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Molekulární mechanismy zodpovědné za polaritu a morfogenezi mechové buňky *Physcomitrella patens*

Molecular mechanisms of cell polarity and morphogenesis in moss *Physcomitrella patens*

Bachelor thesis

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Prohlášení:

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V Praze, 14.05.2015

Podpis

Abstract

Plant cells are able to establish polarity and expand by tip growth. Polarized cells often embrace functions important for plant viability. The process of tip growth requires actin cytoskeleton in collaboration with a number of accessory proteins. The position of the intensively expanding region is provided by microtubules and the function of signalling proteins. Polarized secretion regulates the structural properties and subsequently the shape of the cell wall. Some components of the secretory and signalling pathways are highly conserved among eukaryotes, others are found exclusively in the plant kingdom. Though much has been discovered in yeast and animal cells, many mechanisms in plants are yet to be revealed. Model systems performing tip growth, such as root hairs, pollen tubes and protonema cells, enable comparison and thus a complementary overview of the various processes.

Key words:

Physcomitrella patens, protonema, pollen tube, root hair, tip growth, actin, microtubules

Abstrakt

Rostlinné buňky jsou schopné vykazovat polaritu v procesu zvaném vrcholový růst. Polarizované buňky v mnoha případech zastupují funkce důležité pro životaschopnost rostliny. Vrcholový růst vyžaduje funkci aktinového cytoskeletu ve spolupráci s řadou dalších proteinů. Oblast, na které probíhá intenzivní expanse, je dána pozicí mikrotubulů a funkcí signálních proteinů. Polarizovaná sekrece reguluje strukturní vlastnosti a následně i tvar buněčné stěny. Některé podjednotky sekretorických a signalizačních drah jsou společné pro většinu eukaryot, jiné se vyskytují pouze v rostlinné říši. Třebaže u živočichů a kvasinek již mnohé bylo zjištěno, většina mechanismů v rostlinách zůstala neodhalena. Modelové systémy vykazující polarizovaný růst, mezi něž patří kořenový vlásek, pylová láčka nebo protonema, umožňují srovnání a tím i komplementární přehled různých procesů.

Klíčová slova: Physcomitrella patens, protonema, pylová láčka, kořenový vlásek, actin, mikrotubuly

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Content

1. Introduction

Polarized growth is common to both animal and plant cells. Surprisingly, molecular mechanisms are based on similar principals. Growth is a dynamic process and thus it is most important to understand the extreme modularity of structures, such as cytoskeleton or the cell wall. It has been confirmed that polarized growth requires elaborate signalling and thus polarization confirms the complexity of living systems (Cove, 2000). In plants, tip growth began to evolve most strikingly in response to environmental changes related to water-land transfer. Polarized cells often form organized filamentous structures and play essential roles such as nutrient uptake, water absorption and transport, anchoring to substrate and reproduction. Mosses stand on the crossroad between aquatic algae and vascular plants. *Physcomitrella patens* serves as a genetic model and subsequently also presents tip growth.

Purpose of this thesis is to summarize the late acknowledgements in plant cell biology characterizing tip growth. Polarized growth in root hairs and pollen tubes will be repeatedly compared with protonema cells. First part of the thesis will focus on general architecture of tip-growing systems with emphasis on actin and actin associated proteins. Second part will describe the cell wall composition via secretory pathway. The last chapter will deal with the problem of plant signalling. The highlighted issues are being currently studied at the department of experimental biology of plants at Charles University.

1.1. Introducing *Physcomitrella*

Physcomitrella patens is a moss with a natural habitat on the muddy banks of temperate parts of the world. It was first introduced as a model genetic system in 1968 (Engel, 1968) in a seminar paper as an auxotrophic mutant. Physcomitrella, for it's unique properties featured a promising model system. Raising interest urged for detailed characterization. Now Physcomitrella is used in comparative studies for determining gene function in evolutionary context and biological processes in land plants.

1.1.1. Life cycle

Mosses (Bryophytaea) show generation alternation but unlike seed plants haploid phase is the dominant stage. The haploid spore germinates into filamentous structures called protonema, which then develop into a simple branched plant body. In suitable conditions, protonema give rise to a gametophore. This organ resembles the leafy shoots of higher plants. The phylloids are even covered with cuticle, allowing it to be more drought resistant (Buda et al., 2013). Rhizoids, potentially also showing tip growth, merge from the base of an adult gametophore (Sakakibara et al., 2003). The sexual organs mature on the top of the gametophyte within the closed phylloid. The male antheridia are flagellate, motile and swim towards the archegonia. Thus the eggs can only be fertilized in moist environment. It can be either self-fertilized or cross fertilized by a different archegonium. The previous is more frequent. The diploid sporophyte is fully dependent on the gametophore. One sporophyte generates between 1000 and 5000 haploid spores by meiosis. A matured sporophyte is spherical and clearly visible. When a spore germinates, it develops into

a new protonemata, initially consists only chloronema. Depending on the environment, the protonema may remain chloronemal or differentiate into caulonema (Menand et al., 2007).

1.1.2. *Physcomitrella* as a model organism

Physcomitrella abounds many benefits. In case of contamination a precious mutant can be rescued. This can be done by isolation of spores and restoring a new generation (Cuming, 2011). The simple morphology of the tissue enables easy protoplast isolation and direct reproduction. A whole new colony can be generated from a single cell, thus all cells of the gametophyte are totipotent. While the protoplast regenerates, it undergoes a series of developmental changes identical with those in a growing spore.
One fact that makes *Physcomitrella* so exceptional for genetic studies, is that the dominant phase is a haploid gametophyte. This helps to identify the mutant lines and enables a simple reproduction without the need of F2 crossing. In addition, a single allele mutation enables the phenotype being directly evident. Mosses have the capability of site-specific recombination, which is frequently employed for precise modification or gene inactivation. Another method of determining gene function is by RNAi. It is possible to silence gene expression or entire gene families or knocking down genes by the approach of RNAi . Methods of reverse genetics can be generally used for *Physcomitrella patens* genome, now it is fully sequenced. Over 100,000 express sequence tags have been identified.
On the other hand, *Physcomitrella* grows slowly, compared to pollen tubes and root hairs. Also the life cycle is long. Visualizing cells might often be complicated for they contain many chloroplasts.

1.1.3. RNAi – method of reverse genetics

Recent advances in epigenetic gene silencing enabled reverse-genetics tool called RNA interference (RNAi). The process is induced by introducing double-stranded RNA (dsRNA) into a cell, which subsequently results in degradation of a specific mRNA sequence. This enables efficient silencing of target genes and thus their function. In vivo degradation mechanism is versatile and evolutionarily conserved across the plant and animal kingdoms (Hannon, 2002).
In moss, RNAi has been employed in many cases, for example silencing of profilin genes. Three profilin genes have been identified in *Physcomitrella* genome. PRFa sequence has been amplified in both directions and created a hairpin structure (CDS). In order to silence all three genes, the construct also contained a 3'UTR sequence highly conserved in all these genes. Using a vector, PRFa CDS-RNAi fragment has been transferred into the cell. It is also possible to create a construct containing other sequences common for all target genes (Vidali et al., 2007).

1.1.4. Gene annotations

http://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Ppatens

1.2. Evolutionary context

Physcosmitrella compared with phylogeny of aquatic algae and terrestrial plants, enables reconstruction of evolutionary changes between the genomes. Step by step, features associated with the conquest of land are being revealed. Comparative studies enable tracing down trends associated with water-land transfer. Firstly, early land plants gradually lost genes essential for aquatic life, such as flagellar gamete. The last common ancestor of land plants also gained more complex photoreception, signalling pathways and hormones such as auxin, ABA, cytokinin, became tolerant to drought, high temperature and radiation. Most importantly, more elaborate transport has been developed and finally the specific reproduction cycle. Some of these innovations may have been created by one or more duplications of the whole genome. Therefore the morphological and physiological adaptations are accompanied by the overall increase in gene family complexity. Comparative studies also enable reconstruction of the evolutionary events that occurred after the split of vascular plants and mosses, which is dated to 450 Mil years ago (Rensing et al., 2008). Higher plants developed advanced signalling pathways, namely through ethylene, gibberellic acid, brassinosteroids (GA), jasmonic acid (JA). Some archaic species developed the ability of vegetative dehydration tolerance. Mosses also preserved dominant haploid phase and motile male gametes. The fact that mosses are dependent on water for sexual reproduction contradicts the classification as a terrestrial plant. However, *Physcomitrella* genome provides a genetic resource for the study of evolutionary reconstruction and gene function of early land plants (Rensing et al., 2008).

2. Architecture and physiology of tip-growing cell

2.1. Root hairs and pollen tubes

Root hairs and pollen tubes serve as a model of tip-growing cells in higher plants. The general architecture illustrates characteristic distribution of organelles within the cell (Fig. 1). At the very apex, endoplasmic reticulum is abundant along with accumulation of secretory vesicles. Lower, 25–30 μm away from the tip, is a region containing Golgi dictyosomes. Mitochondria are in most cases equally distributed. The apical region is called the clear zone for it appears organelle-free. The localization of coated pits 6–15 μm behind the tip defines the site of endocytosis. The zonation of endocytic activity possibly contributes to different composition of proteins in the plasmatic membrane (Derksen et al., 1995). Further back reside the vacuoles and plastids. Though this description applies for most tip-growing cells, layout of the organelles may vary among species (Rounds and Bezanilla, 2013). The pollen tube has been investigated mostly in *Arabidopsis* and tobacco. Also lilly pollen has been an attractive model for it grows at terrific speed (Lovy-Wheeler et al., 2007).

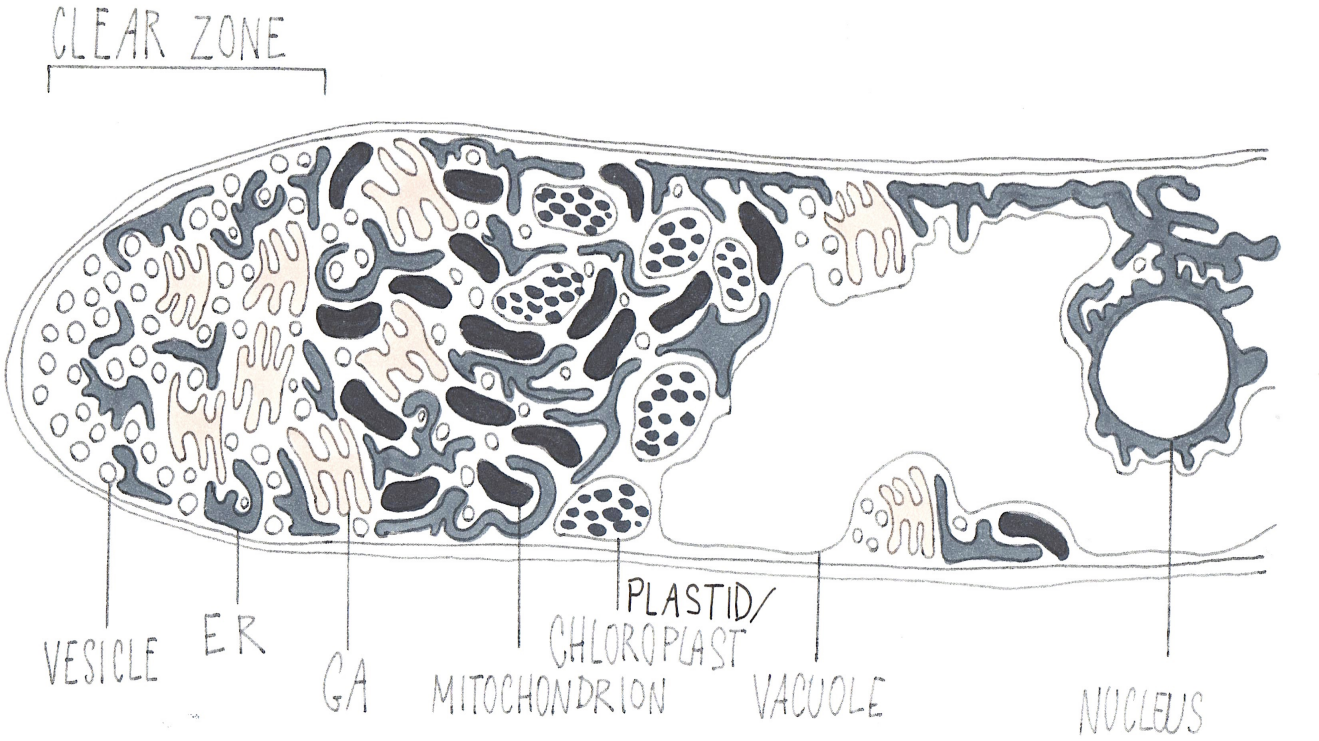

Fig. 1. Schematic picture of a tip-growing cell according to Rounds and Bezanilla (Rounds and Bezanilla, 2013).

2.1.1. Cytoplasmic streaming

It is most important to comprehend the dynamics of this model. Tip-growing cells perform cytoplasmic streaming. This phenomenon gains different appearance. In angiosperms, cells perform the reverse fountain pattern, meaning particles travel along the cortex toward the apex and turn inward just before reaching the clear zone (Fig. 2). The role of streaming is most likely to transport organelles and recycling excess membrane accumulated due to high frequency vesicle deposition. Inhibition of streaming results in reduced growth rates but does not abolish growth entirely (Tominaga et al., 2000), concluding that tip growth is enhanced by cytoplasmic streaming but does not depend on it. In gymnosperms the streaming occurs in an opposite direction. More interestingly, angiosperm streaming is actin-dependent (Cárdenas, 2009) whereas in gymnosperms it seems to be driven by both microfilaments and microtubules (Justus et al., 2004).

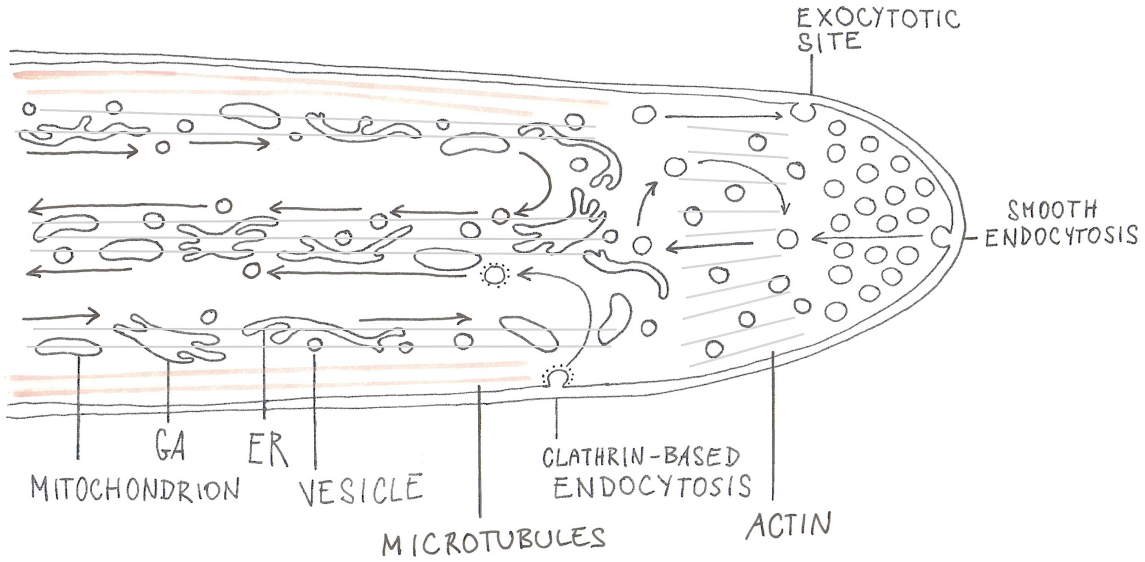

Fig. 2. Schematic picture of cytoplasmic streaming in a pollen tube according to Cai and Cresti, 2010 (Cai and Cresti, 2010).

2.2. Protonema

Protonema perform growth by two main mechanisms: elongation and unequal division. The apical cells exhibit tip growth observed in both chloronema and caulonema cells, contributing to filament elongation (Menand et al., 2007). The side branches originate from sub-apical cells (Fig. 3).

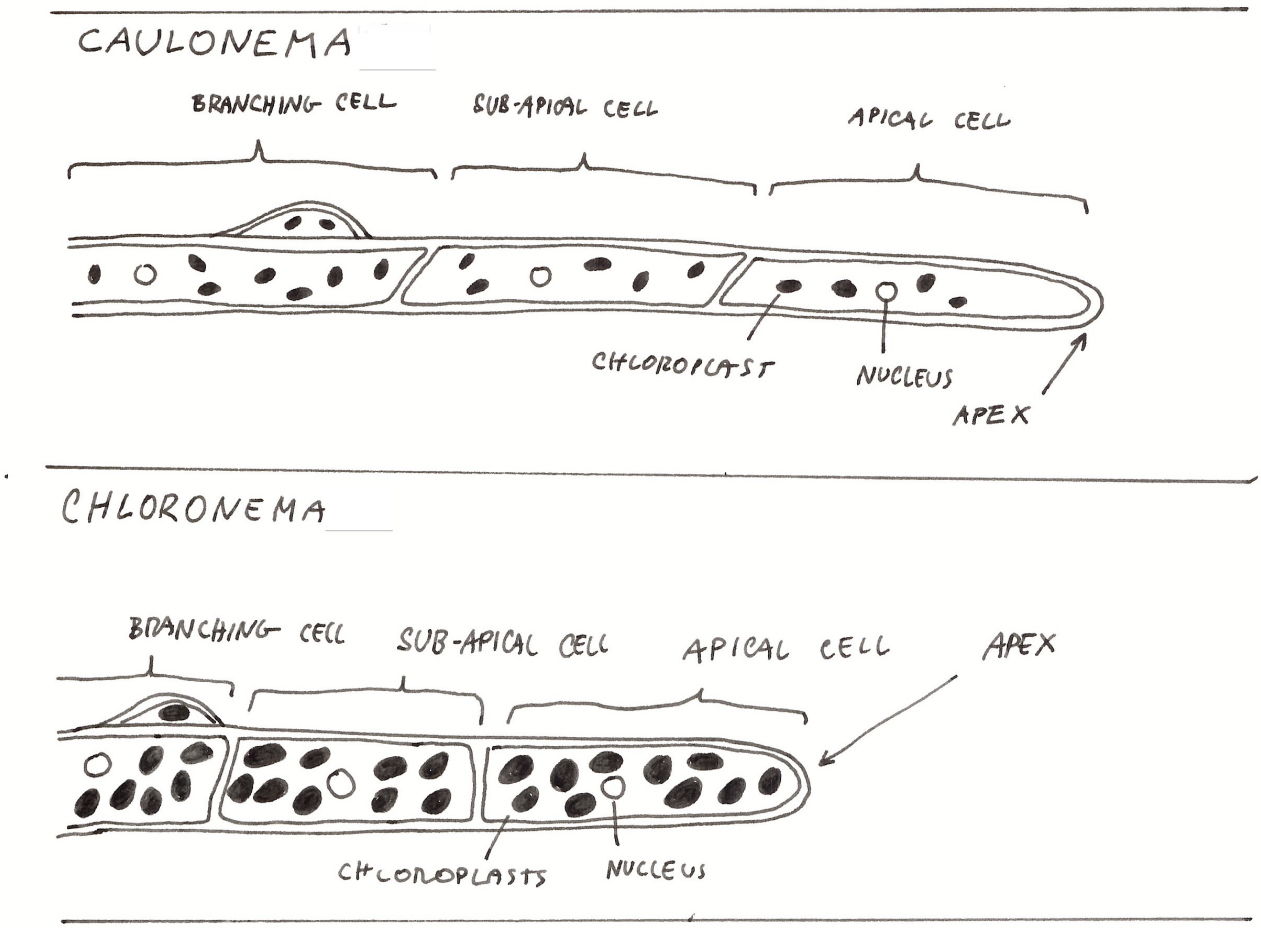

Fig. 3. Comparing the length of chloronema and caulonema cells according to Perroud and Quatrano

- Caulonema
  - sub-apical cell: 180-200 μm
  - tip cell: 400 μm
  - When division takes place the cell wall is formed oblique to the long axis.
- Chloronema
  - sub-apical cell: 75-80 μm
  - tip: 160 μm
  - the partition cell wall is perpendicular to the axis of elongation

(Perroud and Quatrano, 2006a)

2.2.1. Protonema – cell types

Chloronemata exhibit growth at 5,8 ± 0,51 μm h^{-1}(Menand et al., 2007). They can be characterized as photosynthesising cells, full of big chloroplasts and containing one central vacuole. Tip-growing chloronemata show an equal distribution of organelles. The lack of a specific tip-cell organisation along with chloroplast division correlates with the slow growth of chloronema.

Whereas caulonemata, fast growing cells, grow 19.87± 0,51 μm h^{-1} (Menand et al., 2007). These cells

accommodate less chloroplasts. Interestingly, whereas chloronema only grow on light, caulonema expand in light and dark conditions, suggesting their role as colonizers (Cove et al., 2006). Their function includes invasion into new neighbourhoods and nutrient attainment. Caulonema cell type resembles other tip-growing cells such as root hair and pollen tube. Therefore caulonemata are preferable for comparative studies.

2.2.2. Cytoplasmic organization - Protonema

Like higher plants, mosses also exhibit cytoplasmic organisation characteristic for tip-growing cells. These features have been observed in moss *Physcomitrella,* particularly in caulonema cells (Fig. 4). They perform clear zone and vesicle accumulation at the tip. Also axially oriented actin bundles, both longitudinally and crosswise oriented microtubules have been reported (Doonan et al., 1987). Thus caulonema cells serve as a model for studying tip growth. The cytological organization accelerates growth. Chloronema, which perform no such organisation, grow slower, compared to caulonemata (Rounds and Bezanilla, 2013).

The very apex of a caulonema cell contains small electron dense vesicles and smooth endoplasmic reticulum. A layer farther from the apex is an area rich in Golgi bodies and devoid of large organelles such as vacuoles, peroxisomes and chloroplasts. Mitochondria are evenly distributed. The clear zones in caulonema tip-cells are large, presenting an elongation area 30-60 μm long. The next layer alternates in length, contains plastids or amyloplasts, mitochondria, Gologi stacks, ER (both rough and smooth) vacuoles and the nucleus (Tucker et al., 2005).

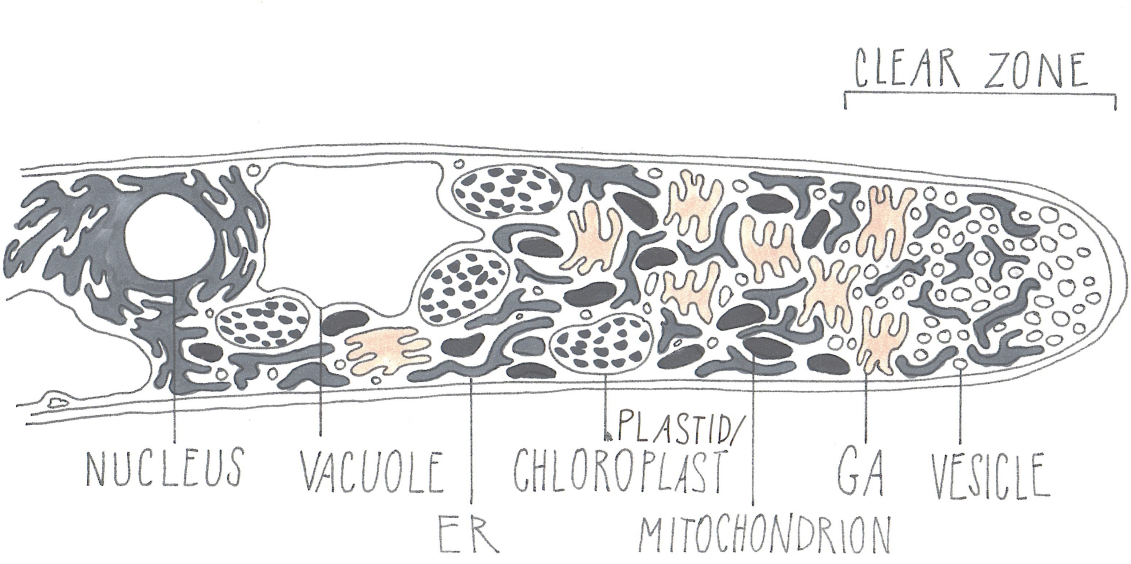

Fig. 4. Schematic picture of caulonema cell according to Tucker et al (Tucker et al., 2005)

2.3. Cytoskeleton

In animal cells actin filaments control cell shape and power cell crawling, whereas microtubules were commonly accepted as tracks for long-distance transport (Pollard and Cooper, 2009). Many molecular mechanisms responsible for the cytoskeletal dynamics have been conserved in most eukaryotes but some alter rapidly in function.

Early pharmacology experiments with plant cytoskeleton using drugs such as Cremart, cytochalasin D, oryzalin, taxol and colchicine allowed first insights into the functions of actin filaments and microtubules. The microtubule disruption leads to frequent side branching but does not abolish growth. In contrast, actin-depolymerizing drugs or latrunculin B, end growth entirely. This indicates two essential roles, microtubules direct site of growth while actin network appears to be fundamental for the growth itself (Doonan et al., 1988)

2.3.1. Microtubules

Microtubules (MTs) are hollow, tubular structures composed of α and β–tubulin dimers. The dimers polymerize into a protofilament and the resulting microtubule is composed of 13 protofilaments. MTs contribute to polarized exocytosis of vesicles, transporting cell wall complexes and ion channels necessary for cellular growth (Doonan et al., 1988). However, the function in higher plants seems to be also organ specific. MT disruption in angiosperm pollen tubes has minor effect on growth (Cai and Cresti, 2010) but has been reported to influence exocytosis and endocytosis. Root hairs showed wavy growth and branched tips, suggesting that MTs direct the site of growth (Bibikova et al., 1999). To compare the effect with other species, MT-depolymerizing drugs have been applied to gymnosperm pollen tubes, which have inhibited cytoplasmic streaming and thus their growth (Justus et al., 2004). Protonema produced curved swollen cells and multiple tips (Doonan et al., 1988).

2.3.1.1. Microtubule structures – pollen tubes and root hairs

Microtubules spread along the plasma membrane and form a cortical array during interphase (Wasteneys, 2002). Remodelling of this array does not occur by moving individual MTs but rather by assembly and disassembly of the existing structures (Lindeboom et al., 2013). Modulation is provided by a wide spectrum of microtubule associated proteins (MAPs) (Gardiner, 2013), kinesins and katanins (Lindeboom et al., 2013). Without centrosomes, plant microtubule arrays are largely self-organized by relative activities of these accessory proteins (Wasteneys, 2002).

In *Arabidopsis* pollen tubes, MTs run along the cortex in the shank and switch to shorter cables that form a mesh in the core of the sub-apical region (Fig. 5). This apical mesh has been visualized by GFP-At-EB1, the plus end binding protein, and described as a basket-structure. It resides about 50–60 μm from the tube apex and consists of axially oriented MTs. Time-lapsee visualization revealed a specific oscillation of this structure. The sub-apical MTs elongate periodically and enter the apical dome. The cortical MTs

also undergo periodical elongation and shrinking, but seem to be stably allocated at the cortex (Cheung et al., 2008). In root hairs, both longitudinal and helical cortical MTs were observed. During root hair formation, an epidermal cell undergoes transition from diffuse growth to highly polarized growth at one specific site, by polarized exocytosis and deposition of cell wall material to the tip. In *Arabidopsis* it appears only MTs parallel to the axis contribute to specific tip growth (Van Bruaene, 2004). The apical structure has not been clearly described (Sieberer et al., 2005).

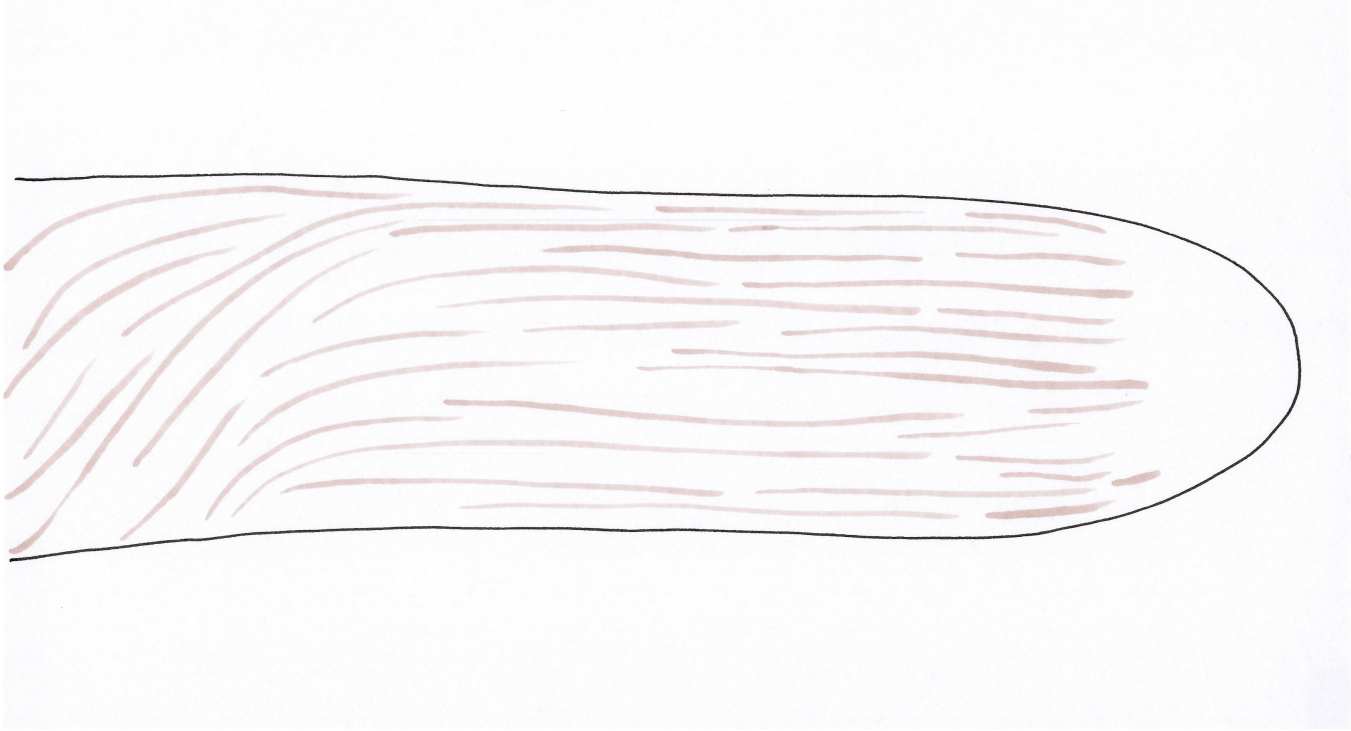

Fig. 5. Schematic picture of microtubule system in a tip-growing tobacco pollen tube inspired by Cheung (Cheung et al., 2008)

2.3.1.2. Microtubule structures - protonema

The interphase protonema cells show both cortical and cytoplasmic MTs (Doonan et al., 1987). They are predominantly longitudinal to the axis of growth but converge at the tip (Doonan et al., 1985). The cortical MTs impose the tubular shape of the protonema (Doonan et al., 1988). Non-cortical MTs pass longitudinally through the core cytoplasm. It has been recently observed that these endoplasmic MTs converge at the tip region and form a bundle (Fig. 6). Visualizing the plus ends by using fluorescently labelled EB1 showed that the bundles are composed of plus ends. Time-lapsee observation revealed that plus ends are distributed evenly within the cell, polymerize mainly in the direction of growth and move towards the apex. The bundle repeatedly forms and collapses, suggesting that generations of MT bundles oscillate dynamically and correlate with the frequency of growth. The conduction towards the focal point has been attributed to specific kinesins KINID1a and KINID1b via attachment with EB proteins (Hiwatashi et al., 2014). Endoplasmic MTs have been studied in order to elucidate the mechanism of MT generation beyond those observed in cortical MT arrays. It is possible that new MTs emerge from the

cytoplasm. A regrowth assay has shown that ϒ-tubulin is the main nucleator but generates MTs in random directions. Branching nucleation has also been observed, interestingly, showing atypical branch angles. The polarization of the network is probably ensured through cross-linking by various MAPs and MT transport mechanisms (Nakaoka et al., 2015).

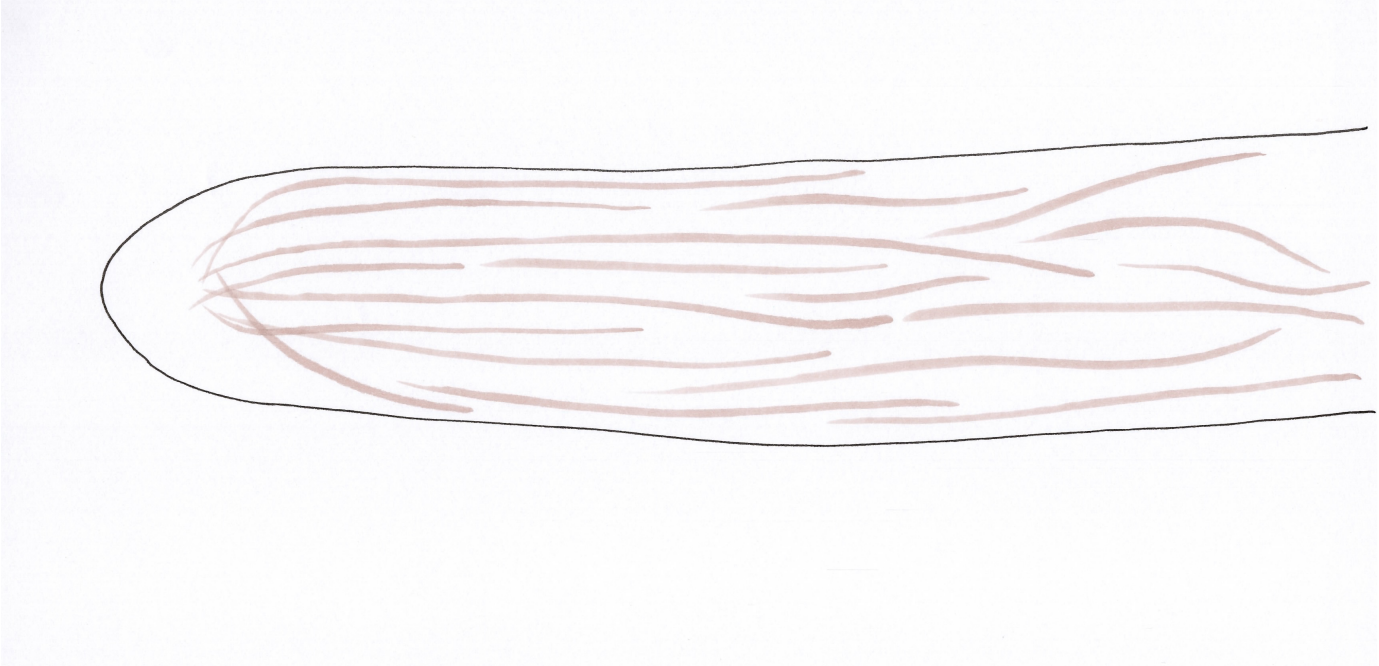

Fig. 6. Schematic picture of microtubule system in a tip-growing caulonema cell inspired by Nakaoka (Nakaoka et al., 2015)

2.3.2. Actin

Actin consists of individual monomers, the globular protein G-actin. When monomers polymerize in a linear manner, forming a filament (F-actin). The process is run by ATP, which binds to the monomers. A model has been proposed for actin stochastic dynamics, grasping the behaviour of actin filament network. The model is based on cellular concentration of actin, biochemical activities investigated by in vitro systems and by reconstructing the mechanism of actin turnover. In a dynamic steady-state regime, defined by the optimal concentration of actin-ATP and actin-ADP, subunits polymerize at the plus end and equally depolymerize at the minus end. This process is called 'tread milling'(Pollard and Borisy, 2003). Most importantly, the model comprises activity of actin binding proteins. The latest studies point out the importance of actin severing and barbed end availability (Henty-Ridilla et al., 2013).

In plants, actin filaments provide tracks on which organelles are transported through the cell, thus operating the structural composition of the cytoplasm. Actin dynamics is also responsible for cytoplasmic streaming, long-distance transport and secretion within the plant cell. In animal cell, this role has been attributed to MTs. However, some studies bring out the role of actin in the vesicle and organelle transport in animal cells. The new model suggests that actin with nucleators and formins develop a network, which

surrounds the compartment. More importantly, the network attaches the vesicle to the plasma membrane and enables it's movement via myosins (Schuh, 2011).

2.3.2.1. Actin structures – pollen tubes and root hairs

Actin filaments are oriented longitudinally along the cortex, as well as the core cytoplasm, which is interwoven mostly by long actin bundles (Derksen et al., 1995). Existence of these bundles correlates with cytoplasmic streaming (Fig. 7.) (Wu et al., 2010). Filaments within the clear zone are much shorter and more dynamic (Cheung and Wu, 2008). It seems apical structures vary among different species. In gymnosperms, the actin bundles are longitudinally oriented at the shank and switch to radial orientation and much fewer filaments at the apex (Anderhag et al., 2000). Lilly pollen tubes form a cortical fringe at the apical region, showing high dynamics and rapid tubulin turnover (Lovy-Wheeler et al., 2005).

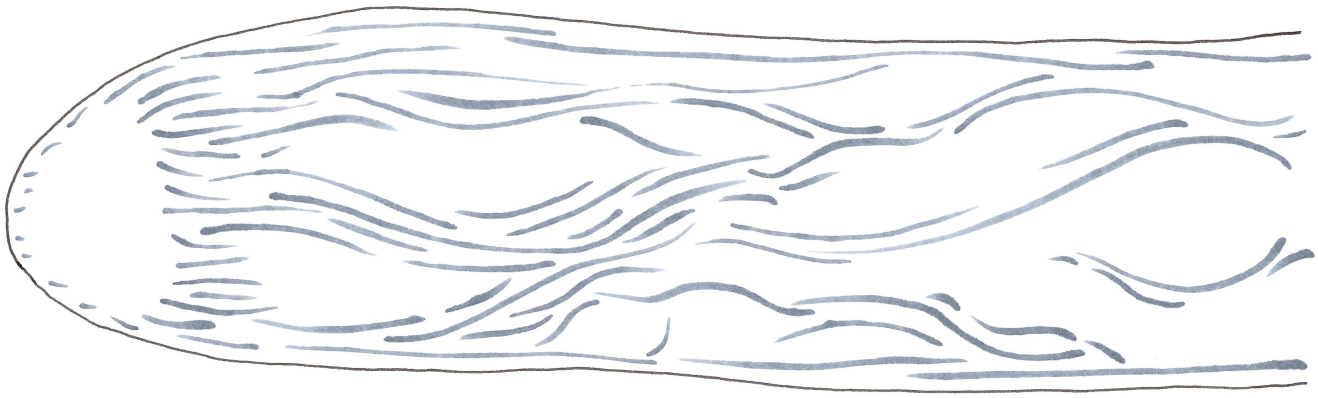

Fig. 7. Schematic picture of actin filaments system in a tip-growing pollen tube inspired by Qu et al. (Qu et al., 2015)

2.3.2.2. Actin structures - protonema

In both protonema cell types the filaments are oriented axially along the cortex. In contrast to pollen tubes and root hairs, moss cells lack notable actin longitudinal filaments, which would promote cytoplasmic streaming. This indicates that cytoplasmic streaming might not be essential for the delivery of building material to the tip but it certainly accelerates the growth in angiosperm tip-growing cells. (Rounds and Bezanilla, 2013)

In caulonema cells the filaments form a focal point at the very apex (Fig. 8). The time-lapse method revealed that filaments emanate from the focal point and reverse back along the cortex. In contrast, chloronema cells do not display such sub-apical organisation. (Vidali et al. 2009).

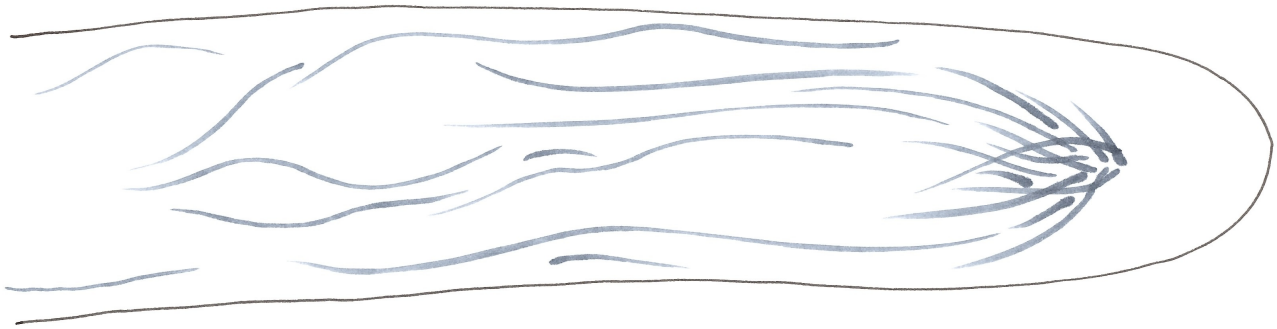

Fig. 8. Schematic picture of actin filaments system in a tip-growing caulonema cell inspired by Vidali (Vidali et al. 2009)

2.3.3. Actin associated proteins

Actin binding proteins are the main factors causing actin filament remodelling. They are responsible for actin dynamic by causing polymerization, depolymerisation, nucleation, severing or translocation of filaments, thus promoting the process of F-actin turnover. Other essential factors are involved in intracellular signalling. Interacting proteins modulate actin network to a wide spectrum of features.

2.3.3.1. Formins - Actin polymerization

Formins are actin binding proteins defined by the presence of conserved formin homology domain FH2 (Higgs, 2005), which promotes actin filament nucleation (Sagot et al., 2002). After nucleating a filament, the FH2 domain remains at the fast-growing filament end, moving processively adding monomers - functioning as a "leaky cap" (Goode and Eck, 2007). The FH1 domain contains a polyproline rich sequence, known to interact with profilin (Chang, 1997).

In plants, formins promote elongation and nucleation of actin. Nucleation occurs at the cell cortex and at the surface of existing filaments. Interestingly, these newly emerged filaments are parallel to the mother filaments and can form bundles. The proper membrane targeting of the FH2 is essential for promoting polarized actin elongation or nucleation. (van Gisbergen et al., 2012)

Three formin families have been identified in plants. Class I formins combine a trans-membrane domain with the FH2 domain. Class II is characteristic for FH2-PTEN architecture. Class III formins contain a RhoGTPase-like domain, which is able to bind to the membrane-anchored GTPase but not activate it. In higher plants the class III formins have not been reported, suggesting that formins class I and class II have substituted the role of a membrane anchor by alternative mechanisms. Bryophyte formins class III contain a Rho GTPase-like domain, similar to opisthokont GBD-FH3 but without the GTPase activating ability.

2.3.3.1.1. Formins - Protonema

In the moss *Physcomitrella*, all three classes have been reported. The function of different formins has been investigated by RNA interference. Loss of all formin genes was lethal for the cells.
Class I formins are encoded by six genes in *Physcomitrella*. RNAi mutants produced small cells but the polarized growth has not been affected. It is the most likely that formins class I participate in cytokinesis, whereas formin class II turned out to be essential for polarized tip growth. The *for2* mutant cells were rounded and lacked any polarized extension. Although actin did accumulate at one end of the cell, the longitudinal actin filaments were entirely disrupted. There are two formin class II genes, both coding product with a (PTEN)-like domain, which serves as a localization signal. Next to it reside the conserved FH1-FH2 domains, which are both necessary for actin polymerization. The experiments showed that replacement of the (PTEN)-like domain with For1 FH1-FH2 domain fails in polarized targeting. Only the combination of (PTEN)-like and For2 FH1-FH2 domain can rescue the phenotype (Vidali et al., 2009). Furthermore, the class II formins PTEN-like domain binds phosphoinositides. This turned out to be necessary for PTEN-like domain activity as a localization signal. Experiments show that the inhibition of PI(3,5)P2 reduced the cortical targeting. There is very little known about the plant PI(3,5)P2. The human PTEN domain is known of binding PI(3,4,5)P3 and converting it to PI(4,5)P2, whereas moss PTEN-like domain binds specifically to PI(3,5)P2. It is generated by FAB1 kinase, which resides at the endomembrane systems but PI(3,5)P2 itself has not yet been found within the membrane. Visualized PI(3,5)P2 binders co-localized with actin dependent trajectories, although direct bond to actin has not been reported. To conclude, the role of PI(3,5)P2 as a signal molecule for binding PTEN-like domain is necessary for polarization of the actin cytoskeleton (van Gisbergen et al., 2012).

2.3.3.2. Actin related protein

Arp2/3 complex is believed to induce the branching of actin filaments in many eukaryotes. One model proposes that Arp2/3 complex initiates nucleation of a daughter filament on the surface of a mother filament at 70° angle. Nucleation by Arp2/3 complex is activated by the Wiskott-Aldrich syndrome protein (WASP) or Scar protein; capping protein and profilin (Blanchoin et al., 2000). The Arp2 and Arp3 subunits can form a surface that mimics a stable actin dimer and promotes filament nucleation (Rodal et al., 2005).

There has been evidence of high functional conservation of the Arp 2/3 complex in many eukaryotes and yet the same function of Arp2/3 complex has not been reported in plants (Yanagisawa et al., 2013). Despite that homologs of all complex members have been identified, no functional Arp2/3 complex has been found in plants. Additionally, functional *Arabidopsis* homologs of Scar/WAVE family Arp2/3 complex activators have been identified that are capable of activating bovine Arp2/3 complex in vitro (Frank et al., 2004) (Basu, 2005). Knocking out the subunits gave varied phenotypes as it is known in other organisms and yet, loss of Arp2/3 complex has a minor effect on overall outgrowth and development of the plant. Perhaps the function of Arp2/3 complex in plants has been taken over by other actin nucleators, such as the formins (Harries, 2005).

The Arp2/3 complex is composed of 7 subunits, which most probably have specific roles. In *Arabidopsis*, the mutation of *ARP3* affects the actin organization especially in cells rapidly expanding by diffuse growth (Mathur, 2003). Root hairs showed wavy growth and reduced length, occasionally multiple tips. It has been suggested that the ARP2/3 plays a minor role in the polarized growth (Rounds and Bezanilla, 2013).

2.3.3.2.1. Actin related protein - Protonema

In *Physcomitrella,* the genes coding ARP2/3 are similar to *Arabidopsis* genes. However, the mutant phenotypes alter in most cases, suggesting the functions vary depending on species.

The loss-of function of *ARP3A* gene obtained by homologous recombination affected the tip growth. Most interestingly, *Pparp3a* mutants developed chloronemal cells but failed to differentiate into caulonema or rhizoids. Chloronema managed to develop short leafy gametophores. F-actin in the mutants visualized by GFP-talin revealed the loss of specific cortical structures. The phenotype has been fully complemented by *Arabidopsis* orthologs, indicating a high degree of evolutionary conservation (Finka et al., 2008).

Loss-of function of *ARPC1* has been obtained by RNA interference. The *Pparpc1* mutants not only lacked caulonemal cell but also failed to form buds and leafy gametophores. Protoplasts were unable to properly establish a polarized outgrowth during regeneration from a single cell. This failure is rescued by *ARPC1* overexpression. A comparison of the intron/exon patterning between *Arabidopsi*s and *Physcomitrella* reveals a conservation of the intron/exon junctions as well as the number of exons (Harries, 2005). Despite this, the impact of *ARPC1* mutation affects *Physcomitrella* phenotype rapidly, in contrast to no visible alteration in *Arabidopsis* mutants (Li, 2003).

Null mutants with deleted *ARPC4* gene displayed reduction of filamentous tip growth, resulting in smaller, denser colonies. It has been discovered that mutant filaments were defective in their response to polarized white light. These observations strongly suggest a specific role of the Arp2/3 complex as a downstream target for signals regulating oriented tip growth (Perroud and Quatrano, 2006b).

Although in higher plants the role Arp2/3 complex has been lost, the experiments show that in moss the

function must have remained. Some studies stress the importance of ARPC4 as the major nucleator (Domozych et al., 2013).

2.3.3.3. Cofilin - depolymerisation

Cofilin is known as actin depolymerizing factor (ADF). Biochemical studies have shown the ability of ADF/Cofilin to bind ADP-actin subunits at the minus end of the filament (Blanchoin and Pollard, 1999). This generates a helical twist and cleft of the filament (McGough, 1997).

In moss, silencing the gene by RNAi causes cytoskeletal aberrations and results in dwarfed unpolarised cells. The phosphorylation of the N-terminus turned out to be essential for cofilin function. This has been shown on *ADF* RNAi mutants with complementation by non-phosphorylatable ADF.

Unlike in *Arabidopsis thaliana*, which has nine members of the ADF family (Dong et al., 2001), there is a single intron-less gene coding the ADF/cofilin in *Physcomitrella.* Moss ADF shares 50–70% identity and 70–80% similarity with other plant ADFs at the amino acid level. Sequence alignment with other ADF/cofilins revealed the high level of conservation of the tertiary structure, thus it is the most probable that the function of ADF/cofilin is conserved in mosses and higher plants (Augustine et al., 2008)

2.3.3.3. AIP1

Actin interacting protein cooperates with ADF and other proteins, causes actin filament remodelling. Mutations in *Arabidopsis* AIP1-1 and AIP1-2 causes higher rate of actin bundling at the apex, resulting in shorter root hairs compare to wild type (Augustine et al., 2011). The mechanism has not yet been described but some studies indicate that AIP1 cooperates closely with ADF and thus contributes to actin dynamics.

There has been found a single gene coding AIP1 in *Physcomitrella.* The exon size is similar to the exons of *AIP1-1* and *AIP1-2* from *Arabidopsis*. Furthermore, the similar exon/intron junctions indicate that the gene structure has been conserved. The moss AIP1 protein sequence shares 58% identity and 78% similarity with the two AIP1 proteins in *Arabidopsis* (Augustine et al., 2011).

In moss, knocking-out the gene by homologous recombination results in defective tip growth. Colony cultivated from a mutant protoplast is smaller than wild type, dense and composed of short filaments. Eventually leaf-like gametophores emerge performing diffuse growth of phylloids. The gametophores show no defects. However rhizoids that emerge from the base are severely stunted. By growing the protonemata in the dark, researchers have achieved the differentiation into caulonema cells. The mutant caulonemata were defective in growth. AIP1 thus probably contributes to tip growth but not to diffuse growth (Augustine et al., 2011).

2.3.3.5. Profilin - polymerization

Profilin contributes to actin polymerization by binding to actin monomers. It can also bind to proteins on their poly-L-proline sequences. Cooperation with these proteins enables complex actin organization. For example, profilin-formin complex induces addition of actin monomers. (Kovar, 2006).

2.3.3.5.1. Profilin - Protonema

There are three genes coding profilin in *Physcomitrella*. Comparing the structure to *Arabidopsis* genes confirmed a high degree of conservation. The three profilin isoforms in moss form a monophyletic group, which is basal to those in higher plants (Vidali et al., 2007).

Loss-of-function has been determined by silencing all three isoforms using RNA interference. The mutant cells were small and round. The mutants did not form normal axially oriented cortical bundles at the sub-apical region. Instead the actin filaments formed a cortical patch. Interestingly, this structure did display polarization. It seems that the lack of profilin does not abolish cell polarization but it is crucial for proper F-actin organization. Mutations have been introduced on two sites of profilin molecule. A mutation in the poly-L-proline binding site weakly rescues profilin RNAi. Whereas a mutation in its actin binding site is unable to rescue utterly, concluding that actin binding site is essential for actin organization (Vidali et al., 2007).

2.3.3.6. Villin – Cross-linking

This protein is one of the actin cross-linkers in plants. In root hairs and pollen tubes, villin organizes actin filaments into bundles, thus being the key factor determining the direction of cytoplasmatic streaming. Villin also strongly contributes to the actin turnover in the cell apex. When actin filaments are nucleated from the apical membrane via membrane anchored formins, these filaments are instantly bundled by villin, causing their rigidity and allowing them to grow from the membrane in a straight manner. Oscillation of calcium gradient occurs in the cell apex. When concentration reaches micromolar levels, the severing activity of villin is activated, allowing actin filaments to be turned over locally or released from their membrane anchors. Free filaments can be used for the construction of actin collars. It is possible that the transport of actin filaments is promoted by class XI myosin (Qu et al., 2013). Moss has similar number of villin coding genes as in *Arabidopsis*. Unfortunately, the function has not yet been fully described.

2.3.3.7. Fimbrin - Cross-linking

Fimbrin is known as a cross-linking protein in higher plants. Mutation in *Arabidopsis* pollen tube AtFIM5 resulted in slower growth, probably caused by defective cytoplasmic streaming. Actin cables in the mutant cells reside in the clear zone, which does not occur in wild type. Their orientation was also altered

(Wu et al., 2010). Unfortunately, the function of fimbrin has not been described in moss.

2.3.3.8. Myosin

Myosins are motor proteins which enable transport of vesicles and organelles along actin filaments. The motion is promoted by conformational change run by ATP hydrolysis. The motor (head) domain bids ATP and enables the association with actin. The neck region is required for dimerization of the protein, for myosins typically functions as a dimer. The tail region binds cargo. Based on their function, 35 types of myosins have been found from different organisms. There are only two classes of myosins in plants. The highly processive myosins class XI enable organelle transport, exocytosis and generates cytoplasmic streaming. The low processivity myosins class VIII functions as a tension sensor-generator (Haraguchi et al., 2014). Comparing the number of genes, class VIII has similar number of families in *Arabidopsis* (5) and in *Physcomitrella* (4), myosin class XI have expanded significantly in *Arabidopsis* (13) compared to *Physcomitrella* (2).

The high processivity myosins XI localize with the apical F-actin structures. Interestingly, the oscillating levels of myosin slightly overcome those of F-actin. Moreover, myosin levels fluctuate in an identical phase with a vesicle marker (Furt et al., 2013). Though the ability to bind F-actin mediates the cargo transport, it does not alter actin dynamics. This has been observed in both higher plants and moss. In *Physcomitrella,* knockout of both genes results in alteration of actin, longitudinal filaments are disrupted and form a rather disoriented array. However, the effect on actin dynamics has been considered as minor (Vidali et al., 2010).

Myosins class VIII localize at the surface of organelles including the endocytic compartments at the tip (Sattarzadeh et al., 2008). Gradual knocking out the five Myosin VIII genes in *Physcomitrella* has affected the growth, resulting in reduced cell size and defective branching. Gametophore development has been significantly delayed in the mutants, which could be returned by applying cytokinins to the medium. It has been suggested that myosins VIII play an important role in hormonal distribution (Wu et al., 2011). Though myosin VIII does most certainly bind to actin, the study did not mention the effect on cytoskeleton.

2.4. Cell wall (CW)

In cells, which undergo diffuse growth, an alignment model describes how the cellulose is incorporated in cell walls which undergo diffuse growth. Cellulose synthase (CESA) complexes are arranged into linear arrays along the transversal microtubules (Baskin, 2001). The CESA rosettes show bidirectional movement along MT tracks, motility is provided by cellulose polymerization (Paredez, 2006). There are 10 CESA isoforms in *Arabidopsis* (Doblin et al., 2002); combination of three different paralogs, CESA1, CESA3, CESA6 forms primary cell wall CESA complex (Desprez et al., 2002). Cellulose is resistant to tensile stress. To achieve cell expansion, other CW components are necessary. Especially at the spherical

tip which undergoes a dynamic change. To allow extensibility, hemicellulose and pectins are deposited to the plasma membrane. The vesicular transport is actin dependent (Voigt et al., 2005). Microtubules probably contribute to the direction of secretion (Bibikova et al., 1999). Though the causal linkage between the cytoskeleton and secretory pathway is evident, the mechanisms in plants are yet to be discovered.

2.4.1. Cell wall – root hairs

In root hairs, cellulose filaments are transversally oriented in the sub-apical region and seem to be laid randomly at the apex. Though cellulose is present, CESA plays a minor role in the cell wall extension. Further investigation revealed that cellulose-synthase-like genes (**CLSD)** might be essential. Supporting this idea, knockout mutants display significant defects in root hair formation. It is most likely that the product of *CLSD1* and *CLSD2* genes is possibly mannan (Yin et al., 2011) or some other alternative of the common β(1–4)-linked glucose (Rounds and Bezanilla, 2013). Recently CLSD3 was also characterized, residing at the tip membrane, also performing alternative synthesis activity (Park et al., 2011).

Analysis of the cell wall revealed another important component, hydroxyproline-rich glycoproteins (**HRGPs**), which undergo proline hydroxylation, catalyzed by prolyl 4-hydroxylases (**P4H**s). HRGPs include extensins (**EXT**s), structural wall polymers with a protein backbone glycosylated on their hydroxyproline residues, oxidatively cross-linked via tyrosine residues (Xu et al., 2008) and arabinogalactan-proteins (**AGP**s). In root hairs, hydroxylation enables rearrangement of the polysaccharide network (Velasquez et al., 2011).

2.4.2. Cell wall – pollen tubes

Many types of AGPs have been found in pollen tubes but their function remains unknown. Secretion of AGPs correlates with the site of pectin deposition at the very tip of pollen tube (Mollet et al., 2002). CLSD probably also contributes to tip growth in pollen tubes which possess none, or only small amounts of cellulose. More cellulose may be found further back from the tip (Rounds and Bezanilla, 2013). Microfibrils are oriented longitudinally and also surrounded by CESA rosettes. Though pollen tubes do not form secondary wall, the primary cell wall is laid in two phases. Firstly pectins are secreted to the apex, secondly callose is deposed to the membrane in the shank (Chebli et al., 2012).

There are four types of pectins, xylogalacturonan (XG), rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II), and homogalacturonan (**HG**). A model has proposes that the HGs are exocytosed to the tip. As soon as they reach the membrane, they are demethoxylated by pectin methyl esterase (**PME)** (Winship et al., 2010). The modified HGs can bind to Ca^{2+}, which triggers cross-linking (Vincent and Williams, 2009). The basic concept suggests that by altering the concentration of enzymes and ions, the cell regulates rigidity of the microfilaments or maximizes the elasticity of the network.

Interestingly turgor within the pollen tube, the main force for cell extension, remains constant even when growth rates change.

2.4.3. Cell wall- protonema

Protonema forms only primary cell wall (Roberts et al., 2012) and the material is very much like in root hairs and pollen tubes. Eight *CESA* genes and three *CESA* pseudogenes have been found in the genome of *Physcomitrella*. In contrast to higher plants, which use specialized isoforms for primary and secondary cell wall, Physcomitrella uses different types of CESA for tip and diffuse growth (Roberts et al., 2012). Interestingly, *Physcomitrella CESA* genes have no orthology with the specialized *CESA*s in angiosperms. The common ancestor of mosses and land plants had a single *CESA* gene (Roberts and Bushoven, 2006). *PpCESA6* is expressed specially in tip-growing protonemal filaments, rhizoids, and axillary hairs (Wise et al., 2011). Furthermore, *Physcomitrella* genome includes eight *CSLD* genes and seems to code proteins involved in all four types of pectins synthesis, as well as their pectin methyl esterases. Esterified pectins were identified in protonema at the sides/shank, rather than at the tip (Kulkarni et al., 2012). Xyloglucan synthases are represented by five members of *CSLC* family in *Physcomitrella* with no orthology to seed plants (Roberts and Bushoven, 2006). Utilization of xyloglucan is believed to correlate with the land colonization (Harholt et al., 2012). AGPs are secreted to the tip as in pollen tubes. Furthermore, knockout mutants showed significant alterations, indicating AGPs critical role for tip growth (Lee, 2005). Extensins seem to be conserved among moss and higher plants (Harholt et al., 2012)

2.4.4. Growth mechanism

How is cell wall structure and mechanics modulated? The basic concept suggests that turgor is the main force pressing against the cell wall (Fig. 9). Rigid cell wall is resistant to deformation and thus keeps the cell's shape. Since osmotic gradient is homogenous within the cell, deformation is regulated precisely by alteration of the mechanical properties of the cell wall specific domains (Winship et al., 2010). Mechanical properties are defined by cell wall composition and by the number and character of cross-links involved. In case of tip growth, low viscosity is required especially at the spherical tip. When the cell wall becomes softer, turgor induces tip growth. Pectin, such as HG, is delivered to the tip via exocytosis. They are incorporated and the wall becomes thicker and softer. Nascent pectins have no cross-links but the following demethoxylation by PME makes the HGs available for Ca^{2}+. This process induces cross-linking and therefore maintains the wall's rigidity.

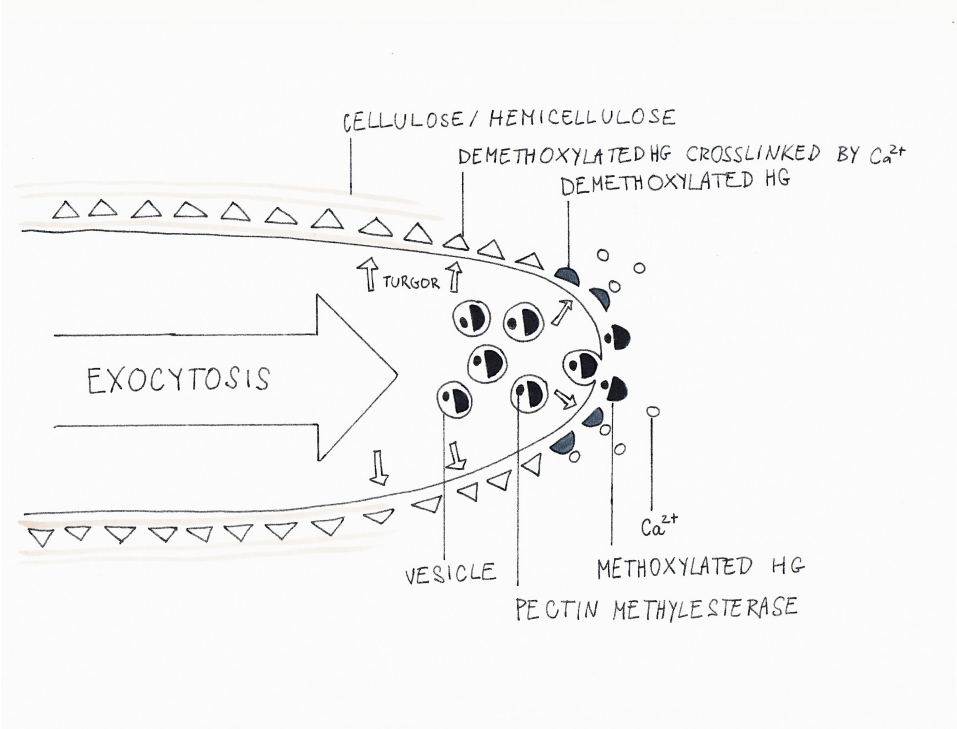

Fig. 9. Schematic model of exocytosis in tip-growing pollen tube according to Rounds and Bezanilla (Rounds and Bezanilla, 2013)

How does cytoskeleton contribute to the pectin deposition? Pectin secretion is targeted to a specific domain of the plasma membrane. Although microtubules are mostly excluded from the growing tip, they contribute strongly to directionality of the transport, cell wall structure and thus the diameter of tip-growing cell (Bibikova et al., 1999). Vesicular transport is provided by actin-myosin interaction (Ojangu et al., 2007) and other actin dependent mechanisms (Voigt et al., 2005). Since exocytosis in plant cells is tightly connected to membrane and cortical cytoskeleton, a linker between vesicle trafficking and cytoskeleton has been intensively sought. A crucial step for exocytosis is signalling. The key components are: exocyst complex, ROP GTPases and SNAREs. How the components of these signalling cascades interact with the cytoskeleton in plants, is still a matter of intensive studies.

2.4.5. Exocyst complex

The exocyst is a complex of eight proteins crucial for vesicle tethering (Fig.10). A basic model suggests that the subunits at the periphery are able to bind vesicle membranes in order to mediate contact with the target membrane. Namely **EXO70** and **SEC3** mark the site of vesicle fusiuon on plasma membrane. These subunits are responsible for the first contact with the target membrane via ROP GTPases, membrane lipids or other molecules, which reside at the target membrane. In *Arabidopsis* SEC3 interacts with ROP GTPases via ICR1/RIP1, an adaptor protein (Lavy et al., 2007). In plants, specific localization of Rop activity defines the site of exocytosis, precisely to the expanding membrane domain (Kost, 2008). The exocyst subunits provide multiple functions. Perhaps this can explain the significant trend in the EXO70 gene evolutionary expansion in land plants. It has been suggested that the microtubule cortical

array provides site for EXO70 interaction, therefore targeting to the domain of pectin deposition. Recent studies confirmed the same mechanism applies to the deposition of the secondary cell wall in xylem (Kulich et al., 2010). Interestingly, mammalian EXO70 was found to bind phosphatidylinositol (Liu et al., 2007). It is a question whereas the plant EXO70 have the same ability. In yeast Exo70 binds the nucleator Arp2/3 complex (Zuo et al., 2006), interaction, which might be also preserved in plants (Synek et al., 2014). The function of EXO70 subunits in *Physcomitrella* has not yet been mapped (Cvrčková et al., 2012) and is currently studied in our lab.

Table 1 | Numbers of exocyst subunit paralogs encoded by the studied plant genomes.

	Sec3	Sec5	Sec6	Sec8	Sec10	Sec15	Exo70	Exo84
A. thaliana	2	2	1	1	1	2	23	3
A. lyrata	2	2	1	1	1	2	23	3
P. trichocarpa	2	2	2	2	2	5	29	8
Solanum sp.	2^2	1^3	1^2	1^4	1^2	2^2	22^2	4^2
V. vinifera	1	1	2	1	1	2	15	3
O. sativa[1]	2(2)	1(1)	1(1)	1(1)	1(1)	4(4)	47	3(3)
S. bicolor	2	1	1	1	1	3	31	3
B. distachyon	2	1	1	1	1	3	27	3
S. moellendorffi	2	1	2	2	2	1	8	2
P. patens	3	3	1	3	3	2	13	7

The complete list of the 392 analyzed genes or proteins including database accession numbers, as well as protein sequences and sequence alignments used in phylogeny calculations, is provided as Supplementary Material.

[1] japonica variety, with numbers for indica in brackets; [2] S. lycopersicon; [3] S. phureja; [4] S. tuberosum.

Fig. 10. A table showing the number of genes coding exocyst subunits in various plant genomes according to Cvrčková et al. (Cvrčková et al., 2012)

2.5. Signalling

2.5.1. Small GTPases

Rab and Rho proteins of plants (ROPs) are families involved in GTPase signalling related to vesicles trafficking, targeting and cytoskeleton dynamics. They both belong to the Ras superfamily of monomeric small GTP binding proteins. These molecules switch to active stage at GTP-bound conformation and inactive in the GDP-bound conformation (Berken, 2006). Regulation is provided by GAPs which increase the Rho GTPase activity and thus inactivate the signalling function. Nucleotide exchange – i.e. the activation - is induced by RhoGEFs which stimulate the signalling (Etienne-Manneville and Hall, 2002).

2.5.1.1. ROP GTPases

ROP GTPases are essential for regulation of the secretory pathway and thus defines the extent of the growing tip (Wasteneys and Ambrose, 2009). The *Physcomitrella* contains 4 genes encoding ROP

GTPases which show a high degree of sequence identity on aminoacid level (99%-100%). Each type is expressed under different physiological and environmental conditions. This idea is supported by the fact, each gene is expressed under sequentially different promoter which enables flexible regulation and fully covers all function by four almost identical genes. *Arabidopsis* genome contains 11 ROP GTPase genes. In contrast to *Physcomitrella*, the *Arabidopsis* ROP GAPs have diverse structures and specialized functions that are expressed according to their need.

Among *Arabidopsis* ROPs, some key regulators of tip growth have been identified. *AtROP1, 3* and *5* are specifically expressed in the pollen tube, whereas *AtROP2* and *4* represent the same functions in root hairs.

Nucleotide sequence analysis revealed that the closest *Physcomitrella* ROP homologs are *AtROP7* and *AtROP8*. There is little known about them, other than they are both specifically, developmentally and physiologically controlled.

In *Physcomitrella* genome, homologues of all *Arabidopsis* genes with key functions in the ROP mediated regulation of tip growth are present, except for ICR (Fig. 11). Both ICR (interactors of constitutively active ROP (Lavy et al., 2007)) and RICs (ROP-interacting CRIB-containing (Wu, 2001)) are the ROP GTPases effectors. Both seem to interact directly with the activated ROP GTPases and function as their downstream regulator in tip-growing cells. The **ICR** family, represented by 5 members in *Arabidopsis*, is conserved in higher plants but does not exist in other eukaryotes (Craddock et al., 2012). AtICR1 binds SEC3 and thus possibly stimulates exocyst localization via activated ROPs (Lavy et al., 2007). This is also essential for polar localization of the auxin efflux carrier PIN1. The absence of ICR in *Physcomitrella* genome indicates that there might be different effectors providing the contact between ROP GTPase pathway and exocyst complex in non-vascular plants.

The **RIC** effectors are mostly involved in cytoskeletal modulation. AtRIC3 and AtRIC4 are essential components of two different signalling pathways with opposite effects on F-actin and MTs organization. Specifically AtRIC3 depolymerizes F-actin by increasing the level of cytosolic Ca^{2+} at the tip of pollen tube (Gu, 2005). AtROP1 provides delicate balance between actin polymerization and depolymerization. *Physcomitrella* genome contains only one *RIC* gene, distinct to *AtRIC*, suggesting that it may play a different, less complicated role (Craddock et al., 2012).

Interestingly, RIC1 acts as a ROP6 effector that promotes the organization of transversely oriented cortical MTs (Fu et al., 2009) which is essential for defining the site of exocytosis and cell wall development, though it is unclear whether it functions as a docking site for exocyst vesicles (Craddock et al., 2012). ICR3 in *Arabidopsis* interacts with a kinesin-13 (Mucha et al., 2010). Their cooperation has been identified as a negative regulator of the secondary cell wall deposition by causing MT depolymerisation. ICR3 also binds MAPs, inducing MT stabilization (Oda and Fukuda, 2012).

	ROP	RIC	ICR
Arabidopsis thaliana	11	11	5
Physcomitrella patens	4	1	0

Fig. 11. Relative sizes of *Physcomitrella*, Arabidopsis ROP signalling gene families according to Eklund (Eklund et al., 2010).

Whether the function of ROP signalling pathways components in moss corresponds to the situation in vascular plants is still unknown. Though the function of ROPs in *Physcomitrella* have not been supported by experimental data, few hypotheses based on sequence analysis have been suggested. The ratio between number of families of upstream regulators of ROP GTPases and the number of ROP GTPase families are much lower in *Arabidopsis* than in *Physcomitrella*, whereas downstream effectors show the opposite trend. Assuming the upstream signalling of *Physcomitrella* ROP GAPs is rather complex, the downstream network is reduced. Alternatively, the downstream pathway of *Physomitrella* might employ effectors different to *Arabidopsis* (Eklund et al., 2010).

2.5.1.2. RAB GTPases

RABs are another family of Ras-related GTPases which are involved in membrane targeting and vesicle transport. In *Arabidopsis* pollen tubes, RAB4A mediates the contact between trans-Golgi and the plasma membrane. The RAB4A is localized to the cell tip in both pollen tubes and root hairs, though knocking out the gene seems to affect the polarized growth in root hairs more as compared to pollen tubes (Rounds and Bezanilla, 2013). RAB4A has been also localized to the tip of protonema cells (Perroud and Quatrano, 2008). Since myosins also contribute to vesicle trafficking, the collaboration with Rab GTPases has been considered as possible. New experiments with *Physcomitrella* confirmed the correlation between fluorescently labeled RABA21 and myosins. These two proteins accumulate at a same phase in the tip of caulonema cells, assuming they bind to the same compartment (Callahan, 2014)

2.5.2. Oscillation and Ca^{2+} Gradient

Growth oscillation is a common feature of pollen tubes, root hairs (Rounds and Bezanilla, 2013) and protonema (Nakaoka et al., 2015). Oscillations have been shown also in Ca^{2+} gradient, ROP activity, cell wall deposition cytoskeletal structures and other components. Comparison of the periods and phases is often employed for determining the causal linkage between individual phenomena. Though using cross-correlation might help identify which oscillation precedes the other, it does not necessarily mean the two phenomena are connected (Rounds and Bezanilla, 2013).

Ca^{2+} is a critical component of signalling cascades. Most important for polarized growth, Ca^{2+} controls actin dynamics via actin binding proteins. High levels of Ca^{2+} induce F-actin depolymerisation and subsequently cause exocytosis (Domozych et al., 2013). In addition, extracellular gradient is necessary for rigidity of pectin in the cell wall (Rounds et al., 2011).

2.5.3. Phytohormones

Unlike in higher plants, cytokinins affect protonema at a single-cell level (Schulz et al., 2000). In addition, the type of cytokinin found in moss is also found in green algae, rather than seed plants (von Schwartzenberg et al., 2007). In Physcomitrella, cytokinins promote formation of a simple meristem and thus the development of gametophore. The single-cell meristem, triangular in shape, gives rise to gametophore, which in contrast displays a tetrahedral apical cell with three cutting faces (Fujita et al., 2008). Cytokinins-deficiency was induced by cytokinins oxidase overexpression. The mutants showed a high number of shorter and wider cells and less buds. Gametophore development was delayed. Interestingly, no sexual organs were developed. Together with auxins, cytokinins modulate the morphology in *Physcomitrella*. Highest auxin gradient has been detected in the tip of the caulonema cells (Jang and Dolan, 2011). A polarized auxin transport system necessary for auxin distribution in higher plants has not been detected in the moss shoot-like gametophyte. Interestingly, the polarized auxin transport has been found in the sporophyte which lacks shoot structures entirely (Fujita et al., 2008). It has also been acknowledged that the PIN transporters are being secreted to the plasma membrane via ROP-dependent clathrin exocytosis (Dhonukshe et al., 2007). Although this has been confirmed in pollen tube tip, in protonema the process of PIN distribution is unknown.

3. Conclusion

Comparing protonema with pollen tubes and root hairs brings out some detail alterations. Time-lapse visualization reveals the different behavior of apical structures. The observation provides a complex insight into the cytoskeletal dynamic which seem to be crucial for understanding the molecular mechanisms.

Since F-actin and microtubules serve as dynamic scaffolding, their function is neatly regulated by actin associated proteins. The most important modulators essential for polarized growth are formins, actin nucleators and myosins, the molecular motors. Localization of these proteins is essential for their function and for the character of polarized growth. Some protein functions seem to be conserved among moss and vascular plants, whereas in the case of Arp2/3 complex, the function varies significantly.

Moreover, studies based on *Physcomitrella* genome enable simplification of the interplay between cytoskeletal structures and the secretory pathway. Signalling is an important moment for the polarized growth. ROPs and Rab GTPases play a key role in these processes.

Though the metazoan and yeast-based models provide a certain clue to protein function, many plant mechanisms remain unknown. The task of the research is to find the missing parts of the puzzle. Perhaps one day it will be possible to comprehend the molecular machinery with a simple informative model.

4. Discussion

Polarized cells – fungal hyphae

Polarized cells can be found across animal, fungi and plant kingdom. For example fungal hyphae expansion resembles plant tip-growth strikingly. Tip of the hyphal cell serves as a deposition site for vesicles delivering glycoproteins and enzymes. Microtubules maintain cytosolic transport and accumulation of vesicles near the apex. This vesicle rich area is called the Spitzelkörper (apical body). Actin provides tracks for myosin-mediated transport and recycling (Steinberg, 2007). Interestingly, Arp2/3 complex plays an important role in the apical actin organization (Machesky and Gould, 1999). Docking and fusion is enabled via exocyst but the site specific deposition is controlled by Ras and Rho GTPases, thus enable polarized secretion. In case of the fungi hyphae, the polarization is induced by external cues, which elevate Ca^{2+} gradient at the apex. The signalling pathways linking Ca^{2+} to the tip-growing mechanism remains a mystery (Brand and Gow, 2009). Tough we can not be sure, whether the molecular mechanisms are the same, the research on fungi provides a clue for unveiling processes in plant kingdom.

Type of growth - protonema

There are three distinct types of cell growth. Firstly, it is the diffuse isodiametric growth defined by expansion equally over the cell surface. Secondly, the oriented diffuse expansion resulting in cell growth in one direction, takes place most predominantly in multicellular tissues, defining the direction of organ growth (Žárský et al., 2009). The alignment model suggests that MTs are oriented perpendicular to the axis of growth and cellulose fibrils are co-aligned constrained by the MTs. Thirdly, tip-growing cells expand on a small specific area (Carol and Dolan, 2002). In tip-growing moss protonema a small group of transversal MTs have been identified, thus diffuse growth could be expected. Perhaps the protonemal cell performs a combination of both types of growth - oriented polarized cell expansion and tip growth. However, some studies have proposed that no diffuse growth was observed. Chloronema and chloronema might grow exclusively by tip growth (Menand et al., 2007).

5. References

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