting factors (NPFs). Upon activation, ARP2 and ARP3 subunits within the complex converge to form the nucleation core. In animals, ARP2/3 is activated by various NPFs, including the WASP (Wiskott–Aldrich syndrome) protein (Zicha et al., 1998), N-WASP (Neuronal WASP), and WAVE (WASP family verprolin-homologous protein), originally described as SCAR (suppressor of the cAMP receptor) in *Dictyostelium discoideum* (Bear et al., 1998). In contrast, plants have lost WASP and WASH proteins, retaining only SCAR/WAVE as the sole NPF, with five members identified in *Arabidopsis* (SCAR1-SCAR4 and WAVE5) (Szymanski, 2005) (Zhang et al., 2008) (Zhang et al., 2013). SCAR/WAVE proteins feature a C-terminal VCA domain, essential

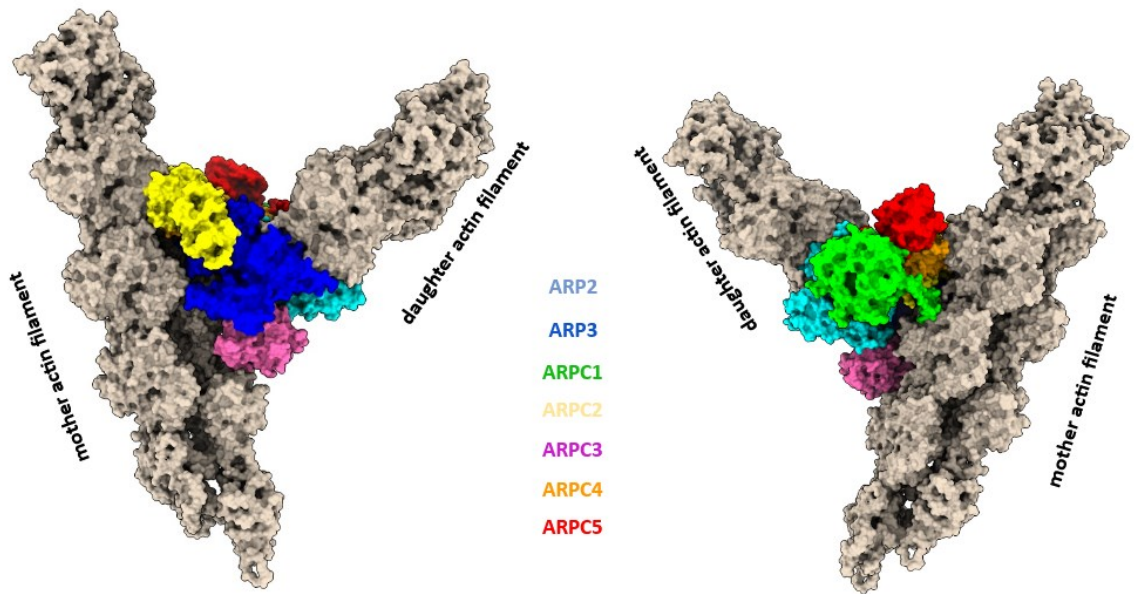
for ARP2/3 activation and actin monomer binding (Machesky et al., 1999). In plants, SCAR/WAVE operates within a multimeric complex (called W/SRC) composed of SCAR1-4, NAP1, BRICK1, ABIL1-4, and PIR1, which regulates its activity and interactions (Szymanski, 2005). Mutations in SCAR/WAVE complex subunits (SCAR1-4, NAP1, BRK1, ABIL and PIR1) exhibit phenotypes comparable with ARP2/3 complex mutants (Yanagisawa et al., 2013). Double mutants of SCAR/WAVE complex and ARP2/3 complex subunits also exhibit comparable phenotypes compared to individual ARP2/3 complex subunit mutants, except ARPC3 one (Deeks et al., 2004) (Bellinvia et al., 2022). In animal cells, the ARP2/3 complex activity is further modulated by the cell's natural inhibitors like Arpin, Coronin, and GMF, but no cell's natural inhibitors of ARP2/3 complex have been identified in *Arabidopsis*. The plants seem to have simplified regulation, with only one known activator complex (SCAR/WAVE) and no known inhibitors.

The major role of the ARP2/3 complex in animal and yeast cells is actin assembly and organisation at the membrane-cytoplasm interface. The ARP2/3 complex is essential for the establishment of lamellipodia by polymerization of actin filaments at the leading edge of the cell and thus for cell motility. It is necessary for cell migration during embryogenesis, chemotaxis of white blood cells, or neuronal axon growth, autophagy, endocytosis, apoptosis, chromatin dynamics, and DNA repair (Svitkina & Borisy, 1999) (Mogilner, 2006) and many more functions (see, e.g., Campellone et al., 2023). In yeast, ARP2/3 is responsible for the organisation of actin patches - dynamic actin structures that are important for endocytosis and cell wall organisation and remodelling (Daugherty & Goode, 2008) (Akram et al., 2020).

The role of the ARP2/3 complex in plants is still largely undocumented. In recent years, a large number of phenotypes associated with plant cell growth and development have been described that may point to exact role for the ARP2/3 complex in plants. The phenotypes of ARP2/3 complex and SCAR/WAVE complex mutations in plants are mostly associated with cell growth, morphogenesis, and cell-to-cell adhesion. ARP2/3 complex and SCAR/WAVE complex mutant plants have distorted trichomes, less lobed cotyledon pavement cells, disrupted cell-to-cell adhesion (Mathur et al., 2003) (Sahi et al., 2018) (Cifrová et al., 2020), root elongation problems (Dyachok et al., 2008), affected root hair initiation domain positioning (Chin et al., 2021), slowed stomatal opening (Zhao et al., 2011) (Jiang et al., 2012), and shorter and wider stems (Sahi et al., 2018). At the intracellular level, ARP2/3 complex and SCAR/WAVE complex mutants have subtle changes of actin and microtubule cytoskeleton arrangement in pavement cells (Cifrová et al., 2020) (Xu et al., 2024), decreased endocytosis (Qin et al., 2021), fragmented vacuolar system (Schiebertová 2013) (Semerák 2016) (García-González et al. 2020), slower autophagic degradation pathway including pexophagy (Wang et al., 2016) (Martinek et al., 2023), disrupted trafficking of auxin transport molecules (García-González et al., 2020) and defects in primary and secondary cell wall deposition (Dyachok et al., 2011) (Yanagisawa et al., 2015) (Sahi et al., 2018). To investigate the specific functions of the ARP2/3 complex in plants, it is essential to study its subcellular localisation. The study of the ARP2/3 complex in plant cells has demonstrated that the ARP2/3 complex is in close proximity with both the actin and microtubule cytoskeleton (Fiserová et al., 2006) (Maisch et al., 2009) (Yanagisawa et al., 2015) (Zhang et al., 2013). Furthermore, subunits of the ARP2/3

complex and components of the SCAR/WAVE complex subunits have been observed to colocalize with diverse organelles such as the nucleus or endoplasmic reticulum (ER) in immunolocalization experiments (Zhang et al., 2013). In our laboratory, we showed that ARPC2-GFP tagged subunit colocalized with microtubules and motile spots of unknown identity (Havelková et al., 2015), which we later uncovered to be peroxisome-associated ARP2/3-positive domain assisting in pexophagy (Martinek et al., 2023). ARP2/3 complex was localised to the tips of growing trichome branches (Yanagisawa et al., 2015). SCAR/WAVE complex subunits showed polarised localizations in root hairs during its initiation phase (Chin et al., 2021). NAP1-GFP was demonstrated to form pressure-induced puncta colocalizing with autophagosome markers (Wang et al., 2016). The number and complexity of ARP2/3 complex localization and mutant phenotypes suggest the complexity of actin-mediated tasks in the plant cells.





**Figure 2.: Activated ARP2/3 complex at a branch junction with mother and daughter actin filament**

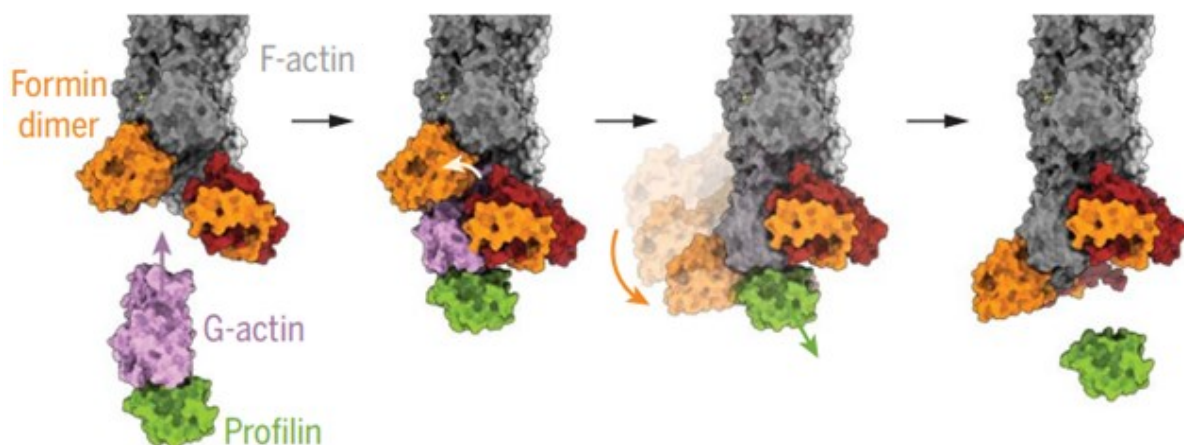
Created with data from (Ding et al., 2022) (they created Single-particle cryo-EM of branched actin filaments by mixing the *Bos taurus* ARP2/3 complex, *Oryctolagus cuniculus* skeletal muscle actin, and the ARP2/3 complex activator VCA domain of N-WASP, PDB: 7TPT), visualised by ChimeraX.

## Formins

The family of formins (formin homology proteins) is a large and evolutionarily conserved group of cytoskeletal regulators expressed through all plant tissues. Their roles are very diverse, including actin filament nucleation, actin filament capping, actin bundling and modulation of microtubule dynamics (Cvrčková et al., 2024). Formin family can be divided into three subfamilies called Class I (11 members), Class II (10 members), and Class III according to their characteristic domain organisation. Class III is documented in algae, mosses, and lycophytes and remains largely unexplored, Class III is absent in angiosperms (Grunt et al., 2008). Class I encodes formins proteins that typically contain a transmembrane domain (except *Arabidopsis* formin 7), and Class II encodes formins proteins without a transmembrane domain, but they typically carry an N-terminal phosphatase and tensin-related (PTEN)-like domain (Cvrčková, 2000) (Cvrčková et al., 2004) (Deeks et al., 2002) (Deeks et al., 2005). Nevertheless, Class II formins have the ability to bind membranes and are recruited to sites of membrane remodelling (van Gisbergen et al. 2012). Notably, Class I possibly can hold cell wall-binding motifs exposed to the extracytoplasmic space. Animal cells encode 15 different formins, whose mutations cause various neurological, immune and cardiovascular disorders. For animal and yeast formins is published that they are forming the dimers which form a ring-like structure around the barbed end of the actin filament. They bind to the barbed end of actin in an asymmetric conformation.

This published structure of the actin-formin-profilin ring complex reveals how formin dimer ring relocation and actin subunit flattening cause the fast releasing of profilin necessary for continual filament elongation. Finally, the ability of different formins to promote filament elongation at different speeds is shown to be due its differences in their actin binding interfaces (for more information about animal and yeast formins see (Oosterheert et al. 2024) or (Palmer et al. 2024)).

Formins are actin nucleators that have been implicated in the generation of linear actin bundles (Blanchoin & Staiger, 2010). The most characteristic and defining feature of formins is the presence of the formin homology domain 1 (FH1) and formin homology domain 2 (FH2). FH1 binds profilin-actin, the major source of actin monomers in cells. FH2 forms a dimeric, ring-like structure that promotes nucleation (Wasserman, 1998) (Moseley et al., 2004) (Xu et al., 2004) (Kovar et al., 2006). Formins can be additionally divided into two groups by their mode of attachment to the barbed end of the actin filaments - processive and nonprocessive formins. Processive formins remain attached to the growing end of the actin filament. They nucleate actin assembly and move along with the barbed end as new actin monomers are added. They are efficient in promoting rapid elongation of actin filaments (Oosterheert et al. 2024) (Palmer et al. 2024). The molecular mechanism of the actin filament elongation by formins is depicted in Figure 3. Nonprocessive formins bind to the actin filament and dissociate after nucleating a certain number of actin monomers. They do not remain attached to the filament's growing end. These formins typically nucleate the formation of new actin filaments but do not support sustained elongation. Instead, they might regulate the initial stages of filament assembly and then release the growing filament end and make space for other actin-binding proteins to take over. Some experimental studies have shown that some formins can exhibit both processive and nonprocessive behaviours under different conditions (Courtemanche, 2018). In addition, in yeast and animal cells, some formins can also depolymerize, sever or bundle actin filaments (Kovar et al., 2006).



**Figure 3.: How the actin filament elongates by actin-formin-profilin ring complex by the "undock-and-lock" mechanism:**

There are 4 proposed models of formin elongation mechanism (Palmer et al. 2024). This depicted model of formin filament elongation is called "undock-and-lock" mechanism - adapted from (Oosterheert et al., 2024): One half of the formin dimer ring is stably bound, the other half is loosely associated with the filament barbed end and is free and capable to capture a new actin subunit. When this new actin subunit arrives, its incorporation into the filament destabilised the formin ring arrangement. As a consequence, part of the formin dimer must undock, move ahead and form a new binding interface with the incorporated actin subunit. Adapted from (Oosterheert et al., 2024), the fission yeast *Cdc12* formins.

The majority of the plant formins biochemically examined so far bind directly to the barbed end of actin filaments and elongate the filament in a non-processive manner, as the FH1-FH2 domain of several plant formins does not alter the rate of actin/profilin addition to barbed ends (Xu et al., 2023). However, *in vitro* single-molecule imaging technology revealed that formin AtFH14 attaches to the actin processively (Zhang et al., 2016). Recently it was also confirmed *in vivo* that plant formins can contribute to the nucleation of side-branched filaments (Xu et al., 2023). Furthermore, plant formins AtFH1, AtFH8, AtFH14, and OsFH5 have been biochemically demonstrated to organise actin filaments into bundles. On the other hand, the AtFH3 and AtFH19 formins do not have bundling activity *in vitro* (Wang et al., 2013). Some formins can connect to and regulate the microtubule network. Notably, AtFH1 can modulate microtubule dynamics (Rosero et al., 2013) (Cifrová et al., 2020). AtFH4, AtFH14, OsFH5 and AtFH16 bind to microtubules (Deeks et al., 2010), (Li et al., 2010) (Zhang et al., 2011), (Wang et al., 2013).

Class I formin: FORMIN 1 (FH1 or AtFH1 gene, At3g25500) is the most expressed formin in *Arabidopsis* and has therefore been the most studied formin family representative. It is known that fluorescently labelled actin polymerized in the presence of AtFH1 (Rosero et al., 2013). AtFH1 detaches from actin filaments soon after nucleation, thus providing the example of a non-processive formin (Blanchoin & Staiger, 2010), furthermore FH1 stay attaches to the side of a preexisting actin filament to nucleate new filaments, suggesting a complicated role for formins in plant cells than just the ability to generate linear filaments (Michelot et al., 2006). The mutant phenotypes of *atfh1* involve larger and less circular pavement cells as a result of defective formation of lobes, cotyledon epinasty, mild trichome morphology defects, and the mutation influences actin and microtubules cytoskeleton (Rosero et al., 2016) (Rosero et al., 2013). Notably, Class I formin FH1 localises at plasmalemma and around plasmodesmata and temporarily it is localised in endomembranes, including secretory and endocytic pathway compartments and the tonoplast (summarised in Cvrčková et al., 2024).

## Actin nucleators and pavement cell shape

The pavement cell morphogenesis is a highly studied process that helps us understand the overall plant cell growth mechanism. Plant cell growth, driven by turgor pressure and localised modifications of the cell wall, is a gradual process that facilitates irreversible expansion in

certain areas or microdomains of the cell wall while restricting it in others (Sapala et al., 2018). The actin cytoskeleton involvement in both diffuse and apical plant cell growth is well-documented (Szymanski & Staiger, 2018). However, the exact molecular mechanisms underlying these processes are still not fully elucidated. It is proposed that actin is critical for the intracellular transport of cell wall materials, thereby enabling cellular growth and morphological changes (Szymanski & Cosgrove, 2009). The cell wall expansion is precisely coordinated between neighboring pavement cells, which results in a closely packed layer of interdigitating pavement jigsaw puzzle-shaped cells with coordinated development of lobes and indentations (necks). Initially, in the embryonic cotyledons, the pavement cells have the shape of cubes with straight cell walls, but as they grow side by side, the cells take on complex, jigsaw-like shapes with lobes and indentations and still maintain their adhesion. The cytoskeleton is important for the pavement cell morphology establishment. The model is that actin is essential in growing lobes to supply secreted components of the expanding cell wall, and microtubules, on the other hand, are located near necks and guide the arrangement of cellulose microfibrils (Sampathkumar et al., 2014). First, the microtubule rearrangement was described as the first observable event and stable determinant of the lobes (Armour et al. 2015). Later it was postulated that long-lived microtubules along the anticlinal wall do not predict the patterns of lobe formation and that lobe initiation is likely controlled by complex interactions among cell geometry, cell wall stress patterns, and transient microtubule networks (Belteton et al. 2018). Additionally, it was proposed that lobes are formed by a localised modification of pectins (Haas et al., 2021) (Cosgrove & Anderson, 2020).

How does the actin cytoskeleton nucleation influence the pavement cells' shape establishment? ARP2/3 complex and SCAR/WAVE complex mutants are known to have more circular (less lobed) epidermal pavement cells (Mathur et al., 2003) (Sahi et al., 2018) (Cifrová et al., 2020). And in agreement with this observation, the plant treated with actin cytoskeleton drugs latrunculin B, cytochalasin D, or jasplakinolide showed compromised pavement cell wall morphology, which was comparable to ARP2/3 complex mutant phenotypes (Holzinger 2022) (Trozzi et al. 2023). Furthermore, the ARP2/3 complex, and its activator complex SCAR/WAVE subunits are among other places localised in the submembrane cortical region at three cell junctions and in the growing lobes (Dyachok et al., 2008) (Qin et al., 2021). Moreover, the ARP2/3 complex is involved in the modulation of auxin transport (Sahi et al., 2018), (García-González et al., 2020), and auxin is a known player in pavement cell morphology establishment (Liu et al., 2021).

The connection between formins and pavement cell shape establishment is not well understood. However, the *Atfh1* mutant exhibits more complex and less circular epidermal pavement cell shapes (Rosero et al., 2016) (Cifrová et al., 2020). Unlike the ARP2/3 complex marker lines, AtFH1-GFP does not show any local enrichment at the lobes or necks of cotyledon pavement cells. Instead, AtFH1 and some other class I formins concentrate at plasmodesmata in epidermal pavement cells (Oulehlová et al., 2019) (Diao et al., 2018). Moreover, several *Arabidopsis* class I formins, such as AtFH5, AtFH1, and AtFH8, localise to the nascent cell plate during cell division, and the application of the formin inhibitor SMIFH2 in tobacco cell cultures disrupts cytokinesis (see, e.g., Cvrčková et al., 2024). The localisation of formins to the cell plate or

near plasmodesmata suggests that this may be their potential pathway for mediating their role in establishing cell shape. But more important and more likely is that AtFH1 influence pavement cells shape by direct interactions with the microtubule and actin cytoskeleton and, when mutated, it alters cytoskeleton structure and dynamics which lead to altered pavement cells shape (Cifrová et al., 2020).

## **Actin nucleators and pollen tube tip growth**

The tip or apical growth is the growth of the cells in one direction - on their tip. Tip growth is used by pollen tubes, root hairs, moss protonema, or fungal hyphae (Geitmann & Emons, 2000). Pollen tubes and root hairs are the two best-characterised tip-growing cell types of seed plants, and the actin cytoskeleton plays a crucial role in their growth. I will concentrate on the role of actin nucleators during pollen tube tip growth in my text.

The pollen tube invasive apical growth takes place to deliver the male gametes to the megagametophyte cells. During the fast but tightly controlled process of pollen tube tip growth, a huge amount of materials for plasma membrane expansion and cell wall synthesis and modifications need to be delivered via secretory vesicle networks to the place of growth (Scholz et al., 2020). To ensure fast and polarised growth and secure stability of the “long like conduit cell shape,” the pollen tube is comprised of three distinct regions: the apical region, which contains the "clear zone" (growth region), the subapical region, which contains the actin fringe (transition region), and the distal part, which is known as the shank region (Zhao et al., 2020). The actin cytoskeleton plays a pivotal yet distinct role in all three regions of pollen tubes. Moreover, actin is a very abundant protein in pollen, accounting for about 2-20% of the total soluble protein in pollen grains, which is generated by five reproductive actin isoforms (ACT1, ACT3, ACT4, ACT11, and ACT12) expressed in *Arabidopsis* pollen (Pawloski et al., 2006). Interestingly, actin monomers are uniformly distributed in the cytoplasm of *Arabidopsis* pollen tubes and can be rapidly redistributed by cytoplasmic streaming, suggesting that actin monomers are readily available for assembly by actin nucleators to actin filaments within the pollen tube (Chang et al., 2017).

The pollen tube actin network can be divided into three main structures: The shank-localised longitudinal actin bundles, the actin fringe, and the fine-branched apical actin structure. Longitudinal actin bundles in the shank region play an important role in pollen tube cytoplasmic streaming generated by the movement of barbed-end directed myosin motors along the shank-localised actin filaments. A distinct actin organisation called the actin fringe (called also actin collar, actin mesh, or actin ring) is located behind the pollen tube tip clear zone. This structure is proposed to act as a physical barrier to exclude large organelles from the pollen tube tip, organising and controlling the precise localization of endo- and exocytotic events, and generating the force to drive cell growth (Stephan, 2017) (Xu & Huang, 2020). Actin filaments are continuously polymerized from the plasma membrane at the tip of a growing *Arabidopsis* pollen tube (Qu et al., 2013). The main players in the nucleation are considered formins in cooperation with their functional partner profilins (Liu et al., 2015) (Lan et al., 2018). From 21

formins found in *Arabidopsis*, several are specifically expressed in pollen tubes, although their loss rarely results in major pollen tube phenotypes due to its functional overlap (“redundancy”). For example, loss of FORMIN 3 and FORMIN 5 leads to defects in the organisation of the actin fringe, causing mild pollen tube growth defects (Cheung et al., 2010) (Lan et al., 2018). Overexpression of FORMIN 3 leads to excessive actin cables formation and pollen tube tip swelling. Moreover, FORMIN 1 is expressed mainly in vegetative tissues, but its ectopic expression in pollen tubes also results in excessive formation of thick bundled actin cables, which leads to the pollen tube tip swelling (Cheung & Wu, 2004). Finally, treatment with the formin inhibitor SMIFH2 impairs actin polymerization from the plasma membrane at pollen tube tips (Qu et al., 2017). Recently, the loss of function of Class II pollen-specific FORMIN 13 resulted in the stimulation of pollen tube growth, while tagged FH13 overexpression inhibited pollen tube elongation (Kollárová et al., 2021). Even though the loss of FORMIN 13 in *Arabidopsis* did not affect plant fertility (Kollárová et al., 2021), the loss of FORMIN 13 in rice caused mild fertility problems (Zhang et al. 2023). Importantly, none of the single formin member mutations cause the pollen tube growth arrest or pollen lethality, the problems with fertility are minor.

Meanwhile, it was repeatedly published that ARP2/3 complex mutations do not dramatically affect pollen tube growth in *Arabidopsis*, which indicated that the ARP2/3 complex is not essential for pollen tube growth and as the main actin nucleator of pollen tube actin network were proclaimed formins (Li et al., 2003), reviewed again in (Xu & Huang, 2020). In contrast, it is known that *Arabidopsis* SCAR/WAVE complex subunits *nap1* and *pir* mutants show slightly decreased fertility (Li et al., 2004). Interestingly, (Ali et al., 2020) showed for *Arabidopsis thaliana* and *Nicotiana tabacum* that the SCAR/WAVE complex subunits, together with myosin XI, control actin filament-based sperm nucleus migration during karyogamy, but independently of the ARP2/3 complex. Additionally, it was demonstrated that tomato (*Solanum lycopersicum*) growing pollen tube tip-localised growth is supported by a spatiotemporal tightly controlled actin cytoskeleton network orchestrated by tomato homologs of ARP2, ARP3, and ARPC4. Moreover, ARP2/3 complex inhibitor, CK-666, slowed down the tomato pollen tube growth (Liu et al., 2020) suggesting a specific role of ARP2/3 complex. Further, *nap1* and *pir1* mutants of *Lotus japonicus* had lower pollen germination and seed production (Yokota et al., 2009), and mutation in ARP2/3 complex subunit ARPC1 of *Lotus japonicus* resulted in short and collapsed seed pods (Hossain et al., 2012). All these results pointed to the important role of the ARP2/3 complex and its activator complex SCAR/WAVE in nucleating and coordinating the actin cytoskeleton during pollen tube growth.

## **Actin nucleators and trichome shape**

Unicellular trichomes are an example of cell whose shape is achieved by both diffuse and tip growth modes (published about cotton trichomes) (Yu et al., 2019) or by tip-biased diffuse growth mode described for *Arabidopsis* trichomes (Yanagisawa et al., 2015). *Arabidopsis* trichomes consist of stalk and usually three pointed branches. However, ARP2/3 complex and SCAR/WAVE complex mutants fail to form this morphologically difficult shape which leads

to a so-called distorted appearance as they have shorter, twisted, bloated, and dull branches and usually also twisted and bloated stalks (Deeks et al., 2004) (Szymanski, 2005). The distorted trichomes are a hallmark phenotype of the ARP2/3 complex mutants, studied for more than 30 years now (Hülkamp et al., 1994).

*Arabidopsis* trichomes differentiation, development, and growth consist of 6 described stages (Szymanski et al., 1998). *Arabidopsis* ARP2/3 complex mutants have normal trichome development until stage five. During stage five, the mutant trichomes undergo uneven diffuse growth, which results in the distorted trichomes shape. Trichomes of ARP2/3 complex mutants have disorganised cytoskeleton (Schwab et al. 2003). Similarly, application of actin drugs latrunculin B, cytochalasin D, jasplakinolide and phalloidin to growing trichomes affected trichome morphology in a similar way as ARP2/3 complex mutants have (Mathur et al., 1999) (Szymanski et al., 1999). This demonstrates the need of precisely regulated actin cytoskeleton structure and dynamics for trichome morphology establishment. The localization studies of the SCAR/WAVE and ARP2/3 complex showed its localization to the tips of growing trichomes (Yanagisawa et al., 2015). The role of microtubules in controlling trichome branching in *Arabidopsis* is well established, as altered microtubule dynamics lead to a reduction in trichome branching and length. The early stages of *Arabidopsis* trichome branch elongation includes highly polarised anisotropic diffuse growth and need transverse alignment organised microtubules and cellulose microfibrils as microtubule-dependent cellulose microfibril patterning is one central feature of polarised diffuse growth of *Arabidopsis* trichomes. The research suggests that the mechanism of *Arabidopsis* trichome morphogenesis bears little or no resemblance to tip growth. It seems that it is instead achieved by highly regulated anisotropic diffuse growth in which spatial gradients in wall thickness are important for shape establishment. The trichome branch apex always contained an obvious microtubule-depleted zone (MDZ), which lacked patterned CESA localisation, but where the activated ARP2/3 complex is located. It was shown that ARP2/3 generates actin networks that have a global influence on the thickness gradient of the cell wall within a branch and a local influence on wall isotropy at the branch tip. Moreover, the importance of Golgi directional motility for cell wall establishment and alternations for trichome branch elongation was shown as large numbers of motile Golgi were present in young ARP2/3 complex mutant branches; however, the directionality of their movement was reduced. The Golgi apparatus plays a crucial role in cell wall establishment by synthesising, modifying, and transporting essential components needed for cell wall formation and maintenance. Specifically, the Golgi is responsible for producing and processing polysaccharides, such as pectins, hemicelluloses, and glycoproteins. Thus controlled Golgi cisternae transport ensures that the cell wall is properly assembled and maintained, contributing to cell structure, remodelling and growth. In summary, the actin network and the microtubule cytoskeleton work together to organise the cytoplasm including Golgi apparatus to pattern the cell wall, and consequently, the cell shape changes. Plants other than *Arabidopsis*, where ARP2/3 complex or activation SCAR/WAVE complex are absent, also manifested distorted trichomes. *Lotus japonicus* plants with a mutation in the *arpc1* subunit (Hossain et al., 2012), soybean *nap1* mutants (Tang et al., 2020), or tomato *arpc1* mutants (Chun et al., 2022) are an example of plants with distorted trichomes.

The involvement of formins in trichome growth is an understudied topic, although, in recent years, we have begun to obtain data suggesting that they play a certain role. Class I formins are known to be differentially regulated during the early stages of single-cell trichome fibre

formation in cotton (Qin et al., 2022). In *Arabidopsis*, AtFH1 localises to young growing trichome tips, and the AtFH1 overexpression can surprisingly result in somewhat misshaped distorted trichome morphology with a reduced number of branches (Oulehlová et al., 2019) (Cifrová et al., 2020).

## **Actin nucleators and the cell wall and cell-to-cell adhesion**

The actin cytoskeleton is integral to various aspects of cell wall formation and maintenance in *Arabidopsis*, from transporting essential materials to guiding synthesis machinery and responding to environmental changes and threats (Smith & Oppenheimer, 2005) (Szymanski & Cosgrove, 2009). Actin cytoskeleton works in concert with microtubules (Schneider et al. 2022) to coordinate the spatial organisation of cell wall synthesis - mainly as the microtubules are the main cytoskeletal players helping the cellulose synthesis machinery (Paredes et al., 2006). Primary plant cell walls of eudicots, like *Arabidopsis thaliana*, are mainly composed of cellulose, hemicellulose, pectins, and structural proteins, while the most abundant components are pectins. Pectins regulate the mechanical properties and stiffness of the cell wall and, during plant development, control cell adhesion, cell wall thickness, and tissue integrity. Pectin influences cell morphology, growth, and cell adhesion (see, e.g., Qiu et al., 2021 and Shin et al., 2021). Besides described phenotypes like distorted trichomes or less lobed pavement cells, ARP2/3 complex subunits mutants and SCAR/WAVE complex mutants have also cell-adhesion defects that are represented by the presence of gaps between epidermal cells of cotyledon, true leaves and hypocotyls (Qiu et al., 2002) (Le et al., 2003) (Mathur et al., 2003) (Zhang et al., 2005). Gaps in true leaves were associated with stomata (Sahi et al., 2018). It is known that ARP2/3 mutants have altered cell wall of trichomes (Yanagisawa et al., 2015) and stems (Sahi et al., 2018), however, the ARP2/3 complex-dependent pathway crucial for proper cell adhesion and cell wall synthesis remain unknown. In particular, the exact pathway by which pectins are delivered from the site of pectin biosynthesis in the Golgi and incorporated into the cell wall is also not known, but the idea that the actin network, together with the ARP2/3 complex, is directly involved is currently under discussion (Shin et al., 2021) (Du et al., 2022).

In contrast, no adhesion defects were reported for ARP2/3 complex subunits or SCAR/WAVE subunits mutants in roots. The absence of functional ARP2/3 complex pathway leads to a decreased primary root growth rate, fewer cell divisions in the meristematic zone, an increased number of cells in the transition zone, and a shorter elongation zone (Dyachok et al., 2011) (Takatsuka et al., 2018). Moreover, BRK1 and SCAR1 subunits of SCAR/WAVE activators were localised in the root tip epidermis up to the elongation zone, predominantly at three-way cell junctions. Furthermore, in *brk1* and *arp3* mutants, actin arrays in the root elongation zone appear disorganised (Dyachok et al., 2008). Finally, the cell walls of *brk1* and *arp2* mutant roots exhibited abnormal structures with unknown composition in some intercellular junctions at the transition between division and elongation zones (Dyachok et al., 2008). Nevertheless, gas chromatography analyses, immunoelectron microscopy using a variety of antibodies experiments, and electron microscopy revealed normal cell wall ultrastructure and composition in most areas of *brk1* and *arp2* mutant root tips (Dyachok et al., 2008). The data showed that

ARP2/3 complex influence root growth and probably root cell wall but its mutation does not cause problems with adhesion. These results suggest specific functions of the ARP2/3 complex in root and above-ground tissues and may imply that plant cell adhesion is mediated differently in above and below-ground plant tissues.

It is reported that formins can bridge the actin cytoskeleton across the plasma membrane and anchor it within the cell wall (Martinière et al., 2011) (Liang et al., 2021), as such it is possible that formins play a role in cell wall synthesis or signalling to and from the cell wall. Nevertheless, to my knowledge, no changes in primary cell wall composition have been reported in *Arabidopsis* formin mutants. However, the rice *formin5 (rmd)* mutant showed together with abnormal cell growth and irregular cell shape establishment also disorganised cell wall surface (Zhang et al. 2011). Nevertheless, how FH5/RMD affects cell wall architecture development remains to be uncovered.

## **Actin nucleators and exocytosis and endocytosis**

Plant cell growth relies on the delivery of membrane-bound vesicles containing membrane lipids, proteins, and exocytotic cargoes, as well as on the endocytic membrane and cargo retrieval. Therefore, exocytosis and endocytosis must be precisely coordinated. The spatial control of plant cell exocytosis and endocytosis is influenced by the cytoskeleton in addition to endomembrane trafficking regulators (see, e.g., Wang & Hussey, 2015 and Orr et al., 2020). Nevertheless, the role of ARP2/3 complex in actin-dependent endocytosis is well described for yeast and animal cells (Campellone et al., 2023), but in contrast in plant cells, the currently prevailing model is that plant endocytosis is actin-independent (Kraus et al. 2024). Recent works by (Narasimhan et al., 2020) and (Johnson et al., 2021) describes that endocytic vesicle formation on the cytoplasmic membrane is independent of actin in plants. Furthermore is shown that actin does not localise to endocytic sites and interfering with actin dynamics does not alter the density of endocytic sites nor does it have a significant effect on endocytic dynamics and flux (Narasimhan et al., 2020). Nevertheless, further detailed inspection revealed that there can be a role for ARP2/3 complex in endocytosis. The participation of the ARP2/3 complex and its activator complex SCAR/WAVE in endomembrane transport was reported by (Qin et al., 2021), who demonstrated that ARP2/3 mutants have decreased endocytic uptake of FM4-64 dye and impaired endocytosis-dependent localization of the AtEH/PEN1 protein. AtEH/PEN1 protein is a subunit of TPLATE complex with the ability to bind actin directly and therefore can assist the newly formed clathrin-coated vesicles move along actin filaments. In addition, AtEH/Pan1 proteins recruit TPLATE complex, AP-2 subunits, clathrin, actin and ARP2/3 proteins to autophagosomes upon induction of autophagy (Wang et al. 2019). Furthermore, our subsequent work demonstrated that the recycling of auxin transporters is impaired in ARP2/3 complex mutants (Garcia González et al., 2020).

ARP2/3 complex involvement in exocytosis was suggested by preliminary experiments in our laboratory; ARP2/3 and an exocyst subunit interacted and colocalized at the cytoplasmic membrane (Jelínková, 2021).

Meanwhile, there is growing evidence suggesting the participation of plant formins in endomembrane trafficking. Cvrčková et al. (2024) proposed that formins are a specific type of 'active cargo'. Active cargoes are proteins that carry out some of their functions during transport, thereby also influencing the fate of the membrane compartments that transport them. Formins, as plants transmembrane actin-binding proteins and actin nucleators, can be seen as cargo molecules that are at the same time active in regulating endomembrane traffic (Cvrčková et al., 2024). Furthermore, the participation of formins in endocytic membrane trafficking in animal cells is very well documented, and it is known that formins participate in both clathrin-independent and clathrin-mediated endocytosis events (see, e.g., Chakrabarti et al., 2021 and Cvrčková et al., 2024). The role of formins in exocytosis is described mainly for pollen tubes.: AtFH5 localises to exocytotic vesicles before pollen tube emergence (Liu et al., 2021). A lily formin LIFH1 modulates pollen tube elongation and also localises to exocytotic vesicles in the actin fringe area (Li et al., 2017). Meanwhile, a model has been proposed for the division of labour between class I and class II formins in *P. patens* tip growth protonemata, with class I formins playing a predominantly exocytotic role, whereas class II counterparts are mainly involved in endocytosis (van Gisbergen et al., 2018) (van Gisbergen et al., 2020). All this recent research exposes and highlights the involvement of the ARP2/3 complex and formins in exocytosis and endocytosis events and signifies the need for further studies of actin nucleators.

*Arabidopsis* possesses ten actin isoforms, one ARP2/3 complex, and twenty-one formins. The interaction between actin nucleators across various cell types and growth modes has long been unclear. However, recent research has started to shed light on these interactions. I hope that my dissertation thesis and the accompanying research papers will lead to a better understanding of actin nucleation systems. In the next chapter, I will outline the aims of my dissertation thesis and provide an overview of the publications included in my thesis. In the discussion section, I will explore and analyse the interactions and networking of actin nucleators from multiple perspectives.

## **DISSERTATION AIMS**

- 1) To determine the difference between the functions of plant actin nucleators ARP2/3 complex and formins in the plant cells.**
- 2) To uncover the mechanism behind the morphological phenotypes of ARP2/3 complex mutant plants.**

# PUBLICATIONS AND MANUSCRIPT ATTACHED TO THIS THESIS

This part of my thesis briefly describes 6 accepted publications about the ARP2/3 complex and/or the role of formins in plants and one unpublished manuscript about the involvement of the ARP2/3 complex in *Arabidopsis* pollen tube growth. The first publication listed is published under my maiden name: "Petra Schiebertova." I am the first author or shared-first authorship of publications number 2, 4, and 7. Publications and the manuscript are added at the end of the thesis.

1. Rosero A., Oulehlová D., Stillerová L., Schiebertová P., Grunt M., Žárský V., and Cvrčková F. (2016). ***Arabidopsis* FH1 Formin Affects Cotyledon Pavement Cell Shape by Modulating Cytoskeleton Dynamics.** *Plant & Cell Physiology* 57 (3): 488–504.
2. Sahi V. P., Cifrová, P., García-González J., Kotannal B., I., Mouillé G., Gineau E., Müller K., Baluška F., Soukup A., Petrášek J., Schwarzerová K. (2018). ***Arabidopsis Thaliana* Plants Lacking the ARP2/3 Complex Show Defects in Cell Wall Assembly and Auxin Distribution.** *Annals of Botany* 122 (5): 777–89.
3. Oulehlová D, Kollárová E, Cifrová P., Pejchar P, Žárský V, Cvrčková F. (2019). ***Arabidopsis* Class I Formin FH1 Relocates between Membrane Compartments during Root Cell Ontogeny and Associates with Plasmodesmata.** *Plant Cell Physiol*, 60(8): 1855–1870
4. Cifrová, P., Oulehlová, D., Kollárová, E., Martinek, J., Rosero, A., Žárský, V., Schwarzerová, K., & Cvrčková, F. (2020). **Division of Labor Between Two Actin Nucleators - Formin FH1 and the ARP2/3 Complex-in *Arabidopsis* Epidermal Cell Morphogenesis.** *Frontiers in Plant Science*, 11, 148.
5. Bellinvia, E., García-González, J., Cifrová, P., Martinek, J., Sikorová, L., Havelková, L., & Schwarzerová, K. (2022). **CRISPR-Cas9 *Arabidopsis* mutants of genes for ARPC1 and ARPC3 subunits of ARP2/3 complex reveal differential roles of complex subunits.** *Scientific Reports*, 12(1), 18205.
6. Martinek, J., Cifrová, P., Vosolsobě, S., García-González, J., Malínská, K., Mauerová, Z., Jelínková, B., Krtková, J., Sikorová, L., Leaves, I., Sparkes, I., & Schwarzerová, K. (2023). **ARP2/3 complex associates with peroxisomes to participate in pexophagy in plants.** *Nature Plants*, 1–16.
7. Martinek, J., Cifrová, P., Kollárová, E., Ničová, K., Garcia-Gonzales, J., Schwarzerová, K. (2024). **Pollen tube cell wall modification employs ARP2/3 complex-dependent endocytosis.** Manuscript in preparation

# 1. *Arabidopsis* FH1 Formin Affects Cotyledon Pavement Cell Shape by Modulating Cytoskeleton Dynamics

Amparo Rosero, Denisa Oulehlová, Lenka Stillerová, Petra Schiebertová, Michal Grunt, Viktor Žárský, Fatima Cvrčková

Plant and Cell Physiology 2016

[doi.org/10.1093/pcp/pcv209](https://doi.org/10.1093/pcp/pcv209)

## Short summary:

This research paper describes the interplay between actin and microtubules in plant cell shape regulation and provides insights into the molecular mechanisms governing cell morphogenesis. The research aims to elucidate the effects of formin FH1 mutations on cell morphogenesis, specifically in cotyledon pavement cells, and how these mutations influence cytoskeletal organisation and dynamics. *fh1* plants exhibited cotyledon epinasty (downward curvature of the leaf), their pavement cells were larger and had more pronounced lobes compared to wild-type plants. *fh1* mutants showed a decreased density and increased stability of actin filament bundles. Conversely, microtubules in these cells displayed enhanced dynamics, and the *fh1* mutants and wild-type plants responded differently to cytoskeletal drugs, particularly the microtubule disruptor oryzalin. The study concludes that FH1 formin plays a critical role in modulating the dynamics of both actin filaments and microtubules, which in turn affects the shape and morphology of cotyledon pavement cells.

**My contribution:** *I contributed to the pavement cell morphology measurements.*

## 2. *Arabidopsis thaliana* plants lacking the ARP2/3 complex show defects in cell wall assembly and auxin distribution

Vaidurya Pratap Sahi, Petra Cifrová, Judith García-González, Innu Kotannal Baby, Gregory Mouillé, Emilie Gineau, Karel Müller, František Baluška, Aleš Soukup, Jan Petrášek, Kateřina Schwarzerová

Annals of Botany 2018

[doi.org/10.1093/aob/mcx178](https://doi.org/10.1093/aob/mcx178)

### Short summary:

In plants, the actin cytoskeleton plays a significant role during the cell wall growth and establishment, which is further essential for plant growth. This research utilised three independent *Arabidopsis thaliana* mutant lines leading to non-functional ARP2/3 complex. The mutants were analysed for various phenotypic changes throughout their development. Key aspects of the study included organ size study, anatomy results, cell wall composition measurements, and auxin distribution analysis. The lack of an ARP2/3 complex led to various developmental abnormalities. These included defects in cotyledon pavement cell morphology and adhesion, vascular bundle formation, and overall plant architecture. The defects suggested a direct link between the ARP2/3 complex-regulated actin cytoskeleton and proper cell wall assembly and function. In inflorescence stems, ARP2/3 mutants displayed significant alterations in mature tissues cell wall composition mainly in pectin amount and in lignin content. Furthermore, the distribution of auxin, a critical plant hormone regulating growth and development, was disrupted in ARP2/3 complex subunits of mutant plants. This disruption was evidenced by altered auxin gradients, which are essential for processes such as cell elongation and differentiation. The study concluded that the ARP2/3 complex is involved in cell wall building in *Arabidopsis thaliana*. The findings highlight the complex interplay between cytoskeletal dynamics and cell wall synthesis, providing insights into the molecular mechanisms underlying plant morphogenesis.

**My contribution:** *I confirmed the ARP2/3 mutant plants by genotyping, performed cotyledons and pavement cells morphology measurements, and analysed the distorted trichomes phenotype. I analysed cell adhesion. I contributed to the stem and hypocotyl morphology and cell wall measurements. I contributed to the experimental design and draft preparation.*

### **3. *Arabidopsis* Class I Formin FH1 Relocates Between Membrane Compartments During Root Cell Ontogeny And Associates With Plasmodesmata**

*Denisa Oulehlová, Eva Kollárová, Petra Cifrová, Přemysl Pejchar, Viktor Žárský, Fatima Cvrčková*

Plant and Cell Physiology 2019

[doi: 10.1093/pcp/pcz102](https://doi.org/10.1093/pcp/pcz102)

#### **Short summary:**

The Class I formin FH1 in *Arabidopsis thaliana* is a membrane-associated actin nucleator important for cytoskeletal dynamics and cell morphology. Our study investigated FH1's localization, dynamics, and functional roles during root cell development, particularly its association with plasmodesmata. Using GFP-tagged FH1, confocal microscopy, and various biochemical assays, we analysed FH1 localization and dynamics in different root cell types. Furthermore, we described the overexpression phenotypes connected with the trihome shapes of GFP-tagged FH1 plants. The interactions between FH1 and plasmodesmata were examined through co-immunoprecipitation and colocalization studies. FH1 was found to relocate between different membrane compartments during root cell ontogeny, showing dynamic changes in its localization patterns. Mutations in FH1 led to altered actin filament organisation and disrupted root cell growth. This study provides new insights into the connection of molecular mechanisms underlying intercellular communication in plants and actin nucleation.

**My contribution:** *I contributed to the cotyledon pavement cells and trichomes morphology measurements.*

## 4. Division of Labor Between Two Actin Nucleators—the Formin FH1 and the ARP2/3 Complex—in *Arabidopsis* Epidermal Cell Morphogenesis

Petra Cifrová, Denisa Oulehlová, Eva Kollárová, Jan Martinek, Amparo Rosero, Viktor Žárský, Kateřina Schwarzerová and Fatima Cvrčková

Frontiers in Plant Science 2020

[doi.org/10.3389/fpls.2020.00148](https://doi.org/10.3389/fpls.2020.00148)

### Short summary:

The ARP2/3 complex and formins are the only two known actin nucleators in plants. This study focused on the roles of the actin nucleators, the ARP2/3 complex, and formin FH1 in the morphogenesis of *Arabidopsis* epidermal cells, particularly pavement cells and trichomes. Mutants lacking FH1, ARPC5, as well as double mutants were used. Phenotypic assessments, cytoskeletal organisation studies using fluorescent markers, and quantification of cell shape were analysed in these mutants. The study revealed that FH1 and the ARP2/3 complex have some complementary and some parallel roles. FH1 predominantly influences microtubule dynamics, while ARP2/3 affects actin organisation. This division of labour underscores the complexity of cytoskeletal regulation in plant cell morphogenesis. In cotyledon pavement cells, mutations in *fh1* increased pavement cell lobing, while *arpc5* mutations decreased pavement cell lobing. Double mutants resemble *arpc5* mutants, indicating ARPC5 is epistatic over FH1 in cotyledons. In true leaf pavement cells, both single and double mutants showed increased cell lobing and size, but to a lesser extent than in cotyledons, suggesting developmental stage-specific roles of actin nucleators. Double mutants exhibited characteristics of both single mutants. Our results showed that ARP2/3 complex and formin FH1 play distinct yet interconnected roles in *Arabidopsis* epidermal cell morphogenesis. Understanding these roles provided another piece of insight into the intricate and complex mechanisms of plant cell shape and function machinery and the role played by actin nucleators.

**My contribution:** *I measured the morphology of cotyledon and leaf pavement cells and analysed the trichome shape and branching in ARP2/3 complex and FH1 mutants; and created double mutants. I analysed the actin and microtubule cytoskeleton structure and dynamics and established and performed cell adhesion analysis. I participated in the experimental design and draft preparation.*

## 5. CRISPR-Cas9 *Arabidopsis* mutants of genes for ARPC1 and ARPC3 subunits of ARP2/3 complex reveal differential roles of complex subunits

*Erica Bellinvia, Judith García-González, Petra Cifrová, Jan Martinek, Lenka Sikorová, Lenka Havelková and Kateřina Schwarzerová*

Scientific Reports 2022

[doi.org/10.1038/s41598-022-22982-8](https://doi.org/10.1038/s41598-022-22982-8)

### Short summary:

The ARP2/3 complex is composed of seven subunits, but the roles of individual subunits, particularly ARPC1 and ARPC3, were not well understood. Our study investigated the specific roles of the ARPC1 and ARPC3 subunits in *Arabidopsis thaliana* by generating CRISPR-Cas9 knockout mutants. We conducted phenotypic analyses of these mutants, including trichome shape analysis, pavement cell shape assessment, and hypocotyl growth assays. We also performed co-immunoprecipitation and confocal microscopy to investigate protein interactions and subcellular localization. The experiments revealed that the loss of the ARPC1 displayed typical ARP2/3 mutant phenotypes, such as distorted trichomes and altered pavement cell shapes. In contrast, *arpc3* mutants did not exhibit trichome or pavement circularity defects, but still showed mild cell adhesion problems. The study found that ARPC3 is not essential for actin network formation in trichomes but is involved in the maintaining of proper cell adhesion between the pavement cells. These differential results highlight the complexity of ARP2/3 functions and suggest that individual subunits contribute uniquely to the ARP2/3 complex's overall role in actin cytoskeleton organisation.

**My contribution:** *I analysed the trichome branch length and number in ARP2/3 complex subunits mutants and rescue lines, cell adhesion of pavement cells of cotyledons and dark-grown hypocotyls in ARP2/3 complex mutants and rescue lines using Ruthenium Red and I measured dark-grown hypocotyl length. I participated in the experimental design and draft preparation.*

## 6. ARP2/3 complex associates with peroxisomes to participate in pexophagy in plants

*Jan Martinek, Petra Cifrová, Stanislav Vosolsobě, Judith García-González, Kateřina Malinská, Zdeňka Mauzerová, Barbora Jelínková, Jana Krtková, Lenka Sikorová, Ian Leaves, Imogen Sparkes & Kateřina Schwarzerová*

Nature Plants 2023

[do.org/10.1038/s41477-023-01542-6](https://doi.org/10.1038/s41477-023-01542-6)

### Short summary:

The ARP2/3 complex is essential for actin filament nucleation, which is important for numerous cellular processes in plants, including morphogenesis and cell-to-cell adhesion. Despite extensive research on ARP2/3 complex role in actin dynamics, its involvement in peroxisome-associated functions in plants remained unexplored. The ARP2/3 complex subunits, tagged with fluorescent proteins, formed motile dot-like structures in the cytoplasm, which were associating with peroxisomes. These ARP2/3-positive peroxisomal domains colocalize with autophagosomes. Furthermore, this colocalization increases under conditions that induce autophagy. Additionally, co-immunoprecipitation with the ATG8f marker confirms the interaction between peroxisome-associated ARP2/3 complex and autophagic machinery. Moreover, mutants lacking functional ARP2/3 complex exhibit increased peroxisome numbers, indicating the ARP2/3 complex role in peroxisome degradation through pexophagy. This study suggests a novel function of ARP2/3 in maintaining peroxisome homeostasis via autophagy.

**My contribution:** *I contributed to the peroxisome's morphology and density analysis, I helped with the experimental design and draft preparation.*

## 7. Pollen tube cell wall modification employs ARP2/3 complex-dependent endocytosis

*Jan Martinek, Petra Cifrová, Eva Kollarová, Klára Ničová, Judith García González, Kateřina Schwarzerová*

Unpublished manuscript - attached at the end of my dissertation thesis.

### ***Short summary:***

Our study provides detailed evidence of ARP2/3 complex role in pollen tube tip growth. We show that ARP2/3 complex and SCAR/WAVE complex mutants have shorter and thicker pollen tubes with reduced clear zones compared to wild-type plants. Additional actin cytoskeleton examination revealed increased actin density and bundling in these mutants. Furthermore, since pectin distribution is delocalized in mutant pollen tube cell wall and endocytosis marker T-PLATE shows reduced dynamics and accumulates at the plasma membrane of mutant pollen tubes, we hypothesise that ARP2/3 complex aids endocytosis of pectin-modifying enzymes in subapical region that is needed for cell wall domains formation in growing pollen tubes.

**My contribution:** *I contributed to the seed and siliques analysis and administered pollen tube and pollen grains shape, germination, growth and morphology experiments. I measured and analysed the pollen tube actin cytoskeleton. I visualised the promoter-GUS activity of ARP2/3 complex subunits and contributed to the analysis of mutant pollen tube cell wall composition. I was in control of the experimental design and draft preparation.*

# DISCUSSION

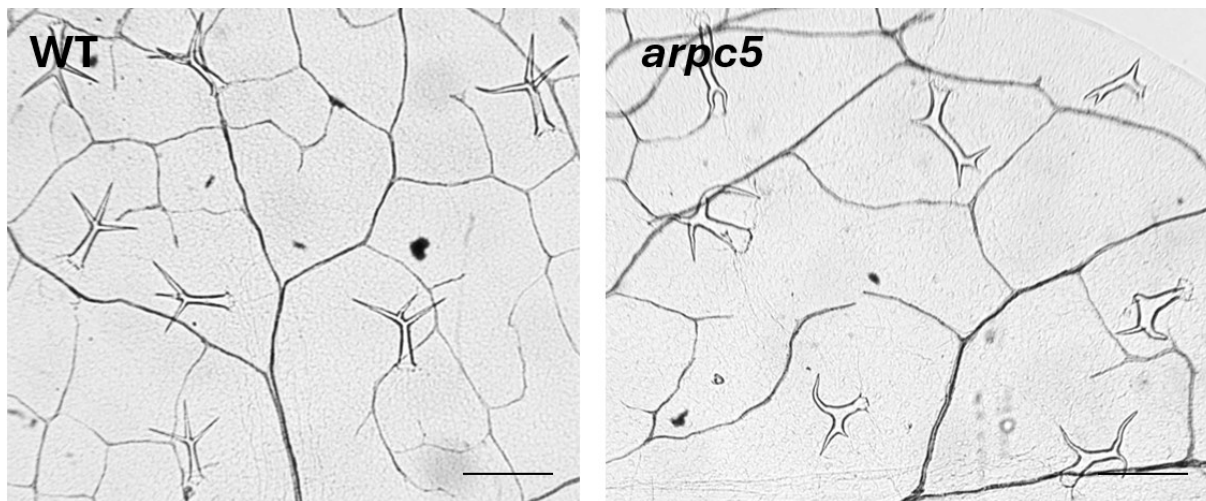
The actin nucleation is essential for the life of animal and yeast cells as the mutations of the ARP2/3 complex or formins can have significant effects, ranging from severe developmental defects to lethality, depending on the specific ARP2/3 complex subunit mutation or formin members involved (Pollard, 2007) (Campellone & Welch, 2010). My PhD research focused on elucidating the role of actin nucleation in plant cells. When a plant cell needs to nucleate or branch actin filaments, it relies on the ARP2/3 complex and/or the formin family (see, e.g., Wang & Mao, 2019 and Yuan et al., 2023). While mutations in either or both of these actin nucleation systems were expected to have severe consequences for plant cells, surprisingly, plants were able to compensate, resulting in no major phenotypic effects (Cifrova et al., 2020). This resilience, however, provides an opportunity: by carefully studying the small but significant phenotypic changes that occur when the ARP2/3 complex and/or formins are mutated, we can identify specific roles for these nucleators and further understand the distinct functions of the actin filaments network.

## **The differences and similarities in functions of plant actin nucleators**

In order to study the functions, similarities and differences of actin nucleators, visualisation of actin filaments is essential. A common approach to studying the dynamic nature of the actin cytoskeleton is the fluorescence microscopy of actin, tagged by fluorescent markers. G-actin directly fused with fluorescent protein does not function well as an actin marker in plant cells (Liu et al., 2004), GFP-tagged actin-binding proteins are used instead, as an indirect marker of the actin network in living plant cells. In our work, we mainly used GFP-tagged Actin Binding Domain 2 of FIMBRIN1 (GFP-FABD2 marker) (Voigt et al., 2005) and the 17-amino acid fragment of yeast ABP140 called “Lifeact” (Riedl et al., 2008). These two markers are the most used ones in the plant actin cytoskeleton research field (Li et al., 2015). It is important to note that the actin markers developed from ABPs can alter the structure and dynamics of the actin network and thus introducing artefacts in the analysis and interpretation of results (see, e.g., Cvrčková & Oulehlová, 2017). As the used actin markers affect the actin cytoskeleton in different ways, it is not possible to compare and discuss the results of two actin network measurements, even when using the same mutants or the same growth conditions but using different actin markers. This makes comparisons of the published results difficult or even impossible, as different scientific teams tend to use different actin markers. In my opinion, the development of a versatile actin cytoskeleton marker that is not exploiting ABPs and could be used by the majority of teams working with the plant cytoskeleton would be highly beneficial to the field. Additionally, it is important to realise that observing actin in different cell types will give varying results, as each plant cell type regulates the actin network differently and likely utilises actin nucleators in distinct ways. Therefore, comparisons between ARP2/3 complex-nucleated actin and form-nucleated actin should be conducted within the same cell

types. Notably, observations should always focus on the same pool of the cytoskeletal network. Finally, it is essential to differentiate between single filament dynamics analyses and observations of the entire actin network structure within the cells, as these approaches can produce very different results.

I would like to start by discussing the actin nucleators functions in the "semi-isolated" cells. The study of "semi-isolated" cells of *Arabidopsis thaliana*, such as trichomes, pollen tubes or root hairs, offers several important advantages, such as their distinct morphology, relatively large size and high number. But most importantly, these cells provide a simplified and accessible model for studying fundamental biological processes without the complexities and relationships introduced by studying the neighbouring cells in tissues. As such, they are an excellent model for studying the structure and dynamic of the cytoskeletal network. In my work I analysed the cytoskeleton of pollen tubes and observed the cytoskeleton in trichomes. Altered or so called "distorted" shape of trichomes observable on leaves and stems of plants lacking ARP2/3 complex or SCAR/WAVE complex subunits has been known for decades (Hülkamp et al., 1994). The important observations and descriptions of distorted shape and actin and microtubule cytoskeleton in trichomes were mainly conducted by Szymanski lab (Yanagisawa et al., 2015). The paper by Yanagisawa (2015) focused on the interplay between the ARP2/3 complex, actin and microtubule cytoskeleton and its influence on cell wall properties and, consequently, trichomes shape establishment. The work shows that the ARP2/3 complex seems to generate actin meshworks at the trichome branch tip, which is crucial for maintaining cell wall anisotropy and promoting tip-biased diffuse growth. Similarly, in our research we showed that overexpression of GFP-tagged FH1 results also surprisingly in a distorted trichome phenotype, while loss-of-function *fh1* mutants exhibit an increase in the number of trichome branches (Oulehlová et al., 2019). Nevertheless, we did not check the cell wall establishment of GFP-tagged FH1 overexpression plants. Furthermore, with using a Lifeact actin marker, we did not observe any significant abnormalities in actin microfilament organisation during early trichome development in single *arpc5* or *fh1* mutants (Cifrova et al., 2020). Additionally, the distorted trichome phenotype in the *fh1/arpc5* double mutant suggests that *arpc5* is epistatic to *fh1* and aligns with the ARP2/3 complex's role in controlling cell wall expansion necessary for proper branch elongation (Cifrova et al., 2020) (Yanagisawa et al., 2015). The trichome cytoskeleton dynamic and structure measurements of *arp2/3/formin1* double mutants are missing, but the individual results indicate that both actin nucleators influence cytoskeleton and are important for trichome shape determination. Moreover, it is confirmed that both nucleators are detectable in developing trichome branch tips in similar places (Yanagisawa et al., 2015) (Oulehlová et al., 2019). It is also important to mention here that applying microtubule (Buschmann et al. 2009) or actin drugs to a developing and growing trichome resulted in trichome morphology comparable to that of ARP2/3 complex or overexpression of GFP-tagged FH1 mutants (Mathur et al., 1999) (Szymanski et al., 1999). Therefore, as both actin nucleators can both nucleate actin and bind microtubules, their effect on trichome shape can be at multiple levels.



**Figure 4.: Visualisation of distorted trichome shapes in the *arpc5* mutant first leaves**

*The 21 DAG cleared first leaves trichomes (methodology described at Cifrova et al., 2020).*

The actin cytoskeleton structure in *arpc5* mutant pollen tubes was visualised with the use of a Lifeact actin marker and described in our attached manuscript (Martinek et al., 2024). We found that *arpc5* mutant plants have a significantly more bundled and denser actin network compared to WT plants. This is the first actin cytoskeleton measurement of ARP2/3 complex mutants in tip-growing cells. In contrast several formins are specifically expressed in pollen tubes (mainly FORMIN 3 and FORMIN 5) and their cytoskeletal network structure is described, although their loss rarely results in major cytoskeleton related phenotypes probably due to functional redundancy. Nevertheless, from published results, it appears that when actin nucleation is aborted in tip-growing pollen tube cells, it consistently results in increased actin cytoskeleton bundling for both actin nucleators. The ARP2/3 complex and formin member double mutants' relationship and interaction are not described in any of the tip growing cells yet. But the good candidate for this experiment is one particular ARP2/3 complex subunit mutant - the ARPC3 subunit mutant. The plant ARP2/3 complex is unique as only exceptionally some of its subunits are encoded by multiple genes. Therefore, when we remove a subunit from the ARP2/3 complex, the complex doesn't have a replacement and do not nucleate actin, the exception is the ARPC3 subunit, as we showed in our studies (Bellinvia et al., 2022) (attached manuscript in preparation - Martinek et al., 2024). ARPC3 mutation interestingly does not change the trichomes or pavement cells morphology but is important for pollen tube growth. Maybe there is a possibility that this subunit is needed to enhance or regulate ARP2/3 complex function in fast and polarised tip growth. Nevertheless, the *fh3/arpc3* or *fh5/arpc3* double mutants could be a right mutant combination to use to elucidate the actin nucleator's role in tip growing cells. Finally, it is also important to realise that the ARP2/3 complex and formins interact during pollen tube growth different sets of actin isoforms - generativ actin isoforms (ACT1, ACT3, ACT4, ACT11, and ACT12) expressed in *Arabidopsis* pollen (Pawloski et al., 2006) compared with actin isoforms in the rest of the *Arabidopsis thaliana* body. Maybe it can also play a role in actin nucleation establishment and specific need of ARPC3 subunit of ARP2/3 complex.

Meanwhile, to prove the ARPC3 subunit specific role in tip growth hypothesis, it could be helpful to explore the root hairs growth of *arpc3* mutants. Root hairs were in early days of plant ARP2/3 complex research described as altered - “the root hair elongation is reduced, and root hairs tend to be wavy” (Mathur et al., 2003) (Li et al., 2003). Nevertheless, there are no more studies to continue with these research directions, except the localization studies of ARP2/3 and SCAR/WAVE complexes subunits during the root hairs initiation phase (Chin et al., 2021). However, in this work *arpc3* subunit mutant was not used. The study of the actin nucleators interplay in pollen tubes, roots and trichome shape establishment is in progress. In my opinion root hair can be another great cell type to study actin nucleators interplay in. Concerning formins role in root hair growth, overexpression of FORMIN 4 and FORMIN 8 resulted in an initiation of ectopically growing root hair tips, and additionally, aberrant expression of AtFH8 resulted in the inhibition of root hair elongation (Yi et al., 2005) (Deeks et al., 2005). Furthermore, rice mutants defective in the OsFH1 have impaired root hair growth (Huang et al., 2013). The study of the actin nucleators during root hairs growth deserves more space, as we have only limited informations and as we can learn from previous experiments we can design our experiments with emphasis on cell wall establishment and interaction of actin and microtubule cytoskeleton.

In contrast to trichomes, pollen tubes and root hairs, the role of the cytoskeleton network in puzzle-shaped cotyledon pavement cells is challenging to study as these interconnected cells grow in a coordinated manner. In our experiments (Cifrová et al., 2020), we examined the cytoskeleton structure and dynamics in cotyledon pavement cells of WT plants, ARP2/3 complex mutants (*arpc5*), and *fh1* mutant. The analysis of actin cytoskeleton visualised by Lifeact marker in WT, *fh1*, *arpc5* and *fh1/arpc5* showed that both *fh1* and *arpc5* mutations increased actin network density. This is in line with findings in pollen tubes and trichomes, where the absence of ARP2/3 also increased bundling. However, the effect of *fh1* mutation contrasts our previous findings in plants expressing the GFP-FABD actin marker, which showed increased bundling and decreased density of microfilaments in *fh1* mutants compared to WT plants (Rosero et al., 2013) (Rosero et al., 2016). It is important to note observed changes in actin organisation were very subtle, and notably, no additive effect of double *fh1/arpc5* mutation was observed. Furthermore, we hypothesised that the *fh1/arpc5* mutants exhibit no major growth or developmental defects due to the functional redundancy of the numerous formin paralogs. In contrast to our results, the single filaments observations in actin epidermal cells from the apical region of 5-day-old etiolated hypocotyls expressing GFP-FABD marker showed the opposite results to our results (Cifrová et al., 2020) as the *arp2* and *arpc2* mutants had reduced actin filament density and bundling (Xu et al., 2023). These differences could be due to the use of a mutation in a different subunit of the ARP2/3 complex or in a different studied tissue (dark-grown hypocotyl and light-grown cotyledon pavement cells) or in the use of different actin markers. Indeed, Xu et al. hypothesised that the opposite results were probably due to the different ARP2/3 subunit mutants used in these studies. But in my opinion, the differences in actin structure measurements are rather due to the fact that both studies analysed different actin structures. Our study focused on actin cytoskeleton array in whole pavement cells and used classical confocal microscopy, while the study of Xu and his colleagues (Xu et al., 2023) used TIRF microscopy and analysed single filament behaviour. Additionally, I would

like to speculate that the the reason for rather minor actin network problems of ARP2/3 complex subunits mutants could be due to the wrong place we were analysing the cytoskeleton in. Booth studies observed and analysed the cytoskeleton near the outer periclinal cell wall, but the problem with adhesions is observable between the cells - in anticlinal cell walls - there the pectin chemistry governs pavement cell morphogenesis (Altartouri et al., 2019), and also the BRK1::YFP and GFP::SCAR1 localize to the cell periphery (Dyachok et al., 2008). In the next attempt to analyse the cytoskeleton in actin nucleator mutants in order to elucidate its role in cell morphology establishment, we should look at the actin cytoskeleton adjacent to the anticlinal cell walls. However, it is important to note that the necessary microscopy techniques for successful cytoskeleton observation are limited. We cannot use TIRF microscopy, and confocal microscopy is also mostly unsuitable for observing the dynamics of the anticlinal cell wall adjacent cytoskeleton network.

What could be behind the *formin1* described pavement cells morphological problems (Rosero et al., 2016) (Cifrová et al., 2020)? The classical model of pavement cell morphogenesis shows that cell shape is determined by the localised loosening or stiffening of the cell wall, with turgor as the driving force behind lobe formation (Bidhendi et al., 2019). In this model microtubules orient the arrangement of cellulose microfibrils in the cell wall of necks to prevent expansion, while actin is essential in growing lobes. We reported that in cotyledons, ARP2/3 complex mutants have less lobed cells, and the *formin1* mutant has more lobed pavement cells. The double mutant - *arpc5/fh1* has less lobed pavement cells, comparable with *arpc5* mutant (Cifrová et al., 2020). It is unlikely that *formin1* mutants have problems with cell wall pectins comparable with ARP2/3 mutants since, as far as I know, there are no problems with the primary cell wall composition or cell-to-cell adhesion in formin mutants. On the other hand, class I formins localise to the cell plate, and application of the formin inhibitor SMIFH2 to tobacco cell cultures disrupts cytokinesis. Moreover, members of the formin family localise to plasmodesmata and also near fungal penetration sites in the formation of defensive callose appositions. These are all places where the cell wall is rich in callose (Cvrčková et al., 2024). Is it possible that formins play a general role in callose-related functions or pathways? Could it be in some manner important for pavement cell shape determination? Also, the pavement shape morphogenesis problems could be related to the organisation of actin and microtubules connected with endocytosis and exocytosis processes (Cvrčková et al., 2024). However, for formins, it has been recently postulated by professor Cvrčková that they contribute to diffuse cell expansion mainly through their effects on the actin and microtubule cytoskeleton, and the observed changes in membrane trafficking may be secondary to changes in the cytoskeleton. Furthermore, the reduced rate of hypocotyl elongation in developing young *atfh1* mutant seedlings has also been attributed to changes in actin organisation (Cui et al., 2023). In further detail, it seems that microtubule but not actin perturbation differentially affects pavement cell development in *fh1* mutant plants (Rosero et al., 2016). This is not the case for the ARP2/3 complex, as its mutation does not affect the microtubule cytoskeleton as much as the FH1 mutation (Cifrová et al., 2020). It needs to be stressed here that formins were traditionally recognized primarily as actin nucleators but now are being identified as versatile multifunctional proteins with various roles (Cvrčková et al., 2024). Furthermore, leaving aside their main function as actin nucleators, both the ARP2/3 complex and formins can bind to the

microtubules (Havelková et al., 2015), ( Li et al., 2010) (Zhang et al., 2011) (Rosero et al., 2013) (Wang et al., 2013), and as functional interactions between actin filaments and microtubules are described as important for driving intracellular transport and growth. Therefore, we propose that the role of the ARP2/3 complex and formins in the plant cell extends beyond their primary role as actin nucleators, and thus we should design our experiments and wrap our hypotheses about their roles in plant cells accordingly.

An alternative method to study actin nucleators is to use the actin nucleator inhibitors CK666 and SMIFH2. However, suprisingly simultaneous treatments with CK-666 and SMIFH2 did not lead to dramatic cytoskeletal changes (Xu et al., 2024). In my opinion this important result shows that use of the actin nucleator inhibitors still needs to be discussed. Even though the acute application of CK-666 to WT plants phenocopied the single filament actin defects observed in ARP2/3 mutants (Xu et al., 2024), it seems suspicious that when added to the medium or dropped on the plants, the CK-666 treatments do not cause distorted trichome shapes, circularity problems or adhesion problems (unpublished results), which are hallmark phenotypes of plants with non-functional ARP2/3 complex. Furthermore, some researches about formin roles in plant cells built on pharmacological experiments utilising the broad-spectrum formin inhibitor SMIFH2, however, in the animal cells field, is just now hypothesised that it might affect additional targets in cells, including myosins (Innocenti, 2023). This was tested in plant cells by (Xu et al., 2024) with use of myosin XI-K and it was not shown to be a valid objection. But it was not tested for all other plant myosins. It also remains unclear whether SMIFH2 can inhibit all 21 formin homologs of plant cells, as nobody tested that.

Finally, do the ARP2/3 complex and formins compete for a common pool of actin monomers, and the loss of one of them favours the activity of the other or do they cooperate? Do they colocalized within the plant cells? The *formin* and *arp2/3* mutant plants showed measurable perturbations in actin network structure in diverse cell types, nevertheless, the measurements are usually just for one actin nucleator and with use of different actin markers. However in our work, we found that ARP2/3 complex and FORMIN1 mutations in cotyledon pavement cells influenced the actin network structure in the same way - similarly increased actin network density (Cifrová et al., 2020). In contrast to these observations, the dynamic of single actin filaments measured for both nucleation systems just recently (Xu et al., 2023) showed opposite results - the actin network occupancy was lower, but was lower again similarly for both actin nucleators. This discrepancy in results between whole cell actin network measurements and single filament measurements needs to be further discussed. Nevertheless, interestingly the use of CK-666 and SMIFH2 inhibitors together in medium also made the actin cytoskeleton denser (Xu et al., 2023). These findings suggest that mutations in both actin nucleators have a similar impact on the actin cytoskeleton, indicating that they do not compete with each other, which implies a collaborative rather than competitive relationship. Subsequently (Xu et al., 2023) showed that the plant formins can also contribute to the nucleation of side-branched filaments, which suggests that actin nucleators can partially share their roles. Moreover, both nucleators are found around plasmodesmata (Van Gestel et al., 2003) (Baluska & Hlavacka, 2005) (Martinière et al., 2011) (Oulehlová et al., 2019), and influence vacuole biogenesis (Schiebertova 2013) (Semerak 2016) (García-González et al. 2020). They are also similarly

localised in the tips of growing trichomes (Yanagisawa et al., 2015), (Oulehlová et al., 2019). Additionally, mutation of the ARP2/3 complex and SCAR/WAVE complex inhibit autophagosome biogenesis (Martinek et al. 2023) and although the involvement of formins in autophagosome biogenesis is not described in plant cells, it is described in opisthokonts (Huett et al. 2009), (Anhezini et al. 2012). To wrap it up, although the ARP2/3 complex and the formins nucleate actin differently and at different rates, create different fractions of the actin network, and are governed by different activation, both actin nucleators can be found at some common sites in the cells where they can meet and cooperate.

## **The ARP2/3 complex and formins are vital for animal and yeast cells, but not for plant cells.**

**Are there other actin nucleators in plants besides the ARP2/3 complex and the formins?  
Do plant cells need actin nucleators at all?**

There has been discussion about the distribution of functions between the ARP2/3 complex and formins in animal and yeast cells for some time now (Pollard, 2007) (Campellone & Welch, 2010) (Breitsprecher and Goode 2013) (Rotty et al. 2013) (Pollard 2016). In animal cells, similarly to the plant cells, the ARP2/3 complex is specialised in creating branched actin networks, and formins are specialised in forming unbranched actin filaments. In animal and yeast cells, the ARP2/3 complex branched actin networks are crucial for maintain cell shape, adhesion, generating pushing forces, vesicle trafficking, cytokinesis, endocytosis, autophagy, apoptosis, chromatin dynamics, DNA repair and are involved in maintaining cell polarity (May, 2001) (Goley et al., 2010) (Campellone et al., 2023). On the other hand, the 15 members on animal formin family play a crucial role during cell division, cell shape maintenance, polarity, intracellular transport, endocytosis, response to environmental stresses and in animal cells in tissue morphogenesis (Campellone & Welch, 2010) (Akram et al., 2020) (X. Liu et al., 2022). In summary, the distinct yet complementary roles of the ARP2/3 complex and formins and their interplay enable animal and yeast cells to dynamically and precisely regulate their actin cytoskeleton. For example, the ARP2/3 complex depletion in fibroblasts leads to increased formin expression and filopodia formation and reduces migration directionality (Dimchev et al., 2021). Furthermore, ARP2/3 and formins balance actin nucleation during cytokinesis in *C. elegans*, with ARP2/3 regulating formin activity (Chan et al., 2019). In conclusion, results with yeast as well as mammalian cells, reveal a more likely competition relationship between the ARP2/3 complex and formin as they need simultaneously reach for a limited pool of actin monomers. Therefore, when the ARP2/3 complex is inhibited, formin-mediated long filament bundles dominate.

In contrast, results from three recently published papers about ARP2/3 complex and formins relationship in plant cells showed that the mutations in both actin nucleators is not lethal and differ from animal and yeast cells (Cifrová et al., 2020) (Qin et al., 2021) (Xu et al., 2023). Since ARP2/3 complex subunits mutations are not essentially life-threatening to the plants (see

figure 5.), it is possible that the plants started to use the ARP2/3 complex for specific functions and either do not need the branched actin network in principle, or are making the branched actin in other ways, maybe with help of formins (Xu et al., 2023). Thus, it is possible that the expanded formin family with extended functions beyond action nucleation has members that provide most of the necessary actin nucleation, branching, bundling and other actin network remodelling. Moreover, individual formin genes may substitute for each other and because no one has analysed multiple formin mutants or other mutant combination with ARP2/3 complex besides AtFH1, we cannot confirm or refute this. SMIFH2 cannot provide the answer, as no one has ever proven that SMIFH2 can inhibit all formins. Moreover, plant stochastic actin dynamics differ from animal actin network behaviour. It is possible that due to the severing and elongation ability and nature of plant actin network, *de novo* nucleation is not that crucial for plant cells. It is also possible that *arp2/3 complex* subunit and *formin1* double mutant plants can efficiently form a sufficient actin network with help of other actin binding proteins that could regulate the amount of actin filaments loose ends and events at them. Finally, it is possible, to safely say that we are not able to observe significant part of the actin cytoskeleton in plants as we mainly have actin markers that are based on proteins that bind polymerized actin, so we may not see important part of dynamic events associated with short actin filament made by few actins monomers. Additionally, results from single actin filament measurements suggest that plants may be hiding from us a completely different system of actin nucleation compared to animal cells (Xu et al., 2023). Simultaneous inhibition of ARP2/3 complex and FORMIN1 increased actin filament nucleation in plant cells, suggesting a unique actin regulation mechanism. Furthermore, it was found that the triple inhibition of ARP2/3 complex, formins, and PROFILIN 1 also resulted in an increased total and *de novo* nucleation frequency, which suggested that a change in the free actin monomer concentration or the function of PROFILIN 1 is not the cause of the increased *de novo* nucleation when both actin nucleators are inhibited. In this light, we can also discuss the possible discovery of new or already known but insufficiently described actin-binding proteins that can nucleate actin filaments, at least in some amount. But more likely, we can hypothesise that for plants, actin nucleators are not essential. Is it possible that plants can grow and live without any actin nucleators at all? Can we hypothesise that plants use ARP2/3 complex and formins to enhance, improve or support specialised and challenging processes such as endocytosis, autophagy, pollen tube growth, or morphology establishment? To answer these questions, we need to knock out all members of the formin family and learn new, more detailed ways of looking at the whole actin cytoskeleton network in plant cells.



**Figure 5.: Comparison of *Arabidopsis thaliana* WT and *arp3* mutant plants vitality**

Taken together, the results suggest that the regulatory mechanisms for actin filament nucleation in plant cells may be unique, revealing a failsafe system that largely preserves actin filament abundance and dynamics even when both nucleators are, to a large extent, inactivated. The situation is opposite in animal and yeast cells. Nevertheless, we can study this unique actin nucleation system in plants and discover what lies behind the ARP2/3 complex and formins family plant-specific roles.

## **Actin nucleators cooperate during pathogen-plant interactions**

The ARP2/3 complex and formins are also involved in defence mechanisms during pathogen-plant interactions, which is not a surprise as the actin cytoskeleton rearrangements are among the first responses to pathogens (Leontovyčová et al., 2020). Plants with defective ARP2/3 complex subunits are described as exhibiting increased susceptibility to pathogens due to compromised signal transduction, defected actin cytoskeleton rearrangements and impaired cellular defence mechanisms (Qi et al. 2017) (Badet et al. 2019) (Sun et al. 2019) (Qin et al. 2021). On the other hand, *Arabidopsis* FORMIN 6 is localised at the plasmalemma of nematode-induced giant cells, but its loss did not change the response to the parasite, which could possibly be due to functional overlap among formins (Favery et al., 2004) (Ma et al., 2021). The FORMIN 4 accumulates at mobile endomembrane compartments adjacent to sites of fungal invasion (Sassmann et al., 2018). Finally, FORMIN 1 and FORMIN 2 are shown to be involved in the defence papilla formation against *powdery mildew* invasion and surprisingly by cooperating with the ARP2/3 complex (Qin et al., 2021). It was shown that simultaneous knockdown of ARP3 and FORMIN 1 abolished actin patch formation, profoundly impaired cell wall deposition, and promoted powdery mildew entry into host cells. These results show for the first time direct evidence of the ARP2/3 complex and formins cooperation during pathogen-plant interaction (Qin et al., 2021). Nevertheless, I would like to stress that even though the

involvement of ARP2/3 in pathogen attack sensing and immune response has been discussed there from several angles, the published papers have never linked problems in the ultrastructure of the cell wall and cell-cell adhesion problems of ARP2/3 complex mutants, in the immune response. I consider that to be an important missing set of experiments. The issues connecting distorted trichome shape and ARP2/3 complex mutants immunity problems are also not discussed, even though the trichomes of *Arabidopsis* produce glucosinolates, which play a pivotal role in defence against herbivores, bacteria and fungi (Zhang et al. 2018). Furthermore, trichomes act as mechanosensory cells that mount an effective immune response against both biotrophic and necrotrophic pathogens (Matsumura et al. 2022). The question is: could the distorted trichomes of ARP2/3 complex mutants still serve as mechanosensory cells or effectively produce glucosinolates? The interplay of actin nucleators during pathogen-plant interactions is a new and open area of research. Many questions remain to be answered.

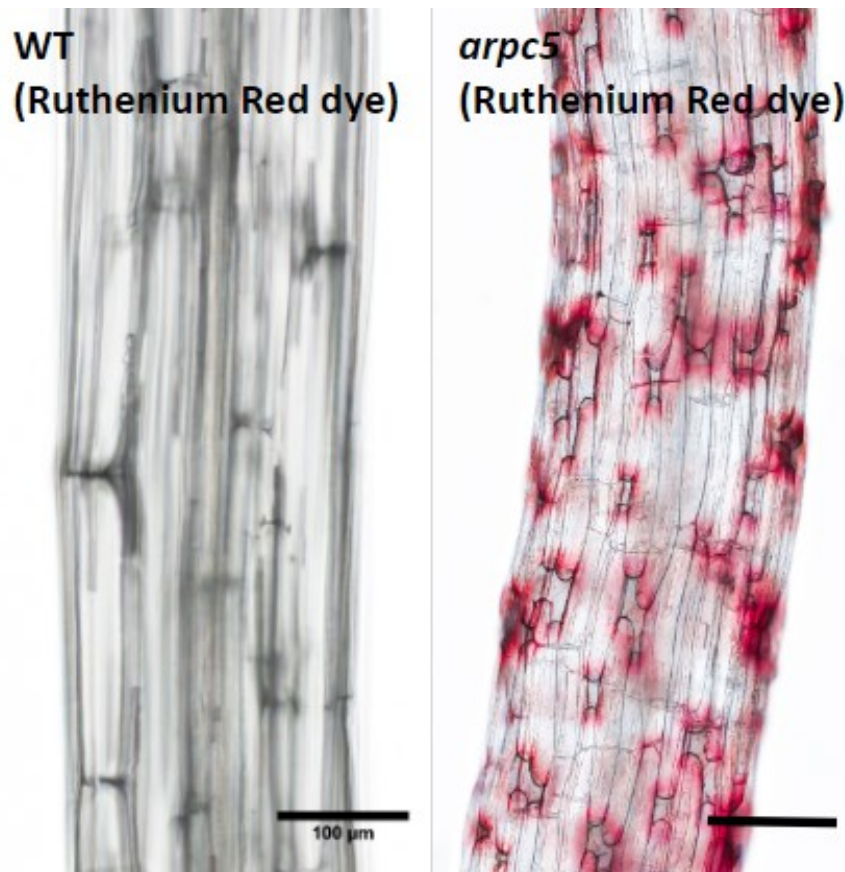
## **What could be behind the morphological and adhesion phenotypes of ARP2/3 complex mutant plants?**

### **Why is the spatial and temporal organisation of cell wall microdomain remodelling by the ARP2/3 complex important?**

Directed and controlled growth of the plant cell wall, and consequently the cells themselves, leads to the formation and stabilisation of plant cell shapes, a process known as cell morphogenesis. Plant cell growth is irreversible due to the static structure of the cell wall (reviewed in Gorelova et al., 2021). In contrast, the cytoskeleton is a highly dynamic network that responds almost immediately to various changes and signals within the plant cells and is under constant remodelling (see, e.g., X. Wang & Mao, 2019 and Yuan et al., 2023). From the perspective of the cell wall, growth occurs in regions where pectin, the most abundant component of the primary cell wall, is being modified (Wolf & Greiner, 2012) (Cosgrove, 2014) (Peaucelle et al., 2015). From the perspective of the plasma membrane, these regions require targeted, dynamic, and precise endocytosis and exocytosis of cell wall components, enzymes, and other proteins. Lastly, from the standpoint of the actin cytoskeleton. Growth regions are where the actin network must polymerize and depolymerize accurately to facilitate targeted exocytosis and endocytosis and enables organelles responsible for the synthesis or degradation of cell wall components to participate effectively (Guo & Yang, 2020). Furthermore, in diffusely growing cells, multiple regions of the cell wall grow simultaneously, necessitating coordinated growth both at the whole-cell level and within the specific cell wall microdomains that are expanding. Nevertheless, understanding the growth machinery in apically growing cells is simpler as it is clear which part of the cell wall is growing (Guo & Yang, 2020). This clarity is one reason we have currently focused on cell elongation and pollen tube growth in our ARP2/3 complex research (manuscript in preparation, Martinek et al., 2024). Furthermore, there are no available markers to identify the growing microdomains of diffusely growing pavement cell walls. Additionally, there are no distinct formations of the actin cytoskeleton adjacent to these growth sites (Armour et al., 2015). Consequently, we cannot easily identify or predict which

cell wall microdomains are growing, and there is no clear connection between the actin cytoskeleton structure and the cell wall growth pattern.

In our work, we tried to uncover the connection between the ARP2/3 complex influencing the actin cytoskeleton, cell wall, and subsequent cell morphology establishment. The connection between the ARP2/3 complex and the cell wall has been already discussed in several studies (Dyachok et al., 2008) (Yanagisawa et al., 2015) (Sahi et al., 2018), (attached manuscript in preparation - Martinek et al., 2024). Our research suggested a hypothesis that the morphological and adhesion defects observed in ARP2/3 complex mutants likely result from the failure to precisely and in right time control cell wall modifications in specific areas, such as cell corners, edges, and vertices rather than changes in the overall cell wall composition. This deduction is supported by our biochemical analysis of cell walls in dark-grown hypocotyls, which exhibit severe adhesion problems without any detectable changes in cell wall composition (Fig 6.) (Sahi et al., 2018), and our observation of pollen tube cells pectins, which are present in the pollen tube cell wall but are delocalized (attached manuscript in preparation - Martinek et al., 2024). Subsequently we realised, that not the ARP2/3 complex subunits themselves, but the activation subunits SCAR1 and BRK1 of ARP2/3 activation complex localise to the specific cell periphery areas of plant cells (Dyachok et al., 2008), which are likely to undergo a modification of pectins connected to growth and morphogenesis. SCAR1 and BRK1 and NAP1 subunits seem to localise to the submembrane regions of the cell wall currently undergoing growth or remodelling such as usually at three cell junctions in the growing lobes and near the stomata aperture (Dyachok et al., 2008) (Qin et al., 2021), on the branch tip of growing trichomes (Yanagisawa et al., 2015) or in polarised localizations in root hairs during its initiation phase (Chin et al., 2021). Notably, when the plant cells stop the growth (trichomes maturation), the GFP signal disappears (Yanagisawa et al., 2015). Therefore, we suggest that the localisation pattern of SCAR1 and BRK1 and NAP1 subunits makes it an ideal marker for plant cell wall rearrangements and growth monitoring.



**Figure 6.: Dark growth hypocotyl adhesion defects**

*The 7DAG dark grown hypocotyl adhesion defects of arpc5 mutant visualised by Ruthenium Red dye treatment (methodology described at Bellinva et al., 2022).*

Additionally, we realised, that the connection between the impaired pectin localization and ARP2/3 complex could be due to impaired endocytosis as is shown by Qin with his team (2021) and in our work – (attached manuscript in preparation - Martinek et al., 2024). The impaired endocytosis in ARP2/3 complex mutants can explain many of the described phenotypes of ARP2/3 complex mutants, such as impaired cell morphogenesis and adhesion, but the ARP2/3 complex has also been shown to play a role in autophagy (Wang et al., 2016) (Wang and Mao, 2019) (Wang et al., 2020) (Martinek et al., 2023). Nevertheless, although endocytosis and autophagy are two rather separate processes, they share several underlying mechanisms and use similar molecular components (Kim et al., 2020) (Dragwidge and van Damme, 2020) (Kraus et al., 2024). One link between endocytosis and autophagy is membrane remodelling. Therefore we hypothesise that cooperating on membrane remodelling is the specific purpose of ARP2/3 complex-mediated nucleation of the actin cytoskeleton and could contribute to the formation of both autophagosomes and endocytic vesicles from source membranes like it is already described for yeast and animal cells (see, e.g., Boulant et al., 2011 and Campellone et al., 2023).

Moreover, I also like to speculate that there can be another possible role of ARP2/3 complex in membrane remodelling which leads to the specialised cell wall microdomain reorganisation and

subsequently plant cell growth. In recent years, another pathway of communication between organelles and the plasma membrane has come to the forefront of interest for cell biologists.: the membrane contacts sites (MCS). It means that membrane-bound structures are interconnected through membrane contact sites, forming an integrated and physically connected network (Eisenberg-Bord et al., 2016) (Pérez-Sancho et al., 2016). Additionally, the membrane contact sites are defined by two membranes with close proximity (< 30 nm) but without fusion (Wang et al., 2023). Finally, the actin cytoskeleton, together with ER-actin bridging proteins and myosin motors, is shown to be the primary force behind ER remodelling (Pain et al., 2023). But although the role of actin-binding proteins in the MCS network is undeniable, the research of proteins that mediate direct interaction between the actin cytoskeleton, ER, PM, and cell wall is at the beginning. However, as the ER-PM contact sites regulate endocytosis as well as autophagy in plants (see, e.g., Wang et al., 2023), there is a possibility that the ARP2/3 complex may play a role in membrane contact site establishment or maintenance. Moreover the AtEHs/Pan1-regulate autophagosomes associate with ER-PM contact sites, and in the same time has the ability to recruit the actin and ARP2/3 complex proteins to autophagosomes upon induction of autophagy (Wang et al., 2023). Exploring this topic and its interconnections is promising for future research, and as the exact pathway of pectin transport to the cell wall is still unknown, the role of membrane contact sites in transporting pectin or pectin-related enzymes to the cell wall seems to me like an intriguing possibility.

In summary, as the ARP2/3 complex partially mediates autophagy and endocytosis, we formulated a hypothesis that the ARP2/3 complex plays a role in targeted membrane remodelling, as it is important for both processes. We concluded that specifically and locally generated actin networks nucleated by ARP2/3 complex are necessary for membrane remodelling of cell wall pectin-based microdomains which are important for plant cells' shape establishment and for cell adhesion.

What is the role of ARP2/3 complex in cell-to-cell adhesion maintenance? It is known that the pectin component of cell walls plays an indispensable role not only in the cell's growth but mainly in the cell-to-cell adhesion. To stay connected, plant cells need to constantly remodel their cell wall and pectin lamella composition during their development. However, the exact pathway of how pectin is delivered to the cell wall is still not understood. The site of pectin biosynthesis is the Golgi apparatus from which pectins are thought to be delivered to the cell surface by vesicles that bud from the medial or trans-Golgi cisternae and are transported to the cell surface by either active transport or diffusion; this pathway may also be used by pectin-modifying enzymes. Nevertheless, a robust characterization of the entire exocytic and endocytic machinery responsible for the transport of pectins has not yet been performed (see Daher and Braybrook 2015 and Baba and Verger 2024).

The disturbed pavement cells adhesion of ARP2/3 complex mutants have been known for more than two decades (see, e.g., Mathur, 2005). The exact reason for these changes remains to be elucidated although the nature of ARP2/3 complex connection to the cell adhesion is currently being discussed (Du et al., 2022). It was shown that changes in pectin structure precede other changes in the cell cortex and cell wall, including cortical microtubule reorientation and

realignment of cellulose deposition in the *Arabidopsis* hypocotyls (Peaucelle et al., 2015). Interestingly, Verger with colleagues (2016) showed that cell adhesion is not directly linked with pectin amount in the cell walls but is rather associated with an altered pectin state (its esterification and de-esterification) related signalling and therefore enzymes related changes of pectins in the cell walls are important to maintain cell adhesion. Additionally it is interesting that pectin biosynthesis maintained by the Golgi apparatus and Golgi transport in hypocotyl cells can be accurately predicted from the actin network topology alone, which suggests a close relationship between actin and Golgi apparatus (Breuer et al., 2017) (Li et al., 2022). Furthermore, it is known that the distorted trichomes phenotype of ARP2/3 mutants is caused by abnormal deposition of the cell wall material connected with reduced directionality of the Golgi cisternae (Yanagisawa et al., 2015). Finally, it is shown that Latrunculin B-mediated depolymerization of actin negatively impacts the uptake of pectins and other polysaccharide components essential for cell wall formation (Baluska et al., 2002) (Sampathkumar et al., 2013), which further supports the importance of actin in the pectin maintenance pathway. All this information leads us to the hypothesis that the actin network, and specially the actin network subpopulation nucleated by the ARP2/3 complex, is directly involved in the pectin/pectin enzymes delivery pathway to the cell wall. But, in our research, we surprisingly did not biochemically detect any changes in cell wall composition or pectin amount in ARP2/3 complex mutants dark-grown hypocotyl cell walls (Sahi et al., 2018). We also found out that neither actin-depolymerizing drugs Latrunculin B and Cytochalasin D nor myosin inhibitor BDM interfered with *Arabidopsis* seedling's hypocotyls or cotyledons pavement cells adhesion. Similarly, no effect in cell adhesion was observed after treatment with either the microtubule-stabilising drug Taxol or the microtubule-depolymerizing drug Oryzalin (unpublished results). Therefore, we hypothesised that ARP2/3 complex mutant-related adhesion problems could be caused not by the changes in the absolute amount of pectin or other cell wall components in the plant tissues but rather by altered localization of pectin or pectin-modifying enzymes to proper places in the cell walls - to the cell wall microdomains. The cell wall microdomains are in literature defined by local differences in cell wall composition. It is shown that spatially and temporally well-organised remodelling of the cell-wall microdomain controls many developmental processes and can define cell expansion and cell morphology as is pollen tube tip growth, as well as epidermal pavement cells shaping and guard cell openings. All the places which need the involvement of pectin-modifying enzymes to fine-tune the loosening/softening versus stiffening properties of pectins within cell wall microdomains (Dauphin et al., 2022). These described places are similar to the places where the ARP2/3 complex activators are published to be localised (Dyachok et al., 2008).

Finally, we noticed that when cotyledon pavement cell adhesion is disrupted, and pavement cells tear apart, the last places where they remain connected are places where they are connected to neighbouring cells via plasmodesmata (unpublished results). The places around plasmodesmata in the cell wall can be also seen as cell wall microdomains with unique cell wall composition enriched in callose and pectic polysaccharides and with the reduction in cellulose content. Pectin with a low degree of esterification is preferentially localised in the areas of the pit fields/plasmodesmata (Knox and Benitez-Alfonso 2014). It could be interesting to analyse

the plasmodesmata distribution, architecture or function in ARP2/3 complex mutants, as it can explain the role of plasmodesmata in cell-to-cell adhesion which was never addressed before.

In our published research, we also addressed a question that has not yet been investigated - the ARP2/3 complex mutants secondary cell walls analysis. We demonstrated that 6-week-old stems of ARP2/3 complex mutants contained less cellulose, less lignin, and a higher amount of homogalacturonan, which is the most abundant pectin subtype in plant cell walls (Sahi et al., 2018). I would like to hypothesise here that the reason why the stems of ARP2/3 complex mutants contain less lignin could be connected with the amount or state of pectin in younger plant tissues. Because when transitioning to secondary cell wall formation, the pectins are largely replaced by lignins, and lignins then take over the role of keeping the cells attached (Atakhani et al., 2022). This shows that the problems with primary cell wall composition of ARP2/3 complex mutants can be and are transmitted to rather complex problems in secondary cell wall compositions. These results also can point out that the ARP2/3 complex is needed for rather specialised and mainly precisely localised changes in the cell wall architecture.

## **Future questions**

In research, good, focused, and thoughtful questions are as important as the answers. I would like to summarise my results and literature review into five main questions that can be the starting point for future actin nucleators research.

- 1. Is it possible to distinguish between the overall functions of the actin cytoskeleton network itself and the functions of subsequent actin filaments nucleated by ARP2/3 complex or formins?**
- 2. Do formins also help to build and maintain the plant cell wall? Maybe in connection with plasmodesmata?**
- 3. What is ARP2/3 complex exact role in membrane remodelling, and how does this influence the cell wall changes? Why the roots of ARP2/3 complex and SCAR/WAVE complex mutants do not show adhesion defects?**
- 4. Neither the ARP2/3 complex nor the SCAR/WAVE complex subunits have a transmembrane domain, how is their association with membranes achieved and regulated?**
- 5. Do plants need actin nucleators to survive and grow at all?**

## CONCLUSION

The actin filament network is a crucial and dynamic structure in plant cells, playing a vital role in plant development, cell growth, morphogenesis, and responses to environmental stimuli. Due to its numerous functions, it is essential to study the actin-binding proteins that maintain the actin network's organisation and activity. Two well-studied actin-binding proteins are the actin nucleators ARP2/3 complex and formins. During my doctoral research, I participated in research and summarised in publications the individual roles of the ARP2/3 complex (Pratap Sahi et al., 2018) and formins (Rosero et al., 2016) as well as their interplay in plant cells (Cifrová et al., 2020). I engaged in studies of localizations of the ARP2/3 complex (Martinek et al., 2023) and AtFH1 in plant cells (Oulehlová et al., 2019) which helped to better understand their roles. Finally, I was involved in studies of the role of the ARP2/3 complex in apical and diffuse growth of plant cells, discovering that the ARPC3 subunit is not essential for diffuse growth in epidermal cells (Bellinvia et al., 2022) but it is needed for elongation of apically growing pollen tubes (attached manuscript in preparation - Martinek et al., 2024).

Over the past four years, three key publications have explored the relationship between the ARP2/3 complex and formins. The research independently described double mutant plants in both actin nucleators and use of actin nucleator inhibitor treatments by CK666 and SMIFH2. The first publication about ARP2/3 complex and formins interplay was written by our laboratory. We found that the ARP2/3 complex and formins have complementary roles in cotyledon pavement cell morphology and act in parallel in other cell types and developmental stages (Cifrová et al., 2020). On the level of single actin filaments observation (Xu et al., 2023) showed that these nucleators cooperate to maintain the homeostatic cortical arrangement of epidermal cells, while (Qin et al., 2021) demonstrated their cooperative role in *Arabidopsis*' resistance to fungal invasion. The obtained data showed that the actin nucleation in plant cells appears to be unique. In plants, actin nucleators, the ARP2/3 complex, and formins are likely to cooperate rather than compete, and their mutations are not lethal. In contrast, studies in yeast and mammalian cells show that the ARP2/3 complex and formins often compete with each other, and mutations in these actin nucleators are typically lethal. We concluded that plants possibly use a failsafe system that largely preserves actin filament abundance and dynamics even when both nucleators are, to a large extent, inactivated.

Furthermore, after almost ten years of research in our laboratory, our understanding of the ARP2/3 complex has shifted from describing seemingly random problems with cell growth and shape to uncovering the overall unifying purpose of the actin filaments nucleated by the ARP2/3 complex. We concluded that the characteristic morphological phenotypes of ARP2/3 complex subunit mutants likely underlie changes in the formation and maintenance of pectin-based cell wall microdomains. To link cell wall microdomain maintenance with the knowledge that the ARP2/3 complex partially mediates autophagy and endocytosis, we further hypothesised that the ARP2/3 complex plays a partial role in membrane remodelling, as it is essential for both processes. In addition, a similar role of the ARP2/3 complex in membrane remodelling has already been described for animal cells and yeast (see, e.g., Chakrabarti et al., 2021 and Campellone et al., 2023). In conclusion, the functional ARP2/3 complex is not essential for the

life of plant cells, as its mutation causes only minor growth and morphology problems, but on the other hand, the functional ARP2/3 complex has an indispensable role associated with specialised membrane and cell wall remodelling, ensuring that the cells stay together during plant growth and can respond properly to stimuli by adjusting the composition of their cell wall.

Looking ahead, I anticipate many more discoveries about the interactions and roles of plant actin nucleators, the ARP2/3 complex, and formins. I am particularly interested in the ARP2/3 complex's role in cell-to-cell adhesion and its connection with the cell wall microdomains biochemistry. Additionally, I wonder if formin-mediated actin nucleation impacts cell wall establishment. Finally, I believe that patience is a crucial skill for scientists, and maintaining a positive attitude while waiting is essential for achieving research goals. I can't wait to learn more about actin nucleation in the future and to boldly go where no one has gone before.

# REFERENCES

## Used software

This thesis was written using the citation tool: <https://paperpile.com/app>

The grammar check was performed by: <https://www.deepl.com/cs/translator> and <https://www.grammarly.com/>

ChimeraX program used to create ARP2/3 model according to the (Ding et al., 2022) available data - Molecular graphics and analyses performed with UCSF ChimeraX, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from National Institutes of Health R01-GM129325 and the Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases. <https://www.rbvi.ucsf.edu/chimerax/>

## Theses and Publications cited

### Theses

Schiebertová, Petra. 2013. "Role Komplexu ARP2/3 v Rostlinné Buňce," Master thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology, <https://dspace.cuni.cz/handle/20.500.11956/51764>.

Němcová, Barbora. 2020. "Aktin a Komplex Arp2/3 v Jádře," Master thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology <https://dspace.cuni.cz/handle/20.500.11956/119605>.

Semerák, Matěj. 2016. "Vliv Arp2/3 Komplexu Na Strukturu Vakuomu, Cytoplasmatické Proudění a Pohyblivost Diktyosomů," Master thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology, <https://dspace.cuni.cz/handle/20.500.11956/81739>.

Martinek, Jan. 2015. "Vizualizace a Analýza Distribuce Komplexu ARP2/3 v Rostlinách," Master thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology, <https://dspace.cuni.cz/handle/20.500.11956/74486>.

Havelková, Lenka. 2010. "Úloha Cytoskeletu v Morfogenezi Rostlinných Buněk," Doctoral thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology <https://dspace.cuni.cz/handle/20.500.11956/34653>.

Šlajcherová, Kateřina. 2011. "Isotypové Složení Aktinu v Rostlinné Buňce," Bachelor thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology , <https://dspace.cuni.cz/handle/20.500.11956/36609>.

Voloshina, Mariia. 2023. "Interakce ARP2/3 S Komponentami Rostlinné Sekretorické Dráhy," Master thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology, <https://dspace.cuni.cz/handle/20.500.11956/185366>.

Kukla, Jakub. 2011. "Funkční Analýza Podjednotek Rostlinného Arp2/3 Komplexu," Master thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology, <https://dspace.cuni.cz/handle/20.500.11956/48434>.

Miklánková, Pavlína. 2015. “Fenotypová Analýza Pokožkových Buniek Rastlín Arabidopsis Thaliana S Narušenou Organizáciou Aktínového Cytoskeletu,” Master thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology, <https://dspace.cuni.cz/handle/20.500.11956/74392>.

Jelínková, Barbora. 2021. “Analýza Lokalizace Endomembránových Markerů v Kortikální Vrstvě Rostlinných Buněk a Jejich Interakce S Komplexem Arp2/3,” Master thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology, <https://dspace.cuni.cz/handle/20.500.11956/150908>.

Dubenecká, Kamila. 2018. “Analýza Dynamiky CESA Komplexů v Rostlinách S Narušeným Cytoskeletem,” Master thesis, supervisor Schwarzerová, Kateřina. Charles University, Faculty of Science, Department of Experimental Plant Biology, <https://dspace.cuni.cz/handle/20.500.11956/102102>.

Fišerová, Jindřiška. 2007. “Following the Arp2/3-Based Processes in Plant Cells,” Doctoral thesis, supervisor Opatrný Zdeněk. Charles University, Faculty of Science, Department of Experimental Plant Biology <https://dspace.cuni.cz/handle/20.500.11956/95189>.

## Publications

Akram, Z., Ahmed, I., Mack, H., Kaur, R., Silva, R. C., Castilho, B. A., Friant, S., Sattlegger, E., & Munn, A. L.

(2020). Yeast as a Model to Understand Actin-Mediated Cellular Functions in Mammals-Illustrated with Four Actin Cytoskeleton Proteins. *Cells*, 9(3). <https://doi.org/10.3390/cells9030672>

Ali, M. F., Fatema, U., Peng, X., Hacker, S. W., Maruyama, D., Sun, M.-X., & Kawashima, T. (2020). ARP2/3-independent WAVE/SCAR pathway and class XI myosin control sperm nuclear migration in flowering plants. *Proceedings of the National Academy of Sciences of the United States of America*, 117(51), 32757–32763.

Altartouri, B., Bidhendi, A. J., Tani, T., Suzuki, J., Conrad, C., Chebli, Y., Liu, N., Karunakaran, C., Scarcelli, G., & Geitmann, A. (2019). Pectin Chemistry and Cellulose Crystallinity Govern Pavement Cell Morphogenesis in a Multi-Step Mechanism. *Plant Physiology*, 181(1), 127–141.

Armour, William J., Deborah A. Barton, Andrew M. K. Law, and Robyn L. Overall. 2015. “Differential Growth in Periclinal and Anticlinal Walls during Lobe Formation in Arabidopsis Cotyledon Pavement Cells.” *The Plant Cell* 27 (9): 2484–2500.

Atakhani, A., Bogdziewicz, L., & Verger, S. (2022). Characterising the mechanics of cell–cell adhesion in plants. *Quantitative Plant Biology*, 3, e2.

Baba, Abu Imran, and Stéphane Verger. 2024. “Cell Adhesion Maintenance and Controlled Separation in Plants.” *Frontiers in Plant Physiology* 2 (February). <https://doi.org/10.3389/fphgy.2024.1369575>.

- Baluska, F., & Hlavacka, A. (2005). Plant formins come of age: something special about cross-walls [Review of *Plant formins come of age: something special about cross-walls*]. *The New Phytologist*, *168*(3), 499–503.
- Baluska, F., Hlavacka, A., Samaj, J., Palme, K., Robinson, D. G., Match, T., McCurdy, D. W., Menzel, D., & Volkmann, D. (2002). F-actin-dependent endocytosis of cell wall pectins in meristematic root cells. Insights from brefeldin A-induced compartments. *Plant Physiology*, *130*(1), 422–431.
- Basu, D., Le, J., Zakharova, T., Mallery, E. L., & Szymanski, D. B. (2008). A SPIKE1 signaling complex controls actin-dependent cell morphogenesis through the heteromeric WAVE and ARP2/3 complexes. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(10), 4044–4049.
- Bear, J. E., Rawls, J. F., & Saxe, C. L., 3rd. (1998). SCAR, a WASP-related protein, isolated as a suppressor of receptor defects in late Dictyostelium development. *The Journal of Cell Biology*, *142*(5), 1325–1335.
- Bellinvia, E., García-González, J., Cifrová, P., Martinek, J., Sikorová, L., Havelková, L., & Schwarzerová, K. (2022). CRISPR-Cas9 Arabidopsis mutants of genes for ARPC1 and ARPC3 subunits of ARP2/3 complex reveal differential roles of complex subunits. *Scientific Reports*, *12*(1), 18205.
- Bidhendi, A. J., Altartouri, B., Gosselin, F. P., & Geitmann, A. (2019). Mechanical Stress Initiates and Sustains the Morphogenesis of Wavy Leaf Epidermal Cells. *Cell Reports*, *28*(5), 1237–1250.e6.
- Blanchoin, L., Boujemaa-Paterski, R., Henty, J. L., Khurana, P., & Staiger, C. J. (2010). Actin dynamics in plant cells: a team effort from multiple proteins orchestrates this very fast-paced game. *Current Opinion in Plant Biology*, *13*(6), 714–723.
- Blanchoin, L., Boujemaa-Paterski, R., Sykes, C., & Plastino, J. (2014). Actin dynamics, architecture, and mechanics in cell motility. *Physiological Reviews*, *94*(1), 235–263.
- Blanchoin, L., & Staiger, C. J. (2010). Plant formins: diverse isoforms and unique molecular mechanism. *Biochimica et Biophysica Acta*, *1803*(2), 201–206.
- Boulant, S., Kural, C., Zeeh, J.-C., Ubelmann, F., & Kirchhausen, T. (2011). Actin dynamics counteract membrane tension during clathrin-mediated endocytosis. *Nature Cell Biology*, *13*(9), 1124–1131.
- Breuer, D., Nowak, J., Ivakov, A., Somssich, M., Persson, S., & Nikoloski, Z. (2017). System-wide organization of actin cytoskeleton determines organelle transport in hypocotyl plant cells. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(28), E5741–E5749.
- Campellone, K. G., Lebek, N. M., & King, V. L. (2023). Branching out in different directions: Emerging cellular functions for the Arp2/3 complex and WASP-family actin nucleation factors. *European Journal of Cell Biology*, *102*(2), 151301.

- Campellone, K. G., & Welch, M. D. (2010). A nucleator arms race: cellular control of actin assembly. *Nature Reviews. Molecular Cell Biology*, *11*(4), 237–251.
- Carrier, M.-F., & Shekhar, S. (2017). Global treadmilling coordinates actin turnover and controls the size of actin networks. *Nature Reviews. Molecular Cell Biology*, *18*(6), 389–401.
- Chakrabarti, R., Lee, M., & Higgs, H. N. (2021). Multiple roles for actin in secretory and endocytic pathways. *Current Biology: CB*, *31*(10), R603–R618.
- Chan, F.-Y., Silva, A. M., Saramago, J., Pereira-Sousa, J., Brighton, H. E., Pereira, M., Oegema, K., Gassmann, R., & Carvalho, A. X. (2019). The ARP2/3 complex prevents excessive formin activity during cytokinesis. *Molecular Biology of the Cell*, *30*(1), 96–107.
- Chang, M., & Huang, S. (2015). Arabidopsis ACT11 modifies actin turnover to promote pollen germination and maintain the normal rate of tube growth. *The Plant Journal: For Cell and Molecular Biology*, *83*(3), 515–527.
- Chang, M., Li, Z., & Huang, S. (2017). Monomeric G-actin is uniformly distributed in pollen tubes and is rapidly redistributed via cytoplasmic streaming during pollen tube growth. *The Plant Journal: For Cell and Molecular Biology*, *92*(3), 509–519.
- Cheung, A. Y., Niroomand, S., Zou, Y., & Wu, H.-M. (2010). A transmembrane formin nucleates subapical actin assembly and controls tip-focused growth in pollen tubes. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(37), 16390–16395.
- Cheung, A. Y., & Wu, H.-M. (2004). Overexpression of an Arabidopsis formin stimulates supernumerary actin cable formation from pollen tube cell membrane. *The Plant Cell*, *16*(1), 257–269.
- Chin, S., Kwon, T., Khan, B. R., Sparks, J. A., Mallery, E. L., Szymanski, D. B., & Blancaflor, E. B. (2021). Spatial and temporal localization of SPIRRIG and WAVE/SCAR reveal roles for these proteins in actin-mediated root hair development. *The Plant Cell*, *33*(7), 2131–2148.
- Chun, J.-I., Kim, S.-M., Jeong, N.-R., Kim, S. H., Jung, C., & Kang, J.-H. (2022). Tomato ARPC1 regulates trichome morphology and density and terpene biosynthesis. *Planta*, *256*(2), 38.
- Cifrová, P., Oulehlová, D., Kollárová, E., Martinek, J., Rosero, A., Žárský, V., Schwarzerová, K., & Cvrčková, F. (2020). Division of Labor Between Two Actin Nucleators-the Formin FH1 and the ARP2/3 Complex-in Arabidopsis Epidermal Cell Morphogenesis. *Frontiers in Plant Science*, *11*, 148.
- Cosgrove, D. J. (2014). Re-constructing our models of cellulose and primary cell wall assembly. *Current Opinion in Plant Biology*, *22*, 122–131.

- Cosgrove, D. J., & Anderson, C. T. (2020). Plant Cell Growth: Do Pectins Drive Lobe Formation in Arabidopsis Pavement Cells? *Current Biology: CB*, *30*(11), R660–R662.
- Courtemanche, N. (2018). Mechanisms of formin-mediated actin assembly and dynamics. *Biophysical Reviews*, *10*(6), 1553–1569.
- Cui, X., Zou, M., & Li, J. (2023). Basally distributed actin array drives embryonic hypocotyl elongation during the seed-to-seedling transition in Arabidopsis. *The New Phytologist*, *240*(1), 191–206.
- Cvrčková, F. (2000). Are plant formins integral membrane proteins? *Genome Biology*, *1*(1), RESEARCH001.
- Cvrčková, F., Ghosh, R., & Kočová, H. (2024). Transmembrane formins as active cargoes of membrane trafficking. *Journal of Experimental Botany*, *75*(12), 3668–3684.
- Cvrčková, F., & Oulehlová, D. (2017). A new kymogram-based method reveals unexpected effects of marker protein expression and spatial anisotropy of cytoskeletal dynamics in plant cell cortex. *Plant Methods*, *13*, 19.
- Cvrčková, F., Rivero, F., & Bavlínka, B. (2004). Evolutionarily conserved modules in actin nucleation: lessons from Dictyostelium discoideum and plants. Review article. *Protoplasma*, *224*(1-2), 15–31.
- Daugherty, K. M., & Goode, B. L. (2008). Functional surfaces on the p35/ARPC2 subunit of Arp2/3 complex required for cell growth, actin nucleation, and endocytosis. *The Journal of Biological Chemistry*, *283*(24), 16950–16959.
- Daher, Firas Bou, and Siobhan A. Braybrook. 2015. “How to Let Go: Pectin and Plant Cell Adhesion.” *Frontiers in Plant Science* 00523
- Dauphin, B. G., Ranocha, P., Dunand, C., & Burlat, V. (2022). Cell-wall microdomain remodeling controls crucial developmental processes. *Trends in Plant Science*, *27*(10), 1033–1048.
- Deeks, M. J., Cvrčková, F., Machesky, L. M., Mikitová, V., Ketelaar, T., Zársky, V., Davies, B., & Hussey, P. J. (2005). Arabidopsis group Ie formins localize to specific cell membrane domains, interact with actin-binding proteins and cause defects in cell expansion upon aberrant expression. *The New Phytologist*, *168*(3), 529–540.
- Deeks, M. J., Fendrych, M., Smertenko, A., Bell, K. S., Oparka, K., Cvrčková, F., Zársky, V., & Hussey, P. J. (2010). The plant formin AtFH4 interacts with both actin and microtubules, and contains a newly identified microtubule-binding domain. *Journal of Cell Science*, *123*(Pt 8), 1209–1215.
- Deeks, M. J., & Hussey, P. J. (2005). Arp2/3 and SCAR: plants move to the fore. *Nature Reviews. Molecular Cell Biology*, *6*(12), 954–964.

- Deeks, M. J., & Hussey, P. J. (2016). Plant Actin Biology. In *eLS* (pp. 1–9). John Wiley & Sons, Ltd.  
<https://doi.org/10.1002/9780470015902.a0021255.pub2>
- Deeks, M. J., Hussey, P. J., & Davies, B. (2002). Formins: intermediates in signal-transduction cascades that affect cytoskeletal reorganization. *Trends in Plant Science*, 7(11), 492–498.
- Deeks, M. J., Kaloriti, D., Davies, B., Malhó, R., & Hussey, P. J. (2004). Arabidopsis NAP1 is essential for Arp2/3-dependent trichome morphogenesis. *Current Biology: CB*, 14(15), 1410–1414.
- Diao, M., Ren, S., Wang, Q., Qian, L., Shen, J., Liu, Y., & Huang, S. (2018). Arabidopsis formin 2 regulates cell-to-cell trafficking by capping and stabilizing actin filaments at plasmodesmata. *eLife*, 7.  
<https://doi.org/10.7554/eLife.36316>
- Dimchev, V., Lahmann, I., Koestler, S. A., Kage, F., Dimchev, G., Steffen, A., Stradal, T. E. B., Vauti, F., Arnold, H.-H., & Rottner, K. (2021). Induced Arp2/3 Complex Depletion Increases FMNL2/3 Formin Expression and Filopodia Formation. *Frontiers in Cell and Developmental Biology*, 9, 634708.
- Ding, B., Narvaez-Ortiz, H. Y., Singh, Y., Hocky, G. M., Chowdhury, S., & Nolen, B. J. (2022). Structure of Arp2/3 complex at a branched actin filament junction resolved by single-particle cryo-electron microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, 119(22), e2202723119.
- Dragwidge, J. M., & VAN Damme, D. (2020). Visualising endocytosis in plants: past, present, and future. *Journal of Microscopy*, 280(2), 104–110.
- Dubenecká, K. (2018). *Analýza dynamiky CESA komplexů v rostlinách s narušeným cytoskeletem*.  
<https://dspace.cuni.cz/handle/20.500.11956/102102>
- Du, J., Anderson, C. T., & Xiao, C. (2022). Dynamics of pectic homogalacturonan in cellular morphogenesis and adhesion, wall integrity sensing and plant development. *Nature Plants*, 8(4), 332–340.
- Dyachok, J., Shao, M.-R., Vaughn, K., Bowling, A., Facette, M., Djakovic, S., Clark, L., & Smith, L. (2008). Plasma membrane-associated SCAR complex subunits promote cortical F-actin accumulation and normal growth characteristics in Arabidopsis roots. *Molecular Plant*, 1(6), 990–1006.
- Dyachok, J., Zhu, L., Liao, F., He, J., Huq, E., & Blancaflor, E. B. (2011). SCAR mediates light-induced root elongation in Arabidopsis through photoreceptors and proteasomes. *The Plant Cell*, 23(10), 3610–3626.
- Eisenberg-Bord, M., Shai, N., Schuldiner, M., & Bohnert, M. (2016). A Tether Is a Tether Is a Tether: Tethering at Membrane Contact Sites. *Developmental Cell*, 39(4), 395–409.
- El-Din El-Assal, S., Le, J., Basu, D., Mallery, E. L., & Szymanski, D. B. (2004). DISTORTED2 encodes an ARPC2 subunit of the putative Arabidopsis ARP2/3 complex. *The Plant Journal: For Cell and Molecular*

- Biology*, 38(3), 526–538.
- Faulkner, C. (2018). Plasmodesmata and the symplast. *Current Biology: CB*, 28(24), R1374–R1378.
- Favery, B., Chelysheva, L. A., Lebris, M., Jammes, F., Marmagne, A., De Almeida-Engler, J., Lecomte, P., Vaury, C., Arkowitz, R. A., & Abad, P. (2004). Arabidopsis formin AtFH6 is a plasma membrane-associated protein upregulated in giant cells induced by parasitic nematodes. *The Plant Cell*, 16(9), 2529–2540.
- Firat-Karalar, E. N., & Welch, M. D. (2011). New mechanisms and functions of actin nucleation. *Current Opinion in Cell Biology*, 23(1), 4–13.
- Fišerová, J. (2007). *Following the Arp2/3-based processes in plant cells*. <https://dspace.cuni.cz/handle/20.500.11956/95189>
- Fiserová, J., Schwarzerová, K., Petrásek, J., & Opatrný, Z. (2006). ARP2 and ARP3 are localized to sites of actin filament nucleation in tobacco BY-2 cells. *Protoplasma*, 227(2-4), 119–128.
- García-González, J., Kebrlová, Š., Semerák, M., Lacek, J., Kotannal Baby, I., Petrásek, J., & Schwarzerová, K. (2020). Arp2/3 Complex Is Required for Auxin-Driven Cell Expansion Through Regulation of Auxin Transporter Homeostasis. *Frontiers in Plant Science*, 11, 486.
- García-González, J., & van Gelderen, K. (2021). Bundling up the Role of the Actin Cytoskeleton in Primary Root Growth. *Frontiers in Plant Science*, 12, 777119.
- Geitmann, A., & Emons, A. M. (2000). The cytoskeleton in plant and fungal cell tip growth. *Journal of Microscopy*, 198(Pt 3), 218–245.
- Goley, E. D., Rammohan, A., Znameroski, E. A., Firat-Karalar, E. N., Sept, D., & Welch, M. D. (2010). An actin-filament-binding interface on the Arp2/3 complex is critical for nucleation and branch stability. *Proceedings of the National Academy of Sciences of the United States of America*, 107(18), 8159–8164.
- Gorelova, V., Sprakel, J., & Weijers, D. (2021). Plant cell polarity as the nexus of tissue mechanics and morphogenesis. *Nature Plants*, 7(12), 1548–1559.
- Grunt, M., Zárský, V., & Cvrcková, F. (2008). Roots of angiosperm formins: the evolutionary history of plant FH2 domain-containing proteins. *BMC Evolutionary Biology*, 8, 115.
- Guo, J., & Yang, Z. (2020). Exocytosis and endocytosis: coordinating and fine-tuning the polar tip growth domain in pollen tubes. *Journal of Experimental Botany*, 71(8), 2428–2438.
- Haas, K. T., Wightman, R., Peaucelle, A., & Höfte, H. (2021). The role of pectin phase separation in plant cell wall assembly and growth. *Cell Surface (Amsterdam, Netherlands)*, 7, 100054.
- Hamant, O., & Traas, J. (2010). The mechanics behind plant development. *The New Phytologist*, 185(2), 369–385.

- Havelková, L. (2010). *Úloha cytoskeletu v morfogenezi rostlinných buněk*.  
<https://dspace.cuni.cz/handle/20.500.11956/34653>
- Havelková, L., Nanda, G., Martinek, J., Bellinvia, E., Sikorová, L., Šlajcherová, K., Seifertová, D., Fischer, L., Fišerová, J., Petrášek, J., & Schwarzerová, K. (2015). Arp2/3 complex subunit ARPC2 binds to microtubules. *Plant Science: An International Journal of Experimental Plant Biology*, 241, 96–108.
- Henty-Ridilla, J. L., Li, J., Blanchoin, L., & Staiger, C. J. (2013). Actin dynamics in the cortical array of plant cells. *Current Opinion in Plant Biology*, 16(6), 678–687.
- Hossain, M. S., Liao, J., James, E. K., Sato, S., Tabata, S., Jurkiewicz, A., Madsen, L. H., Stougaard, J., Ross, L., & Szczygłowski, K. (2012). Lotus japonicus ARPC1 is required for rhizobial infection. *Plant Physiology*, 160(2), 917–928.
- Huang, J., Kim, C. M., Xuan, Y.-H., Liu, J., Kim, T. H., Kim, B.-K., & Han, C.-D. (2013). Formin homology 1 (OsFH1) regulates root-hair elongation in rice (*Oryza sativa*). *Planta*, 237(5), 1227–1239.
- Hülkamp, M., Misra, S., & Jürgens, G. (1994). Genetic dissection of trichome cell development in Arabidopsis. *Cell*, 76(3), 555–566.
- Hussey, P. J., Ketelaar, T., & Deeks, M. J. (2006). Control of the actin cytoskeleton in plant cell growth. *Annual Review of Plant Biology*, 57, 109–125.
- Innocenti, M. (2023). Investigating Mammalian Formins with SMIFH2 Fifteen Years in: Novel Targets and Unexpected Biology. *International Journal of Molecular Sciences*, 24(10).  
<https://doi.org/10.3390/ijms24109058>
- Jelínková, B. (2021). *Analýza lokalizace endomembránových markerů v kortikální vrstvě rostlinných buněk a jejich interakce s komplexem Arp2/3*. <https://dspace.cuni.cz/handle/20.500.11956/150908>
- Jiang, K., Sorefan, K., Deeks, M. J., Bevan, M. W., Hussey, P. J., & Hetherington, A. M. (2012). The ARP2/3 complex mediates guard cell actin reorganization and stomatal movement in Arabidopsis. *The Plant Cell*, 24(5), 2031–2040.
- Johnson, A., Dahhan, D. A., Gnyliukh, N., Kaufmann, W. A., Zheden, V., Costanzo, T., Mahou, P., Hrtyan, M., Wang, J., Aguilera-Servin, J., van Damme, D., Beaurepaire, E., Loose, M., Bednarek, S. Y., & Friml, J. (2021). The TPLATE complex mediates membrane bending during plant clathrin-mediated endocytosis. *Proceedings of the National Academy of Sciences of the United States of America*, 118(51).  
<https://doi.org/10.1073/pnas.2113046118>
- Kandasamy, M. K., McKinney, E. C., & Meagher, R. B. (2002). Functional nonequivalency of actin isoforms in

- Arabidopsis. *Molecular Biology of the Cell*, 13(1), 251–261.
- Kijima, S. T., Staiger, C. J., Katoh, K., Nagasaki, A., Ito, K., & Uyeda, T. Q. P. (2018). Arabidopsis vegetative actin isoforms, AtACT2 and AtACT7, generate distinct filament arrays in living plant cells. *Scientific Reports*, 8(1), 4381.
- Kim, J. H., Jung, H., & Chung, T. (2020). Birth, Growth, Maturation, and Demise of Plant Autophagic Vesicles. *Journal of Plant Biology = Singmul Hakhoe Chi*, 63(3), 155–164.
- Kleine-Vehn, J., & Friml, J. (2008). Polar targeting and endocytic recycling in auxin-dependent plant development. *Annual Review of Cell and Developmental Biology*, 24, 447–473.
- Knox, J. P., & Benitez-Alfonso, Y. (2014). Roles and regulation of plant cell walls surrounding plasmodesmata. *Current Opinion in Plant Biology*, 22, 93–100.
- Kollárová, E., Baquero Forero, A., & Cvrčková, F. (2021). The Arabidopsis thaliana Class II Formin FH13 Modulates Pollen Tube Growth. *Frontiers in Plant Science*, 12, 599961.
- Kotchoni, S. O., Zakhara, T., Mallery, E. L., Le, J., El-Assal, S. E.-D., & Szymanski, D. B. (2009). The association of the Arabidopsis actin-related protein2/3 complex with cell membranes is linked to its assembly status but not its activation. *Plant Physiology*, 151(4), 2095–2109.
- Kovar, D. R., Harris, E. S., Mahaffy, R., Higgs, H. N., & Pollard, T. D. (2006). Control of the assembly of ATP- and ADP-actin by formins and profilin. *Cell*, 124(2), 423–435.
- Kraus, M., Pleskot, R., & Van Damme, D. (2024). Structural and Evolutionary Aspects of Plant Endocytosis. *Annual Review of Plant Biology*. <https://doi.org/10.1146/annurev-arplant-070122-023455>
- Kukla, J. (2011). *Funkční analýza podjednotek rostlinného Arp2/3 komplexu*. <https://dspace.cuni.cz/handle/20.500.11956/48434>
- Lan, Y., Liu, X., Fu, Y., & Huang, S. (2018). Arabidopsis class I formins control membrane-originated actin polymerization at pollen tube tips. *PLoS Genetics*, 14(11), e1007789.
- Le, J., El-Assal, S. E.-D., Basu, D., Saad, M. E., & Szymanski, D. B. (2003). Requirements for Arabidopsis ATARP2 and ATARP3 during epidermal development. *Current Biology: CB*, 13(15), 1341–1347.
- Lenartowska, M., & Michalska, A. (2008). Actin filament organization and polarity in pollen tubes revealed by myosin II subfragment 1 decoration. *Planta*, 228(5), 891–896.
- Leontovyčová, H., Kalachova, T., & Janda, M. (2020). Disrupted actin: a novel player in pathogen attack sensing? *The New Phytologist*, 227(6), 1605–1609.
- Leucci, M. R., Di Sansebastiano, G.-P., Gigante, M., Dalessandro, G., & Piro, G. (2007). Secretion marker proteins

- and cell-wall polysaccharides move through different secretory pathways. *Planta*, 225(4), 1001–1017.
- Liang, P., Schmitz, C., Lacey, B., Ditengou, F. A., Su, C., Schulze, E., Knerr, J., Grosse, R., Keller, J., Libourel, C., Delaux, P.-M., & Ott, T. (2021). Formin-mediated bridging of cell wall, plasma membrane, and cytoskeleton in symbiotic infections of *Medicago truncatula*. *Current Biology: CB*, 31(12), 2712–2719.e5.
- Lian, N., Wang, X., Jing, Y., & Lin, J. (2021). Regulation of cytoskeleton-associated protein activities: Linking cellular signals to plant cytoskeletal function. *Journal of Integrative Plant Biology*, 63(1), 241–250.
- Li, J., Blanchoin, L., & Staiger, C. J. (2015). Signaling to actin stochastic dynamics. *Annual Review of Plant Biology*, 66, 415–440.
- Li, L.-J., Ren, F., Gao, X.-Q., Wei, P.-C., & Wang, X.-C. (2013). The reorganization of actin filaments is required for vacuolar fusion of guard cells during stomatal opening in *Arabidopsis*. *Plant, Cell & Environment*, 36(2), 484–497.
- Li, S., Blanchoin, L., Yang, Z., & Lord, E. M. (2003). The putative *Arabidopsis* arp2/3 complex controls leaf cell morphogenesis. *Plant Physiology*, 132(4), 2034–2044.
- Li, S., Dong, H., Pei, W., Liu, C., Zhang, S., Sun, T., Xue, X., & Ren, H. (2017). LIFH1-mediated interaction between actin fringe and exocytic vesicles is involved in pollen tube tip growth. *The New Phytologist*, 214(2), 745–761.
- Liu, A. X., Zhang, S. B., Xu, X. J., Ren, D. T., & Liu, G. Q. (2004). Soluble expression and characterization of a GFP-fused pea actin isoform (PEAc1). *Cell Research*, 14(5), 407–414.
- Liu, C., Zhang, Y., & Ren, H. (2021). Profilin promotes formin-mediated actin filament assembly and vesicle transport during polarity formation in pollen. *The Plant Cell*, 33(4), 1252–1267.
- Liu, H.-K., Li, Y.-J., Wang, S.-J., Yuan, T.-L., Huang, W.-J., Dong, X., Pei, J.-Q., Zhang, D., McCormick, S., & Tang, W.-H. (2020). Kinase Partner Protein Plays a Key Role in Controlling the Speed and Shape of Pollen Tube Growth in Tomato. *Plant Physiology*, 184(4), 1853–1869.
- Liu, S., Jobert, F., Rahnesan, Z., Doyle, S. M., & Robert, S. (2021). Solving the Puzzle of Shape Regulation in Plant Epidermal Pavement Cells. *Annual Review of Plant Biology*, 72, 525–550.
- Liu, X., Pimm, M. L., Haarer, B., Brawner, A. T., & Henty-Ridilla, J. L. (2022). Biochemical characterization of actin assembly mechanisms with ALS-associated profilin variants. *European Journal of Cell Biology*, 101(2), 151212.
- Liu, X., Qu, X., Jiang, Y., Chang, M., Zhang, R., Wu, Y., Fu, Y., & Huang, S. (2015). Profilin Regulates Apical Actin Polymerization to Control Polarized Pollen Tube Growth. *Molecular Plant*, 8(12), 1694–1709.

- 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.
- Li, Y., Sorefan, K., Hemmann, G., & Bevan, M. W. (2004). Arabidopsis NAP and PIR regulate actin-based cell morphogenesis and multiple developmental processes. *Plant Physiology*, 136(3), 3616–3627.
- Li, Y., Zhang, X., Zhang, Y., & Ren, H. (2022). Controlling the Gate: The Functions of the Cytoskeleton in Stomatal Movement. *Frontiers in Plant Science*, 13, 849729.
- Machesky, L. M., Atkinson, S. J., Ampe, C., Vandekerckhove, J., & Pollard, T. D. (1994). Purification of a cortical complex containing two unconventional actins from *Acanthamoeba* by affinity chromatography on profilin-agarose. *The Journal of Cell Biology*, 127(1), 107–115.
- Machesky, L. M., Mullins, R. D., Higgs, H. N., Kaiser, D. A., Blanchoin, L., May, R. C., Hall, M. E., & Pollard, T. D. (1999). Scar, a WASp-related protein, activates nucleation of actin filaments by the Arp2/3 complex. *Proceedings of the National Academy of Sciences of the United States of America*, 96(7), 3739–3744.
- Maisch, J., Fiserová, J., Fischer, L., & Nick, P. (2009). Tobacco Arp3 is localized to actin-nucleating sites in vivo. *Journal of Experimental Botany*, 60(2), 603–614.
- Martinek, J. (2015). *Vizualizace a analýza distribuce komplexu ARP2/3 v rostlinách*. <https://dspace.cuni.cz/handle/20.500.11956/74486>
- Martinek, J., Cifrová, P., Vosolsobě, S., García-González, J., Malínská, K., Mauzerová, Z., Jelínková, B., Krtková, J., Sikorová, L., Leaves, I., Sparkes, I., & Schwarzerová, K. (2023). ARP2/3 complex associates with peroxisomes to participate in pexophagy in plants. *Nature Plants*, 9(11), 1874–1889.
- Martinière, A., Gayral, P., Hawes, C., & Runions, J. (2011). Building bridges: formin1 of Arabidopsis forms a connection between the cell wall and the actin cytoskeleton. *The Plant Journal: For Cell and Molecular Biology*, 66(2), 354–365.
- Mass, R. L., Zeller, R., Woychik, R. P., Vogt, T. F., & Leder, P. (1990). Disruption of formin-encoding transcripts in two mutant limb deformity alleles. *Nature*, 346(6287), 853–855.
- Mathur, J. (2005). The ARP2/3 complex: giving plant cells a leading edge. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, 27(4), 377–387.
- Mathur, J., Mathur, N., Kernebeck, B., & Hülskamp, M. (2003). Mutations in actin-related proteins 2 and 3 affect cell shape development in Arabidopsis. *The Plant Cell*, 15(7), 1632–1645.
- Mathur, J., Spielhofer, P., Kost, B., & Chua, N. (1999). The actin cytoskeleton is required to elaborate and maintain spatial patterning during trichome cell morphogenesis in Arabidopsis thaliana. *Development*, 126(24), 5559–

5568.

- May, R. C. (2001). The Arp2/3 complex: a central regulator of the actin cytoskeleton. *Cellular and Molecular Life Sciences: CMLS*, 58(11), 1607–1626.
- Ma, Z., Liu, X., Nath, S., Sun, H., Tran, T. M., Yang, L., Mayor, S., & Miao, Y. (2021). Formin nanoclustering-mediated actin assembly during plant flagellin and DSF signaling. *Cell Reports*, 34(13), 108884.
- McDowell, J. M., Huang, S., McKinney, E. C., An, Y. Q., & Meagher, R. B. (1996). Structure and evolution of the actin gene family in *Arabidopsis thaliana*. *Genetics*, 142(2), 587–602.
- Meagher, R. B., McKinney, E. C., & Vitale, A. V. (1999). The evolution of new structures: clues from plant cytoskeletal genes. *Trends in Genetics: TIG*, 15(7), 278–284.
- Mergner, J., Frejno, M., List, M., Papacek, M., Chen, X., Chaudhary, A., Samaras, P., Richter, S., Shikata, H., Messerer, M., Lang, D., Altmann, S., Cyprys, P., Zolg, D. P., Mathieson, T., Bantscheff, M., Hazarika, R. R., Schmidt, T., Dawid, C., ... Kuster, B. (2020). Mass-spectrometry-based draft of the *Arabidopsis* proteome. *Nature*, 579(7799), 409–414.
- Michelot, A., Berro, J., Guérin, C., Boujemaa-Paterski, R., Staiger, C. J., Martiel, J.-L., & Blanchoin, L. (2007). Actin-filament stochastic dynamics mediated by ADF/cofilin. *Current Biology: CB*, 17(10), 825–833.
- Miklánková, P. (2015). *Fenotypová analýza pokožkových buniek rastlín Arabidopsis thaliana s narušenou organizáciou aktínového cytoskeletu*. <https://dspace.cuni.cz/handle/20.500.11956/74392>
- Mogilner, A. (2006). On the edge: modeling protrusion. *Current Opinion in Cell Biology*, 18(1), 32–39.
- Möller, B., Poeschl, Y., Plötner, R., & Bürstenbinder, K. (2017). PaCeQuant: A Tool for High-Throughput Quantification of Pavement Cell Shape Characteristics. *Plant Physiology*, 175(3), 998–1017.
- Moseley, J. B., Sagot, I., Manning, A. L., Xu, Y., Eck, M. J., Pellman, D., & Goode, B. L. (2004). A conserved mechanism for Bni1- and mDia1-induced actin assembly and dual regulation of Bni1 by Bud6 and profilin. *Molecular Biology of the Cell*, 15(2), 896–907.
- Narasimhan, M., Johnson, A., Prizak, R., Kaufmann, W. A., Tan, S., Casillas-Pérez, B., & Friml, J. (2020). Evolutionarily unique mechanistic framework of clathrin-mediated endocytosis in plants. *eLife*, 9. <https://doi.org/10.7554/eLife.52067>
- Němcová, B. (2020). *Aktin a komplex Arp2/3 v jádře*. <https://dspace.cuni.cz/handle/20.500.11956/119605>
- Ofoe, R. (2021). Signal transduction by plant heterotrimeric G-protein. *Plant Biology*, 23(1), 3–10.
- Oma, Y., & Harata, M. (2011). Actin-related proteins localized in the nucleus: from discovery to novel roles in nuclear organization. *Nucleus*, 2(1), 38–46.

- Orr, R. G., Cheng, X., Vidali, L., & Bezanilla, M. (2020). Orchestrating cell morphology from the inside out - using polarized cell expansion in plants as a model. *Current Opinion in Cell Biology*, 62, 46–53.
- Oulehlová, D., Kollárová, E., Cifrová, P., Pejchar, P., Žárský, V., & Cvrčková, F. (2019). Arabidopsis Class I Formin FH1 Relocates between Membrane Compartments during Root Cell Ontogeny and Associates with Plasmodesmata. *Plant & Cell Physiology*, 60(8), 1855–1870.
- Pain, C., Tolmie, F., Wojcik, S., Wang, P., & Kriechbaumer, V. (2023). intER-ACTINg: The structure and dynamics of ER and actin are interlinked. *Journal of Microscopy*, 291(1), 105–118.
- Paredez, A. R., Somerville, C. R., & Ehrhardt, D. W. (2006). Visualization of cellulose synthase demonstrates functional association with microtubules. *Science*, 312(5779), 1491–1495.
- Pawloski, L. C., Kandasamy, M. K., & Meagher, R. B. (2006). The late pollen actins are essential for normal male and female development in Arabidopsis. *Plant Molecular Biology*, 62(6), 881–896.
- Peaucelle, A., Wightman, R., & Höfte, H. (2015). The Control of Growth Symmetry Breaking in the Arabidopsis Hypocotyl. *Current Biology: CB*, 25(13), 1746–1752.
- Pérez-Sancho, J., Tilsner, J., Samuels, A. L., Botella, M. A., Bayer, E. M., & Rosado, A. (2016). Stitching Organelles: Organization and Function of Specialized Membrane Contact Sites in Plants. *Trends in Cell Biology*, 26(9), 705–717.
- Perrin, B. J., & Ervasti, J. M. (2010). The actin gene family: function follows isoform. *Cytoskeleton*, 67(10), 630–634.
- Pollard, T. D. (2007). Regulation of actin filament assembly by Arp2/3 complex and formins. *Annual Review of Biophysics and Biomolecular Structure*, 36, 451–477.
- Pollard, T. D., Blanchoin, L., & Mullins, R. D. (2000). Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annual Review of Biophysics and Biomolecular Structure*, 29, 545–576.
- Pollard, T. D., & Borisy, G. G. (2003). Cellular motility driven by assembly and disassembly of actin filaments. *Cell*, 112(4), 453–465.
- Pratap Sahi, V., Cifrová, P., García-González, J., Kotannal Baby, I., Mouillé, G., Gineau, E., Müller, K., Baluška, F., Soukup, A., Petrášek, J., & Schwarzerová, K. (2018). Arabidopsis thaliana plants lacking the ARP2/3 complex show defects in cell wall assembly and auxin distribution. *Annals of Botany*, 122(5), 777–789.
- Qin, L., Liu, L., Tu, J., Yang, G., Wang, S., Quilichini, T. D., Gao, P., Wang, H., Peng, G., Blancaflor, E. B., Datla, R., Xiang, D., Wilson, K. E., & Wei, Y. (2021). The ARP2/3 complex, acting cooperatively with Class I formins, modulates penetration resistance in Arabidopsis against powdery mildew invasion. *The Plant Cell*,

33(9), 3151–3175.

- Qin, Y., Sun, M., Li, W., Xu, M., Shao, L., Liu, Y., Zhao, G., Liu, Z., Xu, Z., You, J., Ye, Z., Xu, J., Yang, X., Wang, M., Lindsey, K., Zhang, X., & Tu, L. (2022). Single-cell RNA-seq reveals fate determination control of an individual fibre cell initiation in cotton (*Gossypium hirsutum*). *Plant Biotechnology Journal*, *20*(12), 2372–2388.
- Qiu, D., Xu, S., Wang, Y., Zhou, M., & Hong, L. (2021). Primary Cell Wall Modifying Proteins Regulate Wall Mechanics to Steer Plant Morphogenesis. *Frontiers in Plant Science*, *12*, 751372.
- Qiu, J.-L., Jilk, R., Marks, M. D., & Szymanski, D. B. (2002). The Arabidopsis SPIKE1 gene is required for normal cell shape control and tissue development. *The Plant Cell*, *14*(1), 101–118.
- Qu, X., Zhang, H., Xie, Y., Wang, J., Chen, N., & Huang, S. (2013). Arabidopsis villins promote actin turnover at pollen tube tips and facilitate the construction of actin collars. *The Plant Cell*, *25*(5), 1803–1817.
- Qu, X., Zhang, R., Zhang, M., Diao, M., Xue, Y., & Huang, S. (2017). Organizational Innovation of Apical Actin Filaments Drives Rapid Pollen Tube Growth and Turning. *Molecular Plant*, *10*(7), 930–947.
- Riedl, J., Crevenna, A. H., Kessenbrock, K., Yu, J. H., Neukirchen, D., Bista, M., Bradke, F., Jenne, D., Holak, T. A., Werb, Z., Sixt, M., & Wedlich-Soldner, R. (2008). Lifeact: a versatile marker to visualize F-actin. *Nature Methods*, *5*(7), 605–607.
- Romero, S., Didry, D., Larquet, E., Boisset, N., Pantaloni, D., & Carlier, M.-F. (2007). How ATP hydrolysis controls filament assembly from profilin-actin: implication for formin processivity. *The Journal of Biological Chemistry*, *282*(11), 8435–8445.
- Rosero, A., Oulehlová, D., Stillerová, L., Schiebertová, P., Grunt, M., Žárský, V., & Cvrčková, F. (2016). Arabidopsis FH1 formin affects cotyledon pavement cell shape by modulating cytoskeleton dynamics. *Plant & Cell Physiology*, *57*(3), 488–504.
- Rosero, A., Žárský, V., & Cvrčková, F. (2013). AtFH1 formin mutation affects actin filament and microtubule dynamics in Arabidopsis thaliana. *Journal of Experimental Botany*, *64*(2), 585–597.
- Sampathkumar, A., Gutierrez, R., McFarlane, H. E., Bringmann, M., Lindeboom, J., Emons, A.-M., Samuels, L., Ketelaar, T., Ehrhardt, D. W., & Persson, S. (2013). Patterning and lifetime of plasma membrane-localized cellulose synthase is dependent on actin organization in Arabidopsis interphase cells. *Plant Physiology*, *162*(2), 675–688.
- Sampathkumar, A., Krupinski, P., Wightman, R., Milani, P., Berquand, A., Boudaoud, A., Hamant, O., Jönsson, H., & Meyerowitz, E. M. (2014). Subcellular and supracellular mechanical stress prescribes cytoskeleton

- behavior in Arabidopsis cotyledon pavement cells. *eLife*, 3, e01967.
- Sapala, A., Runions, A., Routier-Kierzkowska, A.-L., Das Gupta, M., Hong, L., Hofhuis, H., Verger, S., Mosca, G., Li, C.-B., Hay, A., Hamant, O., Roeder, A. H., Tsiantis, M., Prusinkiewicz, P., & Smith, R. S. (2018). Why plants make puzzle cells, and how their shape emerges. *eLife*, 7. <https://doi.org/10.7554/eLife.32794>
- Sassmann, S., Rodrigues, C., Milne, S. W., Nenninger, A., Allwood, E., Littlejohn, G. R., Talbot, N. J., Soeller, C., Davies, B., Hussey, P. J., & Deeks, M. J. (2018). An Immune-Responsive Cytoskeletal-Plasma Membrane Feedback Loop in Plants. *Current Biology: CB*, 28(13), 2136–2144.e7.
- Schiebertová, P. (2013). *Role komplexu ARP2/3 v rostlinné buňce*. <https://dspace.cuni.cz/handle/20.500.11956/51764>
- Scholz, P., Anstatt, J., Krawczyk, H. E., & Ischebeck, T. (2020). Signalling Pinpointed to the Tip: The Complex Regulatory Network That Allows Pollen Tube Growth. *Plants*, 9(9). <https://doi.org/10.3390/plants9091098>
- Schroer, T. A., Fyrberg, E., Cooper, J. A., Waterston, R. H., Helfman, D., Pollard, T. D., & Meyer, D. I. (1994). Actin-related protein nomenclature and classification. *The Journal of Cell Biology*, 127(6 Pt 2), 1777–1778.
- Semerák, M. (2016). *Vliv Arp2/3 komplexu na strukturu vakuomu, cytoplasmatické proudění a pohyblivost diktyosomů*. <https://dspace.cuni.cz/handle/20.500.11956/81739>
- Shin, Y., Chane, A., Jung, M., & Lee, Y. (2021). Recent Advances in Understanding the Roles of Pectin as an Active Participant in Plant Signaling Networks. *Plants*, 10(8). <https://doi.org/10.3390/plants10081712>
- Slajcherová, K., Fišerová, J., Fischer, L., & Schwarzerová, K. (2012). Multiple actin isotypes in plants: diverse genes for diverse roles? *Frontiers in Plant Science*, 3, 226.
- Smith, L. G., & Oppenheimer, D. G. (2005). Spatial control of cell expansion by the plant cytoskeleton. *Annual Review of Cell and Developmental Biology*, 21, 271–295.
- Staiger, C. J., Sheahan, M. B., Khurana, P., Wang, X., McCurdy, D. W., & Blanchoin, L. (2009). Actin filament dynamics are dominated by rapid growth and severing activity in the Arabidopsis cortical array. *The Journal of Cell Biology*, 184(2), 269–280.
- Stephan, O. O. H. (2017). Actin fringes of polar cell growth. *Journal of Experimental Botany*, 68(13), 3303–3320.
- Svitkina, T. M., & Borisy, G. G. (1999). Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia. *The Journal of Cell Biology*, 145(5), 1009–1026.
- Szymanski, D. B. (2005). Breaking the WAVE complex: the point of Arabidopsis trichomes. *Current Opinion in Plant Biology*, 8(1), 103–112.

- Szymanski, D. B., & Cosgrove, D. J. (2009). Dynamic coordination of cytoskeletal and cell wall systems during plant cell morphogenesis. *Current Biology: CB*, *19*(17), R800–R811.
- Szymanski, D. B., Jilk, R. A., Pollock, S. M., & Marks, M. D. (1998). Control of GL2 expression in Arabidopsis leaves and trichomes. *Development*, *125*(7), 1161–1171.
- Szymanski, D. B., Marks, M. D., & Wick, S. M. (1999). Organized F-actin is essential for normal trichome morphogenesis in Arabidopsis. *The Plant Cell*, *11*(12), 2331–2347.
- Szymanski, D., & Staiger, C. J. (2018). The Actin Cytoskeleton: Functional Arrays for Cytoplasmic Organization and Cell Shape Control. *Plant Physiology*, *176*(1), 106–118.
- Takatsuka, H., Higaki, T., & Umeda, M. (2018). Actin Reorganization Triggers Rapid Cell Elongation in Roots. *Plant Physiology*, *178*(3), 1130–1141.
- Tang, K., Yang, S., Feng, X., Wu, T., Leng, J., Zhou, H., Zhang, Y., Yu, H., Gao, J., Ma, J., & Feng, X. (2020). GmNAP1 is essential for trichome and leaf epidermal cell development in soybean. *Plant Molecular Biology*, *103*(6), 609–621.
- Van Gestel, K., Slegers, H., Von Witsch, M., Samaj, J., Baluska, F., & Verbelen, J.-P. (2003). Immunological evidence for the presence of plant homologues of the actin-related protein Arp3 in tobacco and maize: subcellular localization to actin-enriched pit fields and emerging root hairs. *Protoplasma*, *222*(1-2), 45–52.
- van Gisbergen, P. A. C., Wu, S.-Z., Chang, M., Pattavina, K. A., Bartlett, M. E., & Bezanilla, M. (2018). An ancient Sec10-formin fusion provides insights into actin-mediated regulation of exocytosis. *The Journal of Cell Biology*, *217*(3), 945–957.
- van Gisbergen, P., Wu, S.-Z., Cheng, X., Pattavina, K. A., & Bezanilla, M. (2020). In vivo analysis of formin dynamics in the moss *P. patens* reveals functional class diversification. *Journal of Cell Science*, *133*(3). <https://doi.org/10.1242/jcs.233791>
- Verger, S., Chabout, S., Gineau, E., & Mouille, G. (2016). Cell adhesion in plants is under the control of putative O-fucosyltransferases. *Development*, *143*(14), 2536–2540.
- Vidali, L., & Bezanilla, M. (2012). *Physcomitrella patens*: a model for tip cell growth and differentiation. *Current Opinion in Plant Biology*, *15*(6), 625–631.
- Vidali, L., Burkart, G. M., Augustine, R. C., Kerdavid, E., Tüzel, E., & Bezanilla, M. (2010). Myosin XI is essential for tip growth in *Physcomitrella patens*. *The Plant Cell*, *22*(6), 1868–1882.
- Voigt, B., Timmers, A. C. J., Samaj, J., Müller, 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.
- Volkman, N., Amann, K. J., Stoilova-McPhie, S., Egile, C., Winter, D. C., Hazelwood, L., Heuser, J. E., Li, R., Pollard, T. D., & Hanein, D. (2001). Structure of Arp2/3 complex in its activated state and in actin filament branch junctions. *Science*, 293(5539), 2456–2459.
- Voloshina, M. (2023). *Interakce ARP2/3 s komponentami rostlinné sekretorické dráhy*. <https://dspace.cuni.cz/handle/20.500.11956/185366>
- Wang, J., Zhang, Y., Wu, J., Meng, L., & Ren, H. (2013). AtFH16, [corrected] an Arabidopsis type II formin, binds and bundles both microfilaments and microtubules, and preferentially binds to microtubules. *Journal of Integrative Plant Biology*, 55(11), 1002–1015.
- Wang, P., Duckney, P., Gao, E., Hussey, P. J., Kriechbaumer, V., Li, C., Zang, J., & Zhang, T. (2023). Keep in contact: multiple roles of endoplasmic reticulum-membrane contact sites and the organelle interaction network in plants. *The New Phytologist*, 238(2), 482–499.
- Wang, P., Gao, E., & Hussey, P. J. (2020). Autophagosome Biogenesis in Plants: An Actin Cytoskeleton Perspective. *Trends in Plant Science*, 25(9), 850–858.
- Wang, P., & Hussey, P. J. (2015). Interactions between plant endomembrane systems and the actin cytoskeleton. *Frontiers in Plant Science*, 6, 422.
- Wang, P., Richardson, C., Hawes, C., & Hussey, P. J. (2016). Arabidopsis NAP1 Regulates the Formation of Autophagosomes. *Current Biology: CB*, 26(15), 2060–2069.
- Wang, X., & Mao, T. (2019). Understanding the functions and mechanisms of plant cytoskeleton in response to environmental signals. *Current Opinion in Plant Biology*, 52, 86–96.
- Wasserman, S. (1998). FH proteins as cytoskeletal organizers. *Trends in Cell Biology*, 8(3), 111–115.
- Wolf, S., & Greiner, S. (2012). Growth control by cell wall pectins. *Protoplasma*, 249 Suppl 2, S169–S175.
- Xu, L., Cao, L., Li, J., & Staiger, C. J. (2023). Cooperative actin filament nucleation by the Arp2/3 complex and formins maintains the homeostatic cortical array in Arabidopsis epidermal cells. *The Plant Cell*. <https://doi.org/10.1093/plcell/koad301>
- Xu, Y., & Huang, S. (2020). Control of the Actin Cytoskeleton Within Apical and Subapical Regions of Pollen Tubes. *Frontiers in Cell and Developmental Biology*, 8, 614821.
- Yanagisawa, M., Desyatova, A. S., Belteton, S. A., Mallery, E. L., Turner, J. A., & Szymanski, D. B. (2015). Patterning mechanisms of cytoskeletal and cell wall systems during leaf trichome morphogenesis. *Nature Plants*, 1, 15014.

- Yanagisawa, M., Zhang, C., & Szymanski, D. B. (2013). ARP2/3-dependent growth in the plant kingdom: SCARs for life. *Frontiers in Plant Science*, *4*, 166.
- Yi, K., Guo, C., Chen, D., Zhao, B., Yang, B., & Ren, H. (2005). Cloning and functional characterization of a formin-like protein (AtFH8) from Arabidopsis. *Plant Physiology*, *138*(2), 1071–1082.
- Yokota, K., Fukai, E., Madsen, L. H., Jurkiewicz, A., Rueda, P., Radutoiu, S., Held, M., Hossain, M. S., Szczyglowski, K., Morieri, G., Oldroyd, G. E. D., Downie, J. A., Nielsen, M. W., Rusek, A. M., Sato, S., Tabata, S., James, E. K., Oyaizu, H., Sandal, N., & Stougaard, J. (2009). Rearrangement of actin cytoskeleton mediates invasion of *Lotus japonicus* roots by *Mesorhizobium loti*. *The Plant Cell*, *21*(1), 267–284.
- Yuan, G., Gao, H., & Yang, T. (2023). Exploring the Role of the Plant Actin Cytoskeleton: From Signaling to Cellular Functions. *International Journal of Molecular Sciences*, *24*(20). <https://doi.org/10.3390/ijms242015480>
- Yu, Y., Wu, S., Nowak, J., Wang, G., Han, L., Feng, Z., Mendrinna, A., Ma, Y., Wang, H., Zhang, X., Tian, J., Dong, L., Nikoloski, Z., Persson, S., & Kong, Z. (2019). Live-cell imaging of the cytoskeleton in elongating cotton fibres. *Nature Plants*, *5*(5), 498–504.
- Zhang, C., Mallery, E. L., Schlueter, J., Huang, S., Fan, Y., Brankle, S., Staiger, C. J., & Szymanski, D. B. (2008). Arabidopsis SCARs function interchangeably to meet actin-related protein 2/3 activation thresholds during morphogenesis. *The Plant Cell*, *20*(4), 995–1011.
- Zhang, C., Mallery, E. L., & Szymanski, D. B. (2013). ARP2/3 localization in Arabidopsis leaf pavement cells: a diversity of intracellular pools and cytoskeletal interactions. *Frontiers in Plant Science*, *4*, 238.
- Zhang, S., Liu, C., Wang, J., Ren, Z., Staiger, C. J., & Ren, H. (2016). A Processive Arabidopsis Formin Modulates Actin Filament Dynamics in Association with Profilin. *Molecular Plant*, *9*(6), 900–910.
- Zhang, X., Dyachok, J., Krishnakumar, S., Smith, L. G., & Oppenheimer, D. G. (2005). IRREGULAR TRICHOME BRANCH1 in Arabidopsis encodes a plant homolog of the actin-related protein2/3 complex activator Scar/WAVE that regulates actin and microtubule organization. *The Plant Cell*, *17*(8), 2314–2326.
- Zhang, Z., Zhang, Y., Tan, H., Wang, Y., Li, G., Liang, W., Yuan, Z., Hu, J., Ren, H., & Zhang, D. (2011). RICE MORPHOLOGY DETERMINANT encodes the type II formin FH5 and regulates rice morphogenesis. *The Plant Cell*, *23*(2), 681–700.
- Zhao, L., Rehmani, M. S., & Wang, H. (2020). Exocytosis and Endocytosis: Yin-Yang Crosstalk for Sculpting a Dynamic Growing Pollen Tube Tip. *Frontiers in Plant Science*, *11*, 572848.
- Zhao, Y., Zhao, S., Mao, T., Qu, X., Cao, W., Zhang, L., Zhang, W., He, L., Li, S., Ren, S., Zhao, J., Zhu, G.,

- Huang, S., Ye, K., Yuan, M., & Guo, Y. (2011). The plant-specific actin binding protein SCAB1 stabilizes actin filaments and regulates stomatal movement in Arabidopsis. *The Plant Cell*, 23(6), 2314–2330.
- Zicha, D., Allen, W. E., Brickell, P. M., Kinnon, C., Dunn, G. A., Jones, G. E., & Thrasher, A. J. (1998). Chemotaxis of macrophages is abolished in the Wiskott-Aldrich syndrome. *British Journal of Haematology*, 101(4), 659–665.