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Role of exocyst complex in growth and development of moss *Physcomitrella patens*

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Summary of

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Abstract:

During the course of evolution the early land plants gained extensive innovations that can be seen in modern day plants. The polar growth is an ancient feature of eukaryotic cells and is one of preadaptations that helped plants in successful colonization of land. The polar growth in plants regulates not only the direction of cell expansion and structural properties of cell wall but especially also the orientation of cell division, and is governed by various factors, including the exocyst complex. The exocyst is a well conserved vesicle tethering multi-subunit complex involved in tethering of secretory vesicles to the target membrane.

The essential role of the exocyst complex in regulation of various cellular processes in Angiosperms is now well documented. Here I present results of a doctoral project that contributed to phylogenetic analyses of the land plant exocyst complex and especially to uncovering functions of three moss exocyst subunits, namely EXO70 (isoform *PpEXO70.3d*), SEC6 and SEC3 (isoforms *PpSEC3A* and *PpSEC3B*) in the model organism *Physcomitrella patens*.

Various knock-out (KO) mutants in several moss exocyst subunits (*Ppexo70.3d, Ppsec6, Ppsec3a* and *Ppsec3b*) show pleiotropic defects directly or indirectly linked to the cell polarity regulation. Cell elongation and differentiation, cytokinesis, cuticle formation, response to auxin (phytohormone) are impaired in these mutants, resulting in different strength of developmental deviations ranging from inability to develop gametophores to more subtle morphologic deviations linked to different degree of dwarfism in gametophores. Importantly, the exocyst genes are required for completion of the full moss life cycle including sexual reproduction. While a KO mutation in the single-copy subunit PpSEC6 results in lethality, KO mutants of multi-member subunit families are not lethal – *Ppexo70.3d* (one of thirteen *EXO70* paralogs) is sterile due to defective egg cell development, and *Ppsec3* mutants (three *SEC3* paralogs) show partial defects in sporophytes and spore development.

These results show that the exocyst complex function in cellular morphogenesis is not only conserved in moss *P. patens,* and that the exocyst has a crucial role in the moss life cycle, but they also indicate a functional importance of the multiplication of exocyst genes in this representative of basal land plants.

This work was supported by EU Marie Curie Network (No. 238640 PLANTORIGINS), MSMT (NPUI LO1417) and GACR/CSF (15-14886S).

Abstrakt:

První suchozemské rostliny získaly v průběhu svého vývoje rozsáhlá evoluční vylepšení platná i u dnešních moderních rostlin. Polární růst je pradávnou vlastností eukaryotických buněk a jednou z preadaptací, které pomohly rostlinám při úspěšné kolonizaci souše. Polární růst u rostlin určuje nejen směr expanze buněk, strukturní vlastnosti buněčné stěny, ale také orientaci buněčného dělení. Řízení polárního růstu se účastní různé faktory, včetně komplexu exocyst. Exocyst je evolučně konzervovaný poutací komplex, který se skládá z osmi podjednotek, a účastní se poutání (angl. tethering) sekretorických váčků k cílové membráně.

Zásadní role komplexu exocyst v různých buněčných procesech u krytosemenných rostlin je v současnosti dobře dokumentována. V této práci prezentuji výsledky doktorandského projektu, který přispěl k fylogenetické analýze komplexu exocyst u suchozemských rostlin, a zejména k objasnění funkcí tří podjednotek exocystu, konkrétně EXO70 (isoforma *PpEXO70.3d*), SEC6 a SEC3 (isoformy *PpSEC3A* a *PpSEC3B*), u modelového mechu *Physcomitrella patens*.

Několik *knock-out* (KO) mutantů tohoto mechu v různých podjednotkách exocystu (Ppexo70.3d, Ppsec6, Ppsec3a and Ppsec3b) vykazuje pleiotropní defekty, které jsou přímo či nepřímo propojeny s regulací buněčné polarity. Narušen je dlouživý růst a diferenciace buněk, cytokineze, tvorba kutikuly a odpověď na fytohormon auxin, což má za následek různě silné vývojové defekty od neschopnosti vytvářet gametoforů až po malé morfologické odchylky vedoucí k zakrnělému vzrůstu gametoforů. Důležité je, že tyto geny jsou nezbytné pro dokončení životního cyklu mechu, včetně pohlavního rozmnožování. Zatímco KO mutace PpSEC6 (podjednotka kódovaná jediným gene) je letální, KO mutanti podjednotek kódovaných více geny letální nejsou – mutant *Ppexo70.3d* (jeden z třinácti paralogů *EXO70*) je sterilní kvůli defektu při vývoji vaječné buňky a mutanti *Ppsec3* (tři paralogy *SEC3*) vykazují dílčí poruchy ve vývoji sporofytu a spor.

Výsledky uvedené v této práci ukazují, že funkce komplexu exocyst u mechu *P. patens* je konzervována v procesech buněčné morfogeneze, že exocyst hraje klíčovou roli v životním cyklu mechu, ale také naznačují funkční význam znásobení genů kódujících podjednotky exocystu u tohoto zástupce prvních suchozemských rostlin.

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1. AIMS OF THE PROJECT

- To contribute to the reconstruction of the process of evolution of the exocyst complex in plants, with special focus on EXO70 and SEC3 subunits
- To generate and analyze mutants in selected EXO70 paralogs of *P. patens* and find their role in the moss life-cycle
- To characterize the phenotype of *Ppsec6* mutants and study involvement of PpSEC6 in *Physcomitrella patens* cell morphogenesis
- To determine the involvement of SEC3 subunit in polar growth and secretion in moss *P. patens*

2. INTRODUCTION

1.1. Introduction to model plant *Physcomitrella patens*

1.1.1 Evolutionary position of bryophytes

Colonization of land by plants took place *approx*. 470-450 million years ago (MYA), and is an event of central importance to life on land. Present day land plants evolved from multicellular algae of fresh water, related to the extant charophyte groups Charales or Coleohaetales. Both charophytes and embryophytes, collectively also known as "streptophytes" form a monophyletic group (Figure 1.1), which is sister to other green algae – chlorophytes. The three extant bryophyte lineages (liverworts, mosses and hornworts) separated just before the linage ancestral to present day tracheophytes, and liverworts are considered to be the earliest divergent clade, with mosses forming a sister group to the clade of hornworts and tracheophytes (Qui *et al.,* 2006). However, the position of hornworts relative to the mosses + liverworts clade and to tracheophyte is not yet clear. The study conducted by Wicket *et al*., (2014) showed a clade with mosses and liverworts as sister to tracheophytes, while hornworts appear to be a sister to all other (non-hornwort) land plants.

After migrating from water to terrestrial environment, the early land plants underwent several adaptions which led to numerous morphological, cellular and physiological changes in plants. Polar development of plants based on polar cell growth would have been one of the several factor that allowed plants to develop and flourish on land. Bryophytes were among the first plants to conquer the land. The vital land characters primarily evolved in the bryophyte clade, which hence provides excellent model to understand the early events in evolution of land plants (reviewed in Ligrone *et al.,* 2012).

1.1.2. *Physcomitrella patens* as a model plant

Mosses occur in many extreme habitats such as Antarctic tundra to deserts, and also form important components of tropical systems, boreal forests and woodlands in temperate zone. Despite their small size, mosses have huge impact on various ecosystems and are essential contributors to complex biological cycles. Various moss species e.g., *Funaria hygrometrica*,

Physcomitrella patens, *Ceratodon purpureus*, *Sphagnum,* have been developed as model systems, among which *P. patens* is the most developed and widely used model moss.

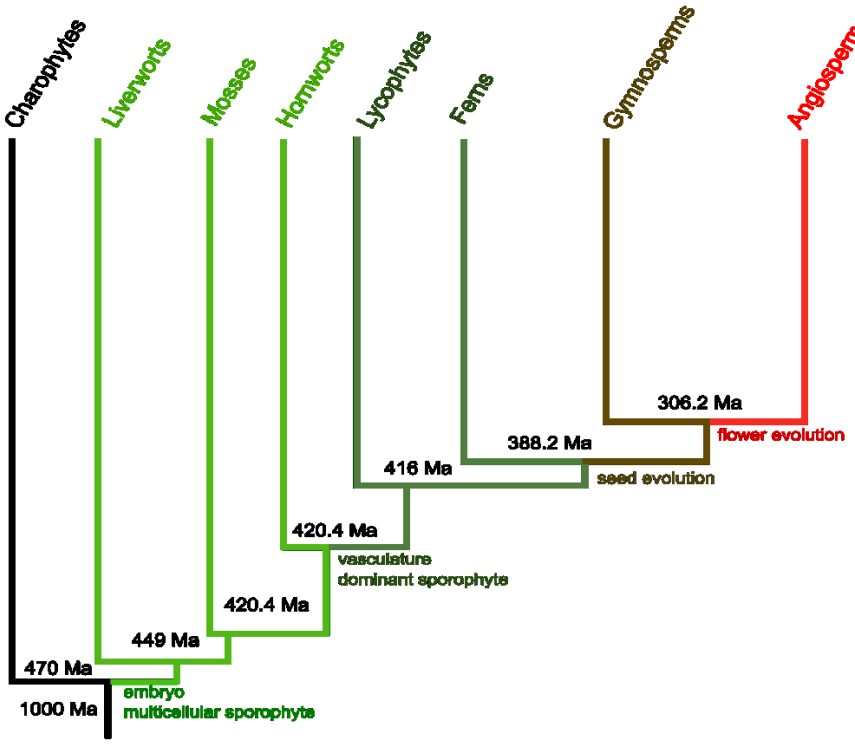

Figure 1.1 Phylogenetic relationships between the major groups of extant plants. Key events that occurred during plant evolution are indicated. The estimated divergence times are indicated in millions of year ago (Ma). Adapted from: Clarke *et al*., 2011; *Pires* & *Dolan,* 2012.

Physcomitrella patens (Hedw.) Bruch & Schimp, also known as spreading earthmoss, is a non-vascular land plant belonging to phylum Bryophyta. It is found growing on the exposed banks of ponds, lakes and rivers and finishes its life cycle by producing sexual organs and then sporophytes at lowered temperatures and short days.

For more than two decades, the moss *P. patens* has been employed as an alternative model suitable for plant cell biology studies. It has relatively small genome size of approximately 511 Mbp size, consisting of 27 chromosomes, and is first fully sequenced genome of a bryophyte (Rensing *et al*., 2008). *P. patens* genome is about four times larger than *A. thaliana* (approx. 135 Mbp, consisting of 5 chromosomes only!), but has smaller average gene family size than that in *A. thaliana*, meaning that the moss has a high number of unique genes.

Homologues of more than 66% of Arabidopsis thaliana genes are present in *P. patens* gametophytes and >90% of the most closely related homologues of *P. patens* gametophytic transcripts occur in vascular plants, suggesting that gametophytes and sporophytes use similar gene sets (Nishiyama *et al.*, 2003). *P. patens* has many advantages to be used as a model plant. Along with fully sequenced genome, it has short generation time (4-8 weeks) and small stature (1-5 mm) with relatively simple body organization and development. It can be easily propagated vegetatively, and undergoes homologous recombination which allows generating targeted knockout mutants with ease (Kamisugi *et al*., 2005). Due to the excellent regeneration capacities of its tissues, the mutants with arrest in any developmental stage can be easily propagated (Cove, *et al*., 2006; Prigge & Bezanilla, 2010). Along with this, performing various molecular techniques in *P. patens* is now a routine. These all features make *P. patens* excellent organism for reverse genetic studies, esp. for developmental and physiological process from evolutionary perspective.

1.1.3. Polarized tip growth in moss *P. patens*

Most of the plant cells grow by diffuse growth, while some grow by depositing cell wall material in a highly polarized manner at the tip of the cell- known as tip growth. Root hairs, pollen tubes, rhizoids, moss protonemata, etc. are a few examples where the cell grows by tip growth. Both gymnosperms and angiosperms rely on tip-growing pollen tubes only for their sexual reproduction, while mosses rely completely on tip growth for their development, starting from spore germination, to protonemata and rhizoid development. Actin appears to be central to the process of tip growth in mosses (Vidali *et al*., 2007; Vidali *et al*., 2010). The mutants in moss Arp2/3 subunits show major defect in tip growth of moss protonemal filaments (Harries *et al*., 2005; Perroud & Quatrano, 2006; Finka *et al*., 2008). Similar to ARPC4, BRICK1- a subunit of Wave/SCAR, is present at the apex of tip-growing cells and is required for localization of apically associated factors (Perroud & Quatrano, 2008). There are several reports showing coordinated action of myosin and actin function at the cell's apex to maintain polarized growth in protonemal cells (Vidali *et al*., 2010; Furt *et al*., 2013)

The exocyst targets the secretory vesicles by coordinating with the actin cytoskeleton cables. EXO70 and SEC3 proteins are associated with plasma membrane (PM) via PI(4,5)P2 and interact with activated RHO GTPases, while the other members of the complex are associated

with the secretory vesicles. In yeast, Exo70 is shown to interact with ARPC1 subunit of Arp2/3 complex (Liu *et al*., 2012). This indicates the interaction of exocyst, actin and GTPases together may mediate the polarized tip growth in mosses as well.

1.2. Exocyst Complex

1.2.1. General overview

Exocytosis is the ultimate and fundamental step in polar growth and development of a cell, and the process, both spatially and temporally, is under tight control. Various tethering complexes are responsible for movement of vesicles, in different pathways, in a plant cell (reviewed in Vukašinović & Žárský, 2016).

Exocyst, also known as the Sec6/8 complex, is an evolutionarily conserved, octameric protein vesicle tethering complex consisting of SEC3, SEC5, SEC6, SEC8, SEC10, SEC15, EXO70 and EXO84 subunits (TerBush *et* al., 1996; Eliáš *et al.,* 2003, Heider & Munson, 2012; Wu & Guo, 2015). It was first identified during a yeast genetic screen for secretory mutants (Novick *et al*., 1980; Novick *et al*., 1981), and later was shown to be present also in metazoans and plants (Hsu *et al*., 1998; Eliáš *et al*., 2003).

Proper targeting of the secretory vesicles is essential for fulfillment of various biological processes in plant's life such as: cell wall biogenesis, cytokinesis, polar growth, deposition of extracellular material including development of a cell wall. The exocyst complex tethers the secretory vesicles, and comes in action before formation of SNARE protein complex takes place.

1.2.2. Structure and function of the exocyst complex

In yeast, exocyst forms a stable, elongated structure, suggesting rod-like architecture of its subunits, which is attributed to the tandem helical bundles that are tightly packed together in a side by side fashion (Figure 1.2) (Hsu *et* al., 1998; Munson & Novick, 2006; Heider *et al*., 2016). The molecular weight of complex itself is approximately 750 kDa with its subunits ranging between 70 – 150 kDa (TerBush *et al*., 1996). The structural data suggest that the N and C termini of the helical bundle repeats are positioned at the opposite direction of rod (Sivaram *et al*., 2006).

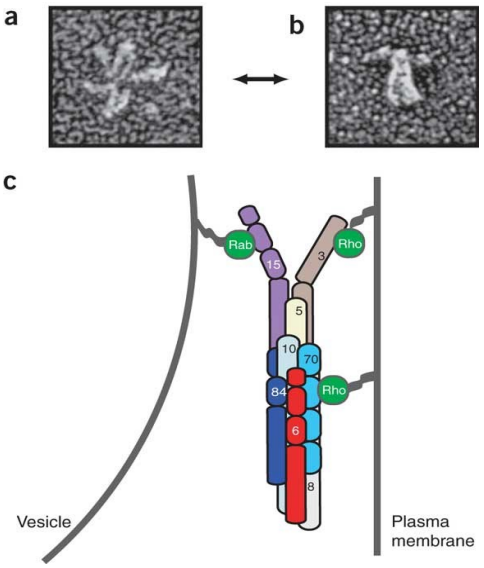

Figure 1.2. A model for the assembled exocyst complex. Quick-freeze EM of purified mammalian brain exocyst complexes either unfixed (a) or fixed with glutaraldehyde (b). (c) Schematic representation of the yeast exocyst complex hypothesizing that each of the exocyst subunits has an elongated helical bundle structure and that they pack together to form a structure similar to that in (b). Taken from: *Munson & Novick*, 2006.

Recently, Picco *et al*., (2017) reconstructed the 3D architecture of yeast exocyst complex *in vivo* and proposed the working model for exocyst complex in vesicle tethering. According to this model, different subunits of the complex are arranged in dimers and except for Sec10, all are attached to the core of the complex by their N-termini, with C-termini projecting outward, while Sec10 which has exactly inverted organization in the complex (Figure 1.3). During the event of fusion, exocyst is positioned at the side of the membrane contact site. The complex binds the vesicle with Sec10-Sec15 dimer and the membrane with Sec3 and Exo70 simultaneously, while the Sec6 interacts with the SNARE complex by its C-terminus. There can be maximum of ~20 complexes at the site taking part in the event of fusion.

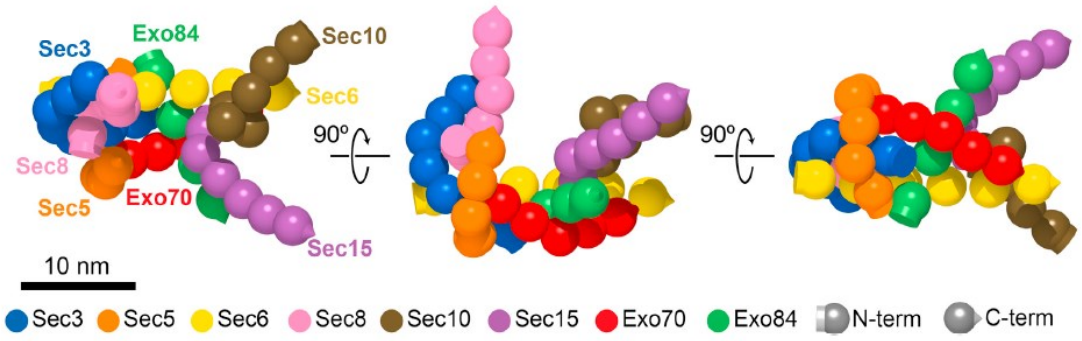

Figure 1.3. The 3D architecture of yeast exocyst complex (Picco *et al*., 2017)

Recently, Heider *et al*. (2016) mapped the connectivity between the subunits of the complex showing that there are two stable modules of four subunit each (Sec3-5-6-8 and Sec10-15-Exo70-84) within the complex and that the presence of most of the exocyst subunits are critical for the integrity and stability of the complex.

The exocyst complex mediates the tethering of secretory vesicle at the polarized micro-domains on plasma membrane and thus regulates the cell polarity (reviewed in Wu & Guo, 2015). In yeasts, both Sec3- through its N-terminally located PH-domain, and Exo70 have been shown to interact with membrane phospholipid PI(4,5)P2, thus marking the site of vesicle fusion on the PM (He *et al*., 2007; Zhang *et al*., 2008).

The role of exocyst in polarized exocytosis is well studied in yeast, mammals and plants (reviewed in Wu & Guo, 2015; Vukašinović & Žárský, 2016). In plants, the germination and growth of pollen tube is controlled by the exocyst complex (Cole *et al*., 2005; Hála *et al*., 2008; Bloch *et al*., 2016; Synek *et al*., 2017), while deletion of *SEC3* causes failure in root hair elongation in maize (Wen *et al*., 2005). The exocyst is involved in various biological processes in plants, like seed coat deposition, secondary cell wall formation, callose ring deposition in trichomes, casparin strips formation etc. and also have role in response to pathogens (Kulich *et al*., 2010, 2015; Vukašinović *et al*., 2016; Lothar *et al.,* 2017; Pečenková *et al*., 2011). The need of constitutive recycling of PIN's (an auxin efflux carrier protein), between PM and endosomal compartments, is carried out by exocyst complex (Drdová *et al*., 2013; Tan *et al*., 2016).

During the process of cytokinesis there is high demand of cell wall material to be deposited in between the two daughter cells. Throughout the whole process of cell plate formation and maturation, the exocyst complex participates intimately in secretory vesicle fusion, and thus plays an important role during cytokinesis in plants (Fendrych *et al*., 2010).

3. RESULTS

Paper 1: Evolution of the Land Plant Exocyst Complexes

Fatima Cvrčková, Michal Grunt, Radek Bezvoda, Michal Hála, Ivan Kulich, Anamika Rawat, Viktor Žárský

Summary: Exocyst is an evolutionarily conserved vesicle tethering complex functioning especially in the last stage of exocytosis. Homologs of its eight canonical subunits – Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 – were found also in higher plants and confirmed to form complexes *in vivo*, and to participate in cell growth including polarized expansion of pollen tubes and root hairs. Here we present results of a phylogenetic study of land plant exocyst subunits encoded by a selection of completely sequenced genomes representing a variety of plant, mostly angiosperm, lineages. According to their evolution histories, plant exocyst subunits can be divided into several groups. The core subunits Sec6, Sec8, and Sec10, together with Sec3 and Sec5, underwent few, if any fixed duplications in the tracheophytes (though they did amplify in the moss *Physcomitrella patens*), while others form larger families, with the number of paralogs ranging typically from two to eight per genome (Sec15, Exo84) to several dozens per genome (Exo70). Most of the diversity, which can be in some cases traced down to the origins of land plants, can be attributed to the peripheral subunits Exo84 and, in particular, Exo70. As predicted previously, early land plants (including possibly also the Rhyniophytes) encoded three ancestral Exo70 paralogs which further diversified in the course of land plant evolution. Our results imply that plants do not have a single "Exocyst complex" – instead, they appear to possess a diversity of exocyst variants unparalleled among other organisms studied so far. This feature might perhaps be directly related to the demands of building and maintenance of the complicated and spatially diverse structures of the endomembranes and cell surfaces in multicellular land plants.

Paper 2: The *Physcomitrella patens* exocyst subunit EXO70.3d has distinct roles in growth and development, and is essential for completion of the moss life cycle

Anamika Rawat, Lucie Brejšková, Michal Hála, Fatima Cvrčková, Viktor Žárský

Summary:

- Exocyst, an evolutionarily conserved secretory vesicles tethering complex, spatially controls exocytosis and membrane turnover in fungi, metazoans and plants. The exocyst subunit EXO70 exists in multiple paralogs in land plants, forming three conserved clades with assumed distinct roles. We report functional analysis of the first moss exocyst subunit to be studied, *Physcomitrella patens PpEXO70.3d* (Pp1s97_91V6), from the so far poorly characterized EXO70.3 clade.
- Following phylogenetic analysis to confirm presence of three ancestral land plant EXO70 clades outside angiosperms, we prepared and phenotypically characterized loss of function *Ppexo70.3d* mutants and localized PpEXO70.3D *in vivo* using GFP-tagged protein expression.
- Disruption of *PpEXO70.3d* caused pleiotropic cell elongation and differentiation defects in protonemata, altered response towards exogenous auxin, increased endogenous IAA levels, along with defect in bud and gametophore development. During-mid archegonia development, abnormal egg cell is formed and subsequently collapses, resulting in mutant sterility. Mutants exhibited altered cell wall and cuticle deposition, as well as compromised cytokinesis, consistent with the protein localization to the cell plate.
- Despite some functional redundancy allowing survival of moss lacking *PpEXO70.3d*, this subunit has an essential role in the moss life cycle, indicating sub-functionalization within the moss EXO70 family.

Manuscript 3: Moss SEC6 exocyst subunit is essential for growth and development

Lucie Brejsková, Michal Hála, Anamika Rawat, Soukupová Hana, Fabien Nogué, Viktor Žárský

Summary:

Cell polarity regulation during cell division and cell expansion plays important roles in plant growth and morphogenesis and relies on the cooperation between cytoskeleton and secretory pathways. Octameric complex exocyst is a phylogenetically conserved exocytotic vesicles tethering factor, functioning as an effector of Rho and Rab GTPases at the plasma membrane. In contrast to most other land plants exocyst subunits, the *SEC6*, a core exocyst subunit, is encoded by only one paralogue in genomes of *Physcomitrella patens* and *Arabidopsis thaliana*. Arabidopsis SEC6 loss-of-function mutation causes male gametophytic lethality.

Here we show that attempts to produce the full disruption of *PpSEC6* by targeted gene replacement did not result in any moss plant regenerated. However accidentally we generated two independent mutant strains with only partial deletion at the C´-terminus of the *PpSEC6* coding locus displaying pleiotropic developmental deviations. Mutants display diminished rate of caulonema filaments elongation - in contrast to normally growing chloronema cells which were however resistant in respect to auxin induced transition to caulonema. Gametophore buds were initiated mostly from chloronema cells exhibited disordered cell file organization with cross wall perforations and were arrested in the 8-10-cell stage of development. Complementation of both mutant lines with *PpSEC6* and *AtSEC6* cDNA succesfully rescued WT gamethophore development. Induction of reproduction resulted in sexual organs differentiation; however sporophyte formation and production of viable spores was achieved only in lines complemented by moss SEC6. This indicates a partial functional conservation of SEC6 exocyst subunit between land plant lineages with possible specific moss SEC6 molecular features necessary to successfully finish the whole moss life cycle. Our results demonstrate an essential role of the moss SEC6 exocyst subunit in growth and development of *P. patens*.

Manuscript 4: The *PpSEC3* genes regulate the sporophyte formation and perispore deposition, giving insight into spore development in early land plants

Anamika Rawat, Klára Aldorfová, Lucie Brejková, Juraj Sekereš, Fatima Cvrčková, Viktor Žárský

Summary:

Polar growth, driven by the controlled exocytosis, is crucial for broad range of biological processes in living organisms. Exocyst, an evolutionary conserved secretory vesicle tethering complex, functions in later stages of exocytosis and targets the secretory vesicle on plasma membrane, just prior to fusion. In yeast the exocyst subunit Sec3 and also Exo70 interacts with the plasma membrane, thus marking the site for vesicle fusion. Here we report the functional role of two SEC3 genes (*PpSEC3A* and *PpSEC3B*) in moss *P. patens*.

Out of three SEC3 paralogs present in *P. patens*, we prepared single as well as double mutants in two of the genes that had interesting expression patterns in the moss tissue, esp. sporophytes. The mutants were phenotypically characterized to understand the role of *SEC3* in moss *P. patens*.

Knock-outs of *SEC3A* and *SEC3B* resulted in unusual protonemal growth when initiated from protoplasts, indicating loss of directional cues during the polar growth in *Ppsec3* mutants. The initiation of cell growth during the stem cell formation in phyllids was altered in *Ppsec3a* and *Ppsec3ab*. Mutants also exhibited defective sporophytes and spores. Only a fraction of mutant spores was viable and exhibited compromised perispore layer formation. The N-term PH-domain of *PpSEC3A* showed positive interaction with membrane PI(4,5)P2 under *in vitro* conditions.

Taken together our results show that the role of *SEC3* in regulating the polar growth is conserved, and that it is one of the factor needed for proper construction of spore wall and spore viability in moss *P. patens*.

4. CONCLUSION

Over the last decade, exocyst, an evolutionary conserved tethering complex, has been studied extensively in plants. These studies so far were focused primarily on Arabidopsis, rice, maize etc. i.e. angiosperms and up to now there are no reports on its function in non-angiosperm plant lineages.

Our phylogenetic analysis of the exocyst complex in land plants indicates that it has undergone immense diversification, due to genome duplication events (not just the only factor!) in the course of plant evolution which has resulted in multiple gene families of exocyst subunits, esp. in case of EXO70 subunit. We confirmed the existence of the exocyst complex subunits not only in angiosperms but also in gymnosperms and other plants from basal groups of plant lineages.

Mosses, due to their position in the phylogeny, present an excellent model for evo-devo studies. Using this system, we are able to show that exocyst complex is an important factor for polar growth, secretion and development in mosses as well. The tip growth, secretion of surface cuticle, cell morphogenesis and transition, cell division etc. are just few of those processes that require intensive polarized exocytosis. Moss exocyst mutants displaying defects in these processes, along with the others, point towards the lack of polarized exocytosis. Based on our results we can say that exocyst truly is a conserved complex which has played an important role during the course of land plant evolution.

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6. *Curriculum vitae*

Anamika Ashok Rawat

Date of Birth: 24.03.1986 Country: India

since 2011: post-graduate student in the laboratory of RNDr. Viktor Žárský, CSc, Prague, Czech Republic, working on a project to find out the role of exocyst complex in polar growth of moss *Physcomitrella patens*

2009: Master of Science in Botany from Sardar Patel University, India. Dissertation on molecular characterization of *fusarium oxysporum* f. Sp. *Ciceris* and identification of a marker for wilt resistance in chickpea (*Cicer arietinum* L.)

2007: Bachelor of Science in Botany from South Gujarat University, India

Publications:

Rawat A, Brejková L, Hála M, Cvrčková F, Žárský V (2017). The *Physcomitrella patens* exocyst subunit EXO70.3d has distinct roles in growth and development, and is essential for completion of the moss life cycle. *New Phytologist* doi: 10.1111/nph.14548

Vukašinović N, Oda Y, Pejchar P, Synek L, Pečenková T, Rawat A, Sekereš J, Potocký M, Žárský V (2016). Microtubule-dependent targeting of the exocyst complex is necessary for xylem development in Arabidopsis. *New Phytolgist* 213: 1052-1067

Cvrčková F, Grunt M, Bezvoda R, Hála M, Kulich I, Rawat A, Žárský V (2012) Evolution of the land plant exocyst complexes. *Front. Plant Sci.* 3:159

Brejsková L, Hála M, Rawat A, Hana Soukupová, Fabien Nogué, Žárský V. Moss SEC6 exocyst subunit is essential for growth, differentiation and development. (Under prep.)

Rawat A, Aldorfová K, Brejková L, Sekereš J, Cvrčková F, Žárský V. The *PpSEC3* genes regulate the sporophyte formation and perispore deposition, giving insight into spore development in early land plants (Under prep.)

Presentations at the conferences:

Anamika Rawat, Lucie Brejšková, Michal Hala, Fatima Cvrčkova and Viktor Žárský, "Exocyst functions in moss *Physcomitrella patens* development: exemplified by the regeneration and sporogenesis" poster presentation at Annual Moss Meeting MOSS2016, 2nd – 5th September 2016, Leeds U.K.

Anamika Rawat, Lucie Brejšková, Michal Hala, Fatima Cvrčkova and Viktor Žárský, "Evolution of EXO70 subunit of exocyst complex and its role in morphogenesis of *Physcomitrella patens*" poster presentation at EMBO workshop: New model systems for early land plant evolution (w16-05), 22th – 24th June 2016, Vienna Austria.

Anamika Rawat, Lucie Brejšková, Fatima Cvrčkova and Viktor Žárský, "An insight into the evolutionary perspective of EXO70, a subunit of exocyst complex and its role in morphogenesis of *Physcomitrella patens*" oral presentation at Society for Experimental Biology (SEB) meeting, 30th June – 3rd July 2015, Prague Czech Republic.

Anamika Rawat, Lucie Brejšková, Fatima Cvrčkova and Viktor Žárský, "An insight into the evolutionary perspective of EXO70, a subunit of exocyst complex and its role in morphogenesis of *Physcomitrella patens*", oral presentation at 12th Phd Student Conference of Plant Experimental Biology, 4th – 5th September 2014, Olomouc Czech Republic.

Anamika Rawat, Lucie Brejšková, Michal Grunt, Hana Soukupova, Michal Hála, Fatima Cvrckova and Viktor Žárský, "EXO70 subunits of the exocyst complex – their evolution and function in the morphogenesis of *Physcomitrella patens*", poster presented at ANNUAL MOSS MEETING, 17th-19th June 2013, Prague Czech Republic.

Workshop:
Summer 2012: EMBO Practical Course on 3D Developmental Imaging, Oeiras, Portugal

2009: National workshop on Electron Microscopy, jointly conducted by BR Doshi School of Biosciences and Sophisticated Instrumentation Centre for Advanced Research and Testing (SICART), Gujarat, India

Scientific stays:
Spring 2012: Short term stay (4 weeks) in the laboratory of Prof. Liam Dolan, University of Oxford, England

Winter 2013: Short term stay (4 weeks) in the laboratory of Prof. Liam Dolan, University of Oxford, England

Spring 2017: Stay (4 months) in the laboratory of Prof. Heribert Hirt, KAUST, Saudi Arabia

Awards and Fellowships:
2011-2014: Marie-Curie Early Stage Researcher (ESR) Fellowship under the project PLANTORIGINS

2010: National Scholarship Program of the Slovak Republic

2009: Prof. I L Kothari Gold medal (M.Sc.)

2007: University Gold medal (B.Sc.)