# Controlled auxin biosynthesis and transport are important for developmental decisions in the early diverging land plant *Physcomitrella patens*

Eric RA Pederson
Faculty of Natural Resources and Agricultural Sciences
Department of Plant Biology
*Uppsala*

Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2014

Acta Universitatis agriculturae Sueciae
2014:107

Cover: Overlay image of a developing of protonemal filament in the *PpYUCF$_{pro}$::GFP* transcriptional reporter line.
(photo: Eric Pederson)

ISSN 1652-6880
ISBN (print version) 978-91-576-8162-1
ISBN (electronic version) 978-91-576-8163-8

Print: SLU Service/Repro, Uppsala 2014

# Controlled auxin biosynthesis and transport are important for developmental decisions in the early diverging land plant *Physcomitrella patens*


## Abstract

The aim of this thesis was to increase our understanding of the importance of the plant hormone auxin in land plant evolution. The role of a number of auxin regulatory network components in developmental decisions during the haploid phase of the life cycle of the model moss *Physcomitrella patens*, which belongs to the early diverging bryophyte group of land plants, was investigated. We show that the role of SHI/STY transcriptional activators in the regulation of auxin biosynthesis rates in seed plants is conserved in moss. The amount and rates of auxin synthesis are reduced in *PpSHI* knockout mutants, defects in these mutants can be mimicked by reducing active auxin in the *PpSHI* expression domain, and several moss homologues of *YUC* and *TAA1/TAR* genes, which encode the main auxin biosynthesis enzymes in seed plants, were activated in moss when PpSHI2 was transiently expressed. Our data reveal that these moss *YUC* and *TAA1/TAR* genes can induce auxin biosynthesis, and that their expression domains largely overlap with that of the two *PpSHI*s in certain stages of the moss life cycle. *PpSHI2* also directly or indirectly activates homologues of genes involved in polar auxin transport (PAT) in seed plants, *PpPINA*, *PpPINB* and *PpLAXB*. We show that the auxin efflux function of PIN proteins have been conserved during the evolution of land plants, and that they, together with the PpSHI auxin biosynthesis regulators, are important for determining developmentally regulated decisions, such as the switch between two cell types of the filamentous stage in moss. In addition, PIN-mediated distribution of auxin synthesised in the leaves plays an important role in determining a developmentally regulated wave of cell expansion. PpSHI regulated local auxin biosynthesis is crucial for several steps in reproductive organ development, such as egg cell maturation, clearance of a canal to the egg cavity, as well as apical opening of both male and female reproductive organs allowing sperm release and entrance into the egg cavity. If polar auxin transport plays a role during these stages remains to be studied. This demonstrates that regulated distribution of auxin was operational in cell-fate decisions already in the early land plants, and that auxin has played a key role in the evolution of complex land plant structures.



*Keywords: Physcomitrella patens*, moss, auxin, *SHI/STY*-family, auxin biosynthesis, auxin transport, plant evolution, YUCCA, TAA1/TAR, PIN



*Author's address:* Eric Pederson, SLU, Department of Plant Biology,
P.O. Box 7080, 75007 Uppsala, Sweden
*E-mail:* Eric.Pederson@slu.se

The only thing that is constant is change

Heraclitus of Ephesus, a Greek philosopher (c.535 BC - 475 BC)

# Contents

**List of Publications** **6**

**1 Introduction** **9**
1.1 Evolution of Plants 9
1.1.1 Evolution of the sporophyte 13
1.2 *Physcomitrella patens* as a model system 14
1.2.1 Lifecycle 16
1.2.2 Methods involving *Physcomitrella patens* 18
1.3 Auxin homeostasis, perception and signalling 20
1.3.1 Biosynthesis pathways 21
1.3.2 Transport 26
1.3.3 Modifications for storage and degradation 29
1.3.4 Perception and signalling 30
1.4 Auxin regulation of moss growth 32
1.4.1 Auxin homeostasis related homologues in *P. patens* 33
1.4.2 Developmental processes regulated by auxin in *P. patens* 35
1.5 Next generation sequencing 36

**2 Aims of the present study** **39**

**3 Results and Discussion** **41**
3.1 The PpSHI genes are expressed in auxin response sites and affects auxin biosynthesis rates in P. patens (I, II) 41
3.2 The PpSHI genes are required for vegetative and reproductive development or growth in *P. patens* (I,II) 42
3.3 PpSHI2 can activate a multitude of genes, several of which are involved in hormonal regulation (III) 43
3.4 Genes encoding auxin biosynthesis are expressed in sites operlapping with PpSHIs (I, II, IV) 45
3.5 PIN-mediated polar auxin transport works in concert with auxin biosynthesis to create auxin maxima (IV, V) 46

**4 Conclusions** **49**

**5 Future perspectives** **51**

**References** **53**

**Acknowledgements** **75**

# List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Eklund DM, Thelander M, Landberg K, Ståldal V, Nilsson A, Johansson M, Valsecchi I, Pederson ERA, Kowalczyk M, Ronne H & Sundberg E. (2010) Homologues of the *Arabidopsis thaliana SHI/STY/LRP1* genes control auxin biosynthesis and affect growth and development in the moss *Physcomitrella patens*. *Development* 137(3), 1275-1284.

*II* Landberg K, Pederson ERA, Viaene T, Bozorg B, Friml J, Jönsson H, Thelander M & Sundberg E. (2013) The moss *Physcomitrella patens* reproductive organ development is highly organized, affected by the two *SHI/STY* genes and by the level of active auxin in the SHI/STY expression domain. *Plant Physiology* 162, 1406-1419.

III Pederson ERA, Lagercranz U, Thelander M, Landberg K, Schwarzbach S & Sundberg E. (2014) The *Physcomitrella patens* SHI2 protein modulates the expression of genes involved in hormonal homeostasis and cell wall modification when transiently induced. (manuscript).

IV Pederson ERA, Landberg K, Thelander M, Viaene T, Vyas P, Sanchez V, & Sundberg E. (2014) Auxin biosynthesis is developmentally regulated by spatiotemportal control of *TAR* and *YUC* genes in *Physcomitrella patens*. (manuscript).

V Viaene T, Landberg K, Thelander M, Medvecka E, Pederson ERA, Feraru E, Cooper ED, Karimi M, Delwiche CF, Ljung K, Geisler M, Sundberg E, and Friml J. (2014) Directional auxin transport mechanisms in early diverging land plants. *Current Biology,* doi:10.1016/j.cub.2014.09.056

The contribution of Eric Pederson to the papers included in this thesis was as follows:

I Eric participated in the lab work, and genotyped some of the transformants.

II Eric participated in the planning of some parts of the project. He made the IAAL construct, made and genotyped the transformations of this construct, and made the qRT-PCR verification of their expression. He also made some of the expression studies of the reporter lines and made statistics of their expression at different developmental stages.

III Eric participated in planning of the project, did all the labwork, communicated with the SciLife sequencing facility, and continued the bioinformatics work initiated by Ulf Lagercrantz. Eric wrote the first draft of the manuscript.

IV Eric participated in planning of the project, made the majority of the constructs, transformations and genotyping. He also made expression studies and phenotyping of the lines. Eric wrote the first draft of the manuscript.

V Eric made a large number of the transformations to generate the transgenic lines used in this project. He also participated in the analysis of the reporter lines.

‘

# 1 Introduction

In the age of genome sequencing, relationships between species are becoming more transparent as the evolution of genes can be readily addressed by comparative studies in divergent model organisms. In plant science the model species *Arabidopsis thaliana* has so far been at the forefront of most research aimed at the elucidation of gene function to understand development. However, as a result of the genome sequences of many plants being completed, the origin and evolution of genes, and the developmental processes they govern, can now also be mapped. In this thesis the work will largely concern the relationship between the flowering plant *A. thaliana* and the moss *Physcomitrella patens*, two land plant species separated by at least 400 million years of evolution. Despite the evolutionary distance between *P. patens* and *A. thaliana*, they do share the ability to utilize phytohormones such as auxin as signalling molecules to regulate developmental processes. The research presented in this thesis is focused on *P. patens* genes assumed to be important for auxin function based on their similarity to genes in *A. thaliana*. A growing relationship between the SHI/STY family of transcriptional activators and auxin is demonstrated, and a characterization of genes encoding auxin biosynthetic enzymes and auxin efflux carriers in moss is presented.

## 1.1 Evolution of plants

Aquatic plants reminiscent of freshwater Charophycean algae (Streptophyte algae), began colonisation of land around 500-630 million years ago (Pickett-Heaps, 1969; Pickett-Heaps & Marchant, 1972; Stewart & Mattox, 1975; Graham, 1985; Karol *et al.*, 2001; Lewis & McCourt, 2004; Leliaert *et al.*, 2012). The adaptation to terrestrial life was probably gradual, with seasonal variations in access of water. Thus, means to monitor drought and systems for desiccation tolerance was required giving rise to the plants we know and use

today. With time, water acquisition became more important which resulted in not only a complex vascular system but also stomatal regulation and high-conductivity conduits (Sperry, 2003). Eventually, plants adapted further to life on land and evolved systems for underground nutrient and water acquisition, cold tolerance, secondary growth such as wood, various reproduction strategies and seed/spore dispersion. For example *Picea abies*, a spruce tree, can become quite large as a result of a complex vascular system (thus belonging to the tracheophytes, Figure 1A) with secondary growth, and can survive in the northern hemisphere during winter. However, even without some of these adaptations extant species thought to be reminiscent of early land plants, such as the bryophytes, still survive today. The bryophytes are also known as pre-tracheophytes, which refers to the lack of a complex vascular system (Lucas *et al.*, 2013). Comparison of tracheophytes and bryophytes with respect to land colonization shows a clear investment in different survival strategies. Although most mosses (which belong to the bryophytes) can tolerate desiccation, they rely on water for fertilisation. Seed plants have adapted to avoid desiccation by different means such as development of waxy cuticles and rely on an array of elements such as wind and animals for fertilisation.

Within the plant kingdom three clear groups can be separated; rhodophytes or red algae, glaucophytes or fresh water algae and the green plants, which include all land plants as well as charophytes and chlorophytes or green algae (Bowman *et al.*, 2007, Figure 1A). The land plants, also referred to as embryophytes because they all possess a multicellular sporophytic generation starting from an embryo, include angiosperms, gymnosperms, ferns, fern allies and bryophytes. The embryophytes originated from ancestral charophycean algae and this evolutionary relationship is recognized by the use of the collective term streptophyta, which comprise both extant charophytes and embryophytes.

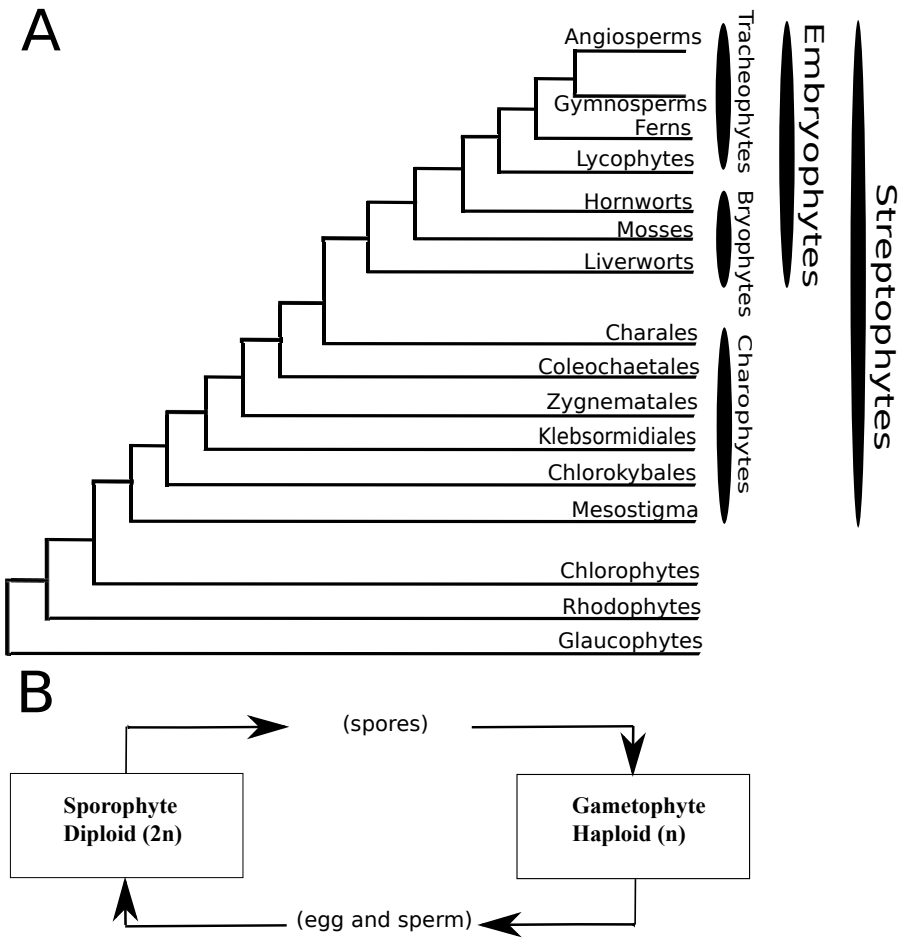


Figure 1. Evolution of the plant kingdom. Adapted from Bowman 2013. The plant kingdom comprises the rhodophytes (red algae), glaucophytes (fresh water algae) and the green plants including embryophytes (land plants), charophytes and chlorophytes (green algae). The embryophytes, including the angiosperms, gymnosperms, ferns, fern allies and bryophytes originated from ancestral charophycean algae and these two groups are together referred to as streptophytes. The paraphyletic group of bryophytes consist of liverworts, mosses and hornworts, the latter of which is believed to be the sister group of the tracheophytes (vascular plants). The ancestors of today's land plants eventually evolved and passed on the alternation of multicellular generations, which is the hallmark of all embryophytes (land plants) today (B).

Among extant charophycean algae, two groups, Coleochaetales and Charales, share certain traits with land plants and are thought to hold the key to how and when plants colonized land (Nishiyama, 2007; Bowman, 2013). Both have infrequent sexual reproduction and poorly understood species

distribution, and have so far only been sparsely studied (Delwiche *et al.*, 2002; Nishiyama, 2007). Based on morphological observations such as the retention and subsequent development of the zygote on the haploid parental body, as occurring in bryophytes, the genus Coleochaete was previously considered as a candidate for closest relative to land plants (Graham, 1985; Mishler & Churchill, 1985). However, more recent sequencing data utilizing the RUBISCO large subunit gene *rbcL*, place Charales as sister taxon to the land plants (Delwiche *et al.*, 2002). Interestingly, Charales is the one group of Charophycean algae that has demonstrated apical growth and also contain some compelling structures, such as rhizoids, which are common in bryophytes (Graham *et al.*, 2000). Despite the fact that many details are still missing, the phylogenetic tree shown in Figure 1 has at present strong support from the scientific community.

Little is known about the earliest land plants as much of the fossil record is missing from that period, but it is thought that bryophytes are a paraphyletic division and sister to all vascular plants (tracheophytes), containing three groups; hornworts, liverworts and mosses (Nishiyama *et al.*, 2004; Banks *et al.*, 2011). The group division is confirmed via mitochondria, chloroplast and nuclear genome data, which also delineates liverworts as the earliest diverging bryophytes (Groth-Malonek *et al.*, 2005; Groth-Malonek & Knoop, 2005; Qiu *et al.*, 2007). Spores collected from the Mid-Ordovician period (470 Ma) are the oldest recorded evidence of land plants and thought to have come from ancestral liverworts (Wellman *et al.*, 2003; Gensel, 2008). In addition, the predicted body plan of early land plants is dorsal-ventral, just as that of liverworts, whereas a radial body plan was acquired by mosses (Mishler & Churchill, 1985). Molecular clock estimates indicate minimum ages of 449 Ma for the divergence of liverworts and 420 Ma for the divergence of mosses (Clarke *et al.*, 2011). During the Carboniferous period (358.9–298.9 Mya) it is thought that mosses played an essential role in formation of modern wetlands (Hübers *et al.*, 2013). Today there are approximately 10,000 moss species that find suitable habitat in most places on Earth (Raven *et al.*, 2005). True mosses are found in the phylum Bryophyta, which contains eight classes including Bryidae (true mosses), which contains the model organism *Physcomitrella patens*. Despite the fact that the moss lineage has evolved for quite a large time frame, the fossil record suggests that the morphology of modern mosses is quite similar to ancient mosses indicating a slow rate of change in morphology (McDaniel & Shaw, 2003). Predecessors belonging to the third group of bryophytes, the hornworts, are believed to constitute the ancestors of vascular plants and are therefore often placed as their sister group in phylogenies.

### 1.1.1 Evolution of the sporophyte

Charophycean algae only produce one diploid cell, the zygote, that without any mitotic divisions directly undergoes meiosis to produce haploid spores (Graham, 1985; Graham *et al.*, 2000). All land plants have a more complex lifecycle involving an alternation of multicellular generations (Figure 1B). The multicellular diploid sporophyte generation, which is a defining feature of land plants, has evolved by including mitotic divisions of the zygote before meiosis, resulting in a multicellular embryo that can develop further before producing haploid spores via meiosis (Graham *et al.*, 2000; Pires & Dolan, 2012). Germinating spores induce the development of the multicellular haploid gametophyte generation producing gametes, which will fuse to produce a diploid sporophyte. During the evolution of land plants, the diploid sporophyte phase has become an increasingly dominant part of the lifecycle, while the haploid gametophyte has decreased and eventually lost its free-living stage (Haig & Wilczek, 2006).

One of the major questions in developmental biology concerns how the sporophyte generation has become dominant in tracheophytes while the first land plants had a dominant gametophyte generation, a trait retained in extant bryophytes. The sole purpose of the sporophyte generation of bryophytes is the production and dispersion of hardy spores, which will eventually start the gametophyte generation. In seed plants such as *A. thaliana* on the other hand, the sporophyte constitute essentially the entire plant body while the enclosed reduced gametophyte generation is utilized only for reproduction. The bryophyte sporophyte is made up of several tissue types, depending on the species in question, but generally lack any real complexity (Graham *et al.*, 2000). Thus, the bryophyte sporophyte is dependent on its gametophyte host and requires both energy and carbon input from it (O'Donoghue *et al.*, 2013). Despite much research the complexity and origin of the ancestral sporophyte remains elusive (Shaw *et al.*, 2011).

A considerable amount of research has been performed to elucidate the origin of the sporophyte building on the hypothesis that pre-existing gene networks of the gametophyte were recruited to the sporophyte generation. If the hypothesis holds true, genes homologous to those that are active in *e.g.* the gametophyte generation of *P. patens* should be regulating similar processes in the sporophyte of seed plants. Indeed, *A. thaliana* and *P. patens* share 84% of the 424 land-plant orthologous gene groups (Banks *et al.*, 2011). Two examples of gene recruitment are the *KNOTTED1-LIKE HOMEOBOX* (*KNOX*) and *ROOT HAIR DEFECTIVE* (*RHD*) genes encoding transcription factors. These genes appear to perform similar functions in the *P. patens* gametophyte generation and the sporophyte generation of *A. thaliana*

(Masucci & Schiefelbein, 1994; Menand *et al.*, 2007; Sakakibara *et al.*, 2008, 2013). RHD transcription factors regulate root hair development in *A. thaliana* while in *P. patens* they regulate the development of the root hair like rhizoids (Menand *et al.*, 2007; Jang *et al.*, 2011). Furthermore, the transition between gametophyte and sporophyte also marks major changes in the transcriptome which suggests that altered transcription factor activity and changes in gene expression is the base for developmental changes involved in the alternation of generations (O'Donoghue *et al.*, 2013). This suggests that evolutionary changes, as a result of reorganisation in developmental gene regulatory networks, are shown in the body plan of multicellular organisms (Erwin & Davidson, 2009) including the growth of both the gametophyte and sporophyte generations.

## 1.2 *Physcomitrella patens* as a model system

The moss *P. patens* and the liverwort *Marchantia polymorpha* are at present time the two main model organisms for long distance comparative studies between bryophytes and seed plants. Both these plants fall into phylogenetic grades of long branches, and the moss *P. patens* in fact shares a more recent common ancestor with the flowering plant *A. thaliana* than with the liverwort *M. polymorpha* (Bowman, 2013). Thus, as these extant taxa have evolved further during several hundred million years, they have most likely lost characters relative to their ancestral species, which they shared with the flowering plant lineage. Similarly, they are likely to have derived other characters after their divergence from the flowering plant lineage. Knowledge of genetic programs shared between species in the different grades may help to understand how ancestral genetic programs have been modified and co-opted during the evolution (Bowman, 2013). As my work is based on comparative studies of *P. patens* and *A. thaliana*, I will focus on the presentation of *P. patens* in this chapter.

Apart from its basal position in the phylogeny of the plant kingdom, there are many practical aspects that make *P. patens* a useful organism for comparative studies. First of all, the *P. patens* genome sequence (480 mega-base pairs) was published in 2008, and since has had problematic stretches re-sequenced several times to include a more complete annotation as well as new information concerning transcript isoforms and non-protein-coding loci (Rensing *et al.*, 2008; Zimmer *et al.*, 2013). This revealed that many gene families present in *A. thaliana* were also present in *P. patens*, and hence most likely also in their common ancestor. This allows for direct comparisons of gene sequences and comparative studies of gene function. The genome

sequence confirmed a relatively recent genome duplication in *P. patens* which had previously been suggested from EST sequencing projects (Lang *et al.*, 2005; Rensing *et al.*, 2008). Genome duplication in plants is not only fairly common but is usually followed by a period of gene loss, a dominant mechanism for speciation (Guo *et al.*, 2013). However, the gene-pairs produced by this late *P. patens* polyploidization event are often quite similar, and suggests a high degree of gene redundancy.

Secondly, totipotency or reprogramming of any cell into a protonemal stem cell state that confers pluripotentiality (Xiao *et al.*, 2012) allows *P. patens* to be blended or protoplasted only to be brought back to a well-differentiated colony from a single cell without passing any callus phase or hormone treatments, making tissue culture under sterile conditions easy. Protonemal tissue can also form from excised leaf pieces, if placed onto basic media.

Thirdly, one important point concerning the use of *P. patens* is its simple and inexpensive laboratory practices. Basic growth parameters for *P. patens* includes constant incubation at 25°C in continuous light on plates containing a low nutrient mixture and agar without the need for sugars or hormones, allowing for simple cultivation practises (Reski, 1998). The media is poured into petri dishes, which can house many individual colonies for months and numerous such dishes can be fitted into one simple growth cabinet. As stated above *P. patens* can be propagated via blending, or simply pulling off a piece, in sterile conditions. It is also possible to grow *P. patens* in liquid cultures depending on your experimental setup.

Fourthly, *P. patens* is easily transformed genetically, which is a prerequisite for comparative studies of gene functions. In addition, a high rate of homologous recombination occurs spontaneously in this species and much effort has been put into understanding the mechanism of gene targeting as well as to exploiting it (Schaefer & Zryd, 1997; Schlink & Reski, 2002; Kamisugi *et al.*, 2005, 2006). Homologous recombination, which is the exchange of nucleotide sequences between similar DNA molecules, allows for gene targeting or the incorporation of foreign DNA into a specific chromosomal location. The frequency of gene targeting in *P. patens* depends on the length of the homologous sequences, and with flanking sequencing being 1 kb a frequency of around 70-80% can be achieved compared to 7-20% when only 400 bp is used (Kamisugi *et al.*, 2005). This should be compared to most other plant species, which only reach an average between $10^{-1}$ to $10^{-3}$% (Hanin & Paszkowski, 2003; Trouiller *et al.*, 2007). By utilizing the DNA repair machinery to induce a double strand break at the target locus, it has recently been possible to reach homologous recombination frequencies around 1% in *A. thaliana* (Fauser *et al.*, 2012). Therefore, gene targeting in *P. patens* remains

considerably more efficient than in most other studied plant species. However, *P. patens* is not the only moss with this ability, as homologous recombination was found in *Ceratodon purpureus*, also belonging to the Bryidae class, though with quite a lower frequency (Trouiller *et al.*, 2007). It is believed that bryophytes in general are well equipped for homologous recombination as *M. polymorpha* has an efficiency approaching 2% (Ishizaki *et al.*, 2013). Consequently, after sequencing of the *P. patens* genome, high-efficient pipelines can now be constructed to locate interesting genes, such as homologues of genes found in flowering plants, and to produce a diverse range of transformants for functional studies of selected genes, using methods, which are discussed in detail below.

### 1.2.1 Lifecycle

The lifecycle of *P. patens* is illustrated in Figure 2. As for all bryophytes, it is dominated by the haploid gametophyte phase. When haploid spores germinate, they produce filamentous protonemal tissue of the type called chloronema. The chloronema cells are highly vacuolated, densely packed with chloroplasts in the peripheral cytoplasm for high photosynthetic capacity, and carry a centrally located nucleus with a striking nucleolus (Reski, 1998; Cove, 2005; Pressel *et al.*, 2008; Furt *et al.*, 2013). A second type of protonemal tissue, called caulonema, differentiates from chloronema tip cells. Caulonema cells have less chloroplasts, but a higher growth rate, and are thus more suited for colonisation of new areas for expansion of the colony. The differences between the chloronema apical cell and its derivatives are very subtle, whereas the apical caulonema cell differs from its derivative cells by carrying a limited amount of plastids (Pressel *et al.*, 2008; Furt *et al.*, 2013). The differentiation of caulonema from chloronema tip cells occurs gradually, why it can be difficult to exactly determine the nature of individual cells. A simple way to distinguish between the protonemal cell types is to locate the oblique cross-walls indicative of caulonema (Wyatt *et al.*, 2008). Expansion in both cell types occurs via tip growth (Jang & Dolan, 2011) and branching occurs soon after initiation of a new filament and continues throughout the life of the colony.

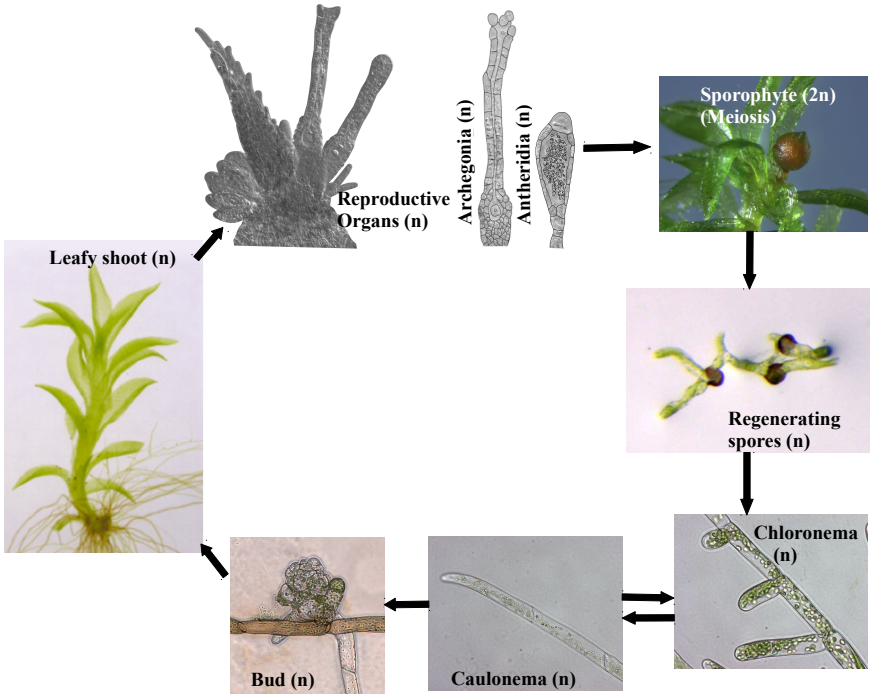


*Figure 2.* The lifecycle of *Physcomitrella patens*.

Leafy shoots or gametophores arise from buds, which are produced as lateral branches of caulonemal filaments (Cove, 2005). Tissues arising from the young gametophore include the stem, root like structures known as rhizoids, leaf-like structures known as microphylls and axillary hairs. The shoot and lateral organs such as microphylls ultimately develop from a single meristematic cell from the bud or the shoot apex, respectively (Harrison *et al.*, 2009). Growth of the stem occurs vertically from the bud it originates from and it contains certain specialized tissues such as a centralized vascular bundle of unknown function (Harrison *et al.*, 2009; Espiñeira *et al.*, 2011). Leaf-like structures exist as basal juvenile and upper adult leaves, the latter of which possess a midrib and serrulate margins (Barker, 2011). Leaf sizes increase dramatically during the early stages of development, which also leads to changes in the outline from approximately rectangular to lanceolate (Barker, 2011). Rhizoids are tip-growing filaments not unlike caulonemal tissue and have a variety of functions such as water transport and attachment to the growth substrate (Jang *et al.*, 2011; Jones & Dolan, 2012). In *P. patens* there are two types of rhizoids; basal rhizoids, which are formed at the base of the shoot and midstem rhizoids, which are formed in more apical regions (Sakakibara *et al.*, 2003).

*P. patens* gametophores will upon inductive conditions corresponding to autumn day length and temperatures enter into the reproductive phase. The female and male reproductive organs, or archegonia and antheridia respectively, are formed on the apex of the gametophore, surrounded by axillary hairs and young leaves. The archegonia form an egg cell within its cavity, to which the sperms formed by the antheridia must swim. Thus, reproduction must take place in a semi-aqueous environment. After fertilisation of the egg, a diploid sporophyte develops from the archegonia position on the gametophore apex as a proto-shoot structure, which remains relatively small and attached to the gametophyte (Glime, 2007). The sporophyte consists of a capsule, which via meiosis produces approximately 4000 haploid spores, and a connecting stalk or seta (Cove, 2000, 2005). The capsule does not have any specialised dehiscence structures but over time will crack and discharge the spores (Courtice *et al.*, 1978). *P. patens* has the ability to both self and cross fertilise (Cove & Knight, 1993).

### 1.2.2 Methods used in *Physcomitrella patens*

Blending or tissue disruption allows *P. patens* to be propagated easily without going through sexual reproduction. Though after some time, the ability to produce sporophytes will diminish (unpublished data). Thus, it is important to allow lab-strains to go through the sexual phase at least once a year in order to ensure the ability to produce sporophytes. Genetic transformation of *P. patens* utilizes protoplasts and though the methods have been described before (Nishiyama *et al.*, 2000), I will briefly present those used in my thesis work.

Sequences of genes for comparative functional studies can often be identified and retrieved based on similarity to seed plant homologues using an online genomic resource (e.g. Phytozome.net or Cosmoss.org). Using targeted homologous recombination it is possible to disrupt or delete genes, add a sequence encoding a tag to an endogenous gene which creates a translational knock-in reporter, or add any kind of construct (over-expressor or transcriptional reporter fusion) to a neutral site as a dump-site knock-in (Figure 3).

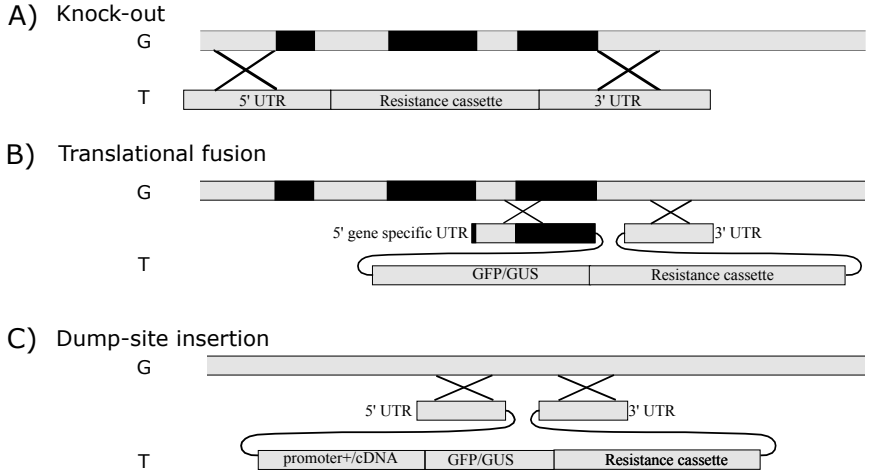


Figure 3. The production of genetically modified moss lines through homologous targeting of transgenic DNA. Knock-out (A) or deletion constructs have up- (5') and downstream (3') target UTR regions, which should be around 0.5-1 kb in length for proper integration. Translational fusion (B) constructs allow the integration of reporter proteins such as GFP and GUS into the native locus by destruction of the native stop codon in the 5' flanking region. They also require a 3' flanking, which, along with the 5' should also be 0.5-1 kb in length. The third approach, named dump-site insertion (C) here, utilizes neutral loci (BS213 or Pp108B) in the genome, where constructs can be integrated without any adverse effects per se. Many different types of constructs can be integrated in this way such as transcriptional promoter fusions, overexpressors, RNAi silencers, translational fusions and different kinds of inducible constructs. The transgene fragment should contain selection markers plus the modifying or reporter add-on(s), and the 0.5-1 kb 5' and 3' homology stretches. The genomic sequence is denoted with a G and the transgene with a T.

For overexpression of genes in *P. patens*, the rice actin promoter or cauliflower mosaic virus 35S promoters are common choices, and both have been used in papers in this thesis (I and V). Inducible expression systems can be very useful when working with e.g. essential genes or when searching for downstream pathways, and the XVE estrogen-inducible system (Nakaoka *et al.*, 2012; Kubo *et al.*, 2013), as well as the heat shock promoter *Gmhsp17.3B* have successfully been utilized as induction tools in *P. patens* (III; Saidi *et al.*, 2005; Aoyama *et al.*, 2012; Lavy *et al.*, 2012). In addition, the *GAL1* promoter has been used to induce gene expression using galactose, while repressing it with glucose (Qudeimat *et al.*, 2008).

In order to produce protoplasts for transformation, filamentous *P. patens* tissue is blended with a homogenizer, and the disrupted tissue is cultivated in cellophane covered plates containing a simple media (Frank *et al.*, 2005; Roberts *et al.*, 2011). Protoplasts are prepared by treating tissue five days after blending with driselase, containing laminarinase, xylanase and cellulase for cell wall degradation. After washing, PEG and heat shock are used to allow for

transformation in hypotonic solution. A large amount of linearized DNA of the plasmid(s) produced from the cloning must be prepared prior to transformation. A week after transformation, selection for stable transformants takes place during an eight-week period to discriminate between stable and unstable transformants. Unstables constitute transformants where the transgene persists extra-chromosomally as opposed to stable transformants, which have the transgene integrated chromosomally (Cove, 2000). The first 4 weeks the transfomants are placed on selection to kill off the non-transformants. Then the potential transformants are taken off selection for two weeks to allow for transgene loss in unstables, then back on selection for 2 weeks to discriminate between stable and unstable transformants. Four different selection markers are used in *P. patens* transformations; NPTII (geneticin), BleoR (zeocin), hygromycin B phosphotransferase (hygromycin) and blasticiden S-deaminase (blasticidin). After selection, the putative stable transformants must be genotyped before they can be used for phenotyping. This is performed by designing primers homologous to sequences inside the construct, usually in the reporter cassette or selection cassette, which together with primers homologous to genomic DNA flanking the recombination sequences will amplify DNA over the integration site. If the genotyped lines differ in phenotype, Southern blot can be used to ascertain which line only carry one copy of the transgene in the correct site.

For characterization of phenotypic changes induced by gene disruption or overexpression or to analyze expression patterns or protein localization it is important to compare tissues that are in the same stage. It is therefore common to blend the tissue several times in order to produce homogenous chloronemal starting material. Tiny balls of tissue are then rolled and placed onto deep petri-dishes with ammonium-free BCD media for 4-5 weeks, and during this period, different stages of protonemal and gametophore development can be studied using a simple light microscope, which often requires dissection of tissue samples. The sexual reproductive phase can be initiated using low temperature (15°C) and short day (8 hour light and 16 hour dark) conditions, which with a bit of water at the right time will facilitate fertilization and sporophyte development.

## 1.3 Auxin homeostasis, perception and signalling

Auxin is the name given to a group of molecules that are crucial regulators of plant growth and development, but can also be found in many other organisms. The majority of research conducted on auxins has been completed in *A. thaliana*, therefore, unless otherwise specified, the research discussed below

will be discussed in terms of *A. thaliana*. Auxins are defined as phytohormones, but also display several characteristics similar to that of a morphogen. For example, establishments of auxin gradients, maximas and minimums are required for the formation of new organs. Streamlined measurements dating back to the turn of the last century attributed to auxins has been vital in the establishment that auxin gradients are important for organeogenesis (Ljung *et al.*, 2001; Esmon *et al.*, 2006). Thus, auxin affects many developmental processes including embryogenesis, seedling growth, vascular patterning, and flower development and serves not only as a signal between closely positioned cells, but as a long distance signalling molecule between tissues and organs.

Indole-3-acetic acid (IAA) is the most important and abundant auxin found in all plants investigated, in free form as well as in conjugates with sugar esters, peptides and amino acids. The IAA molecule contains an indole ring structure coupled to a side chain connected to a carboxyl group. Aside from IAA intermediates and conjugates (described below) there are a few synthetic auxins produced commercially such as 1-naphthalene acetic acid (1-NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), 3,6-dichloro-2-methoxybenzoic acid (dicamba), and 4-amino-3,5,6-trichloropicolinic acid (picloram) which act in a similar manner as IAA (Colins *et al.*, 1978; Delbarre *et al.*, 1996; Ghoshdastidar & Tong, 2013). Despite the similarities, transport of the synthetic molecules occurs differently. 1-NAA requires an active transport out of the cell, but not into the cell, while 2,4-D requires active import, but can be exported out without active transporters (Delbarre *et al.*, 1996).

### 1.3.1 Biosynthesis pathways

IAA is synthesized mainly in young tissues (Ljung *et al.*, 2001, 2005) where it functions locally or is transported to other tissues. However, a complete picture of all auxin biosynthesis pathways in plants is still missing. The four most well characterized auxin pathways use Tryptophan (Trp) as a precursor. In addition, tryptophan-independent pathways do exist such as the indole-3-glycerol phosphate (IGP) pathway. IAA can also be formed through indole-3-butyric acid (IBA), whose own formation is not presently known and could be formed in either a Trp-dependant or -independent pathway (Ludwig-Muller 2000). Tryptophan is produced by the Shikimate pathway situated in the chloroplast through a series of eight enzymatic reactions (Ouyang *et al.*, 2000). The last step allows for the formation of a multitude of molecules including phenylalanine, which constitute a building block for aromatic secondary metabolites (Weaver & Herrmann, 1997). Trp is used for auxin synthesis in the indole-3-pyruvic acid (IPyA) pathway, indole-3-acetaldoxime (IAOx)

pathway, indole-3-acetamide (IAM) pathway and tryptamine (TRA) pathway (Figure 4).

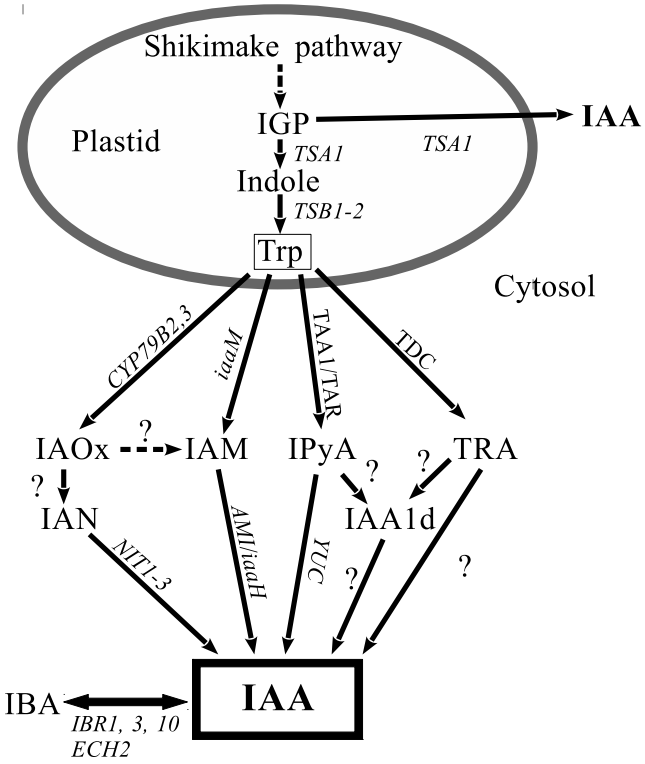


Figure 4. IAA biosynthesis pathways in *A. thaliana*. Enzymes are shown in italics, while pathway intermediates are in bold. Abbreviations: AMI, AMIDASE 1; ECH2, ENOYL-COA HYDRATASE 2; IAA, Indole-3-acetic acid; iaaM, TRYPTOPHAN-2-MONOOXYGENASE; iaaH, indole-3-acetamide hydrolase; IAA1d, 1-Methylindole 3 acetaldehyde; IAOx, Indole-3-acetaldoxime; IAM, indole-3-acetamide; IAN, indole-3-acetonitrile; IBA, Indole-3-butyric acid; IBR, INDOLE-3-BUTYRIC ACID RESPONSE 1, 3, 10;IGP, Indole-3-glycerol phosphate; IPyA, indole-3-pyruvic acid; NIT1-3, NITRILASE 1-3;TRA, tryptamine; TDC, TRYPTOPHAN DECARBOXYLASE; Trp, Tryptophan; TSA1, TONSUKU-associated protein 1; TSB1-2, TRYPTOPHAN SYNTHASE BETA-SUBUNIT 1-2; YUC, YUCCA.

The IPyA auxin biosynthesis pathway is the most important in *A. thaliana* (Stepanova *et al.*, 2008; Tao *et al.*, 2008) and has been suggested to play a main role also in most other embryophytes (Reviewed by Tivendale *et al.*, 2014). The first step involves the conversion of Trp to IPyA followed by a rate-limiting step converting IPyA to IAA (Mashiguchi *et al.*, 2011). Production of IPyA is catalyzed by the action of alliinase tryptophan aminotransferases encoded by *TAA1/TAR* genes identified in several plant species including *A.*

*thaliana* (Stepanova *et al.*, 2008, 2011), *Zea mays* (Chourey *et al.*, 2010; Phillips *et al.*, 2011) and *Pisum sativum* (Tivendale *et al.*, 2012). Mutations in the *TAA1/TAR* genes result in reduced IAA levels and typical phenotypes of auxin depletion (Stepanova *et al.*, 2008; Tao *et al.*, 2008). The activity of the *TAA1/TAR* family members is critical throughout plant development, *i.e.* for proper embryogenesis, seedling growth, flower development, vascular patterning, lateral root formation, tropism, and shade avoidance (Hardtke & Berleth, 1998; Stepanova *et al.*, 2008; Tao *et al.*, 2008). During early stages of embryogenesis *TAA1* expression is restricted to a few apical protodermal cells (Stepanova *et al.*, 2008; Robert *et al.*, 2013), which coincides with the expected site of auxin production feeding auxin via transport to the root poles (Weijers *et al.*, 2006).

Conversion of IPyA to IAA in the IPyA pathway is mediated by the *YUCCA* (*YUC*) gene family members, which encode flavin monooxygenase-like proteins (Zhao *et al.*, 2001; Mashiguchi *et al.*, 2011). A FAD cofactor has been identified which interacts with e.g. YUC6, and uses NADPH and oxygen in the conversion of IPyA to IAA (Dai *et al.*, 2013). IAA levels in *A. thaliana* increase as a result of overexpression of *YUC* genes (Cheng *et al.*, 2006; Kim *et al.*, 2007) whereas IPyA levels increase in multiple *yuc* mutant lines (Mashiguchi *et al.*, 2011). Interestingly, the phenotypic defects of *yuc* mutants cannot be rescued by exogenous IAA application, only by activation of auxin biosynthesis in the *YUC* expression domain, suggesting that local regulation of auxin production is a crucial process (Cheng *et al.*, 2006). There is a great deal of overlap between the *TAA1/TAR* and *YUC* gene expression domains as well as in phenotypes of mutants of multiple members of each family, such as e.g. defective embryos, flowers, vasculature and root growth (Cheng *et al.*, 2007; Won *et al.*, 2011; Chen *et al.*, 2014), characters also connected to loss of auxin perception or auxin transport (Hardtke & Berleth, 1998; Won *et al.*, 2011).

Although there is very little information concerning the transcriptional regulation of the *TAA1/TAR* genes, some upstream regulators of *YUC* genes have been identified. The SHI/STY family of transcriptional activators, which have 10 members in *A. thaliana*, encode a RING finger-like zinc-binding domain with DNA-binding capacity (Kuusk *et al.*, 2006). They play important roles in embryo, leaf and floral organ development as well as in vascularization (Kuusk *et al.*, 2006; Eklund *et al.*, 2010; Baylis *et al.*, 2013). STY1 activates *YUC4* and *YUC8* (Sohlberg *et al.*, 2006; Ståldal *et al.*, 2012) and has been shown to directly bind to a short sequence of the *YUC4* promoter (Eklund *et al.*, 2010). Similarly, PIF4, a member of the phytochrome-interacting factor (PIF) family interacts directly with *YUC8* to control hypocotyl elongation as well as auxin biosynthesis (Li *et al.*, 2012; Sun *et al.*, 2012). PIF4 and its

homologue PIF5 act downstream of the blue light sensor PHOTOTROPIN1 (PHOT1) and directly activate certain Aux/IAA transcriptional repressors (Sun *et al.*, 2013). These two seemingly contradictory pieces of information can only give insight into how complex this pathway must be and that it requires more research. Lastly, a third regulator of auxin biosynthesis known as SPOROCYTELESS/NOZZLE (SPL/NZZ) has been found to repress *YUC2* and *YUC6* transcription (Li *et al.*, 2008). Several phenotypes in the *spl-D* mutant such as fewer lateral roots, simpler venation patterns, and reduced shoot apical dominance overlap with phenotypes found in *yuc* mutants (Li *et al.*, 2008).

It is thought that the IAOx pathway is only found in the *Brassicaceae* family, although not all plant families have been studied so far. The majority of IAOx that is produced is accomplished via the *CYP79B* genes (Figure 3; Sugawara *et al.*, 2009). IAOx was not detected in rice, maize, and tobacco, which do not have apparent *CYP79B* orthologues (Sugawara *et al.*, 2009). The pathway is mainly utilized for the production of glucosinolates or camalexins, which are defence compounds (Mikkelsen *et al.*, 2004; Baskar *et al.*, 2012). In *A. thaliana,* two cytochrome P450's, CYP79B2 and CYP79B3, are involved in the conversion of Trp to IAOx. Overexpression of both cytochromes causes increase of not only IAA but also indole glucosinolates and indole-3-acetonitrile (IAN), while knock-out mutations cause a decrease in all three molecules plus IAM (Zhao *et al.*, 2002; Sugawara *et al.*, 2009). This suggests that production of IAA is a side branch of the main pathway that could also involve IAN and IAM. IAN can be converted to active IAA through the activities of the NIT1 family of nitrolases (Normanly *et al.*, 1997; Vorwerk *et al.*, 2001).

The IAM pathway, which was originally identified in bacteria such as *Agrobacterium tumefaciens,* is now also known as an alternative pathway for IAA synthesis in plants. In bacteria, IAA is produced in a two-step process involving first TRYPTOPHAN-2-MONOOXYGENASE (iaaM) to convert Trp to IAM. Thereafter indole-3-acetamide hydrolase (iaaH) accomplishes the conversion of IAM to IAA. In *A. thaliana* and other Brassicaceae species, IAM is mainly produced from IAOx (Sugawara *et al.*, 2009). However, plant species outside this family are thought to use other enzymes that are similar to the bacterial IAAM genes (Lehmann *et al.*, 2010). Conversion of IAM to IAA occurs via the AMIDASE1 (AMI1) and other members of the same enzyme family (Pollmann *et al.*, 2003). At present it is not known how widespread this pathway is throughout the plant kingdom but AMI1 homologues are found in many plants such as *P. sativum*, *Z. mays* and *Oryza sativa*, among others (Mano *et al.*, 2010). Previous research has exploited the robustness of the bacterial IAAM and IAAH enzymes to study elevated levels of auxin

biosynthesis in plants in specific sites by utilizing specific promoters. For example *YUC1*$_{pro}$*::IAAM* was used to rescue *yuc* mutant lines deficient in IAA (Cheng *et al.*, 2006) whereas *WOX5*$_{pro}$*::IAAH* was used to study the auxin dependant orientation of PINs at the quiescent centre after addition of IAM (Blilou *et al.*, 2005). Lastly, the enzyme IAA-lysine synthetase (IAA-L) conjugates free auxin and can be used in conjunction with a tissue specific promoter to deplete the tissue of auxin (II; Savka *et al.*, 2001).

Finally, in the TRA pathway a cytosolic Tryptophan decarboxylase (TDC) converts Trp to TRA (Fiore *et al.*, 2002). The rest of the pathway is still unresolved. Several earlier studies postulated that the *YUC* genes could be involved in producing *N*-hydroxytryptamine (Zhao *et al.*, 2001; Expósito-Rodríguez *et al.*, 2007; LeClere *et al.*, 2010), a possible precursor of TRA. However more recent studies have scrutinized the proposal as the *YUC* genes were found to be involved in the IPyA pathway (Stepanova *et al.*, 2008, 2011; Tivendale *et al.*, 2010; Ross *et al.*, 2011). In a study involving *P. sativum* no traces of *N*-hydroxytryptamine was found in young tissues, but rather indicated that the TRA auxin biosynthesis pathway to occurs as follows: Trp, tryptamine, IAAld, IAA (Quittenden *et al.*, 2009). However, a more detailed study by the same group could not detect any evidence to support that IAA biosynthesis occur via tryptamine as an intermediate (Tivendale *et al.*, 2010). Therefore this pathways true form is still unclear.

The auxin precursor IBA is almost identical to IAA and is converted to IAA to affect root hair and cotyledon cell expansion during seedling development (Strader *et al.*, 2010). It is not known how IBA is formed but it is hypothesized to be a Trp-dependant process. IBA is thought to be oxidized to IAA via the removal of two side-chain methylene units in a process similar to fatty acid β-oxidation (Zolman *et al.*, 2007, 2008). The candidates for IBA to IAA conversion are four peroxisomal enzymes, three INDOLE- 3-BUTYRIC ACID RESPONSE (IBR) isozymes and one ENOYL–COA HYDRATASE2 (ECH2) (Zolman *et al.*, 2007, 2008; Strader *et al.*, 2011). Seedlings carrying mutations in all four genes encoding these enzymes exhibit decreased free IAA levels leading to wide-ranging auxin-related developmental defects (Strader *et al.*, 2011).

Not much is known about the Trp-independent pathways or how many exist. One such pathway is known as the indole-3-glycerol phosphate (IGP) pathway whose existence was discovered via feeding studies utilizing stable labelled IAA precursors and different tryptophan biosynthesis mutants (Wright *et al.*, 1991; Normanly *et al.*, 1993). It is thought that the conversion from IGP into indole is catalyzed either by the TONSUKU-associated protein 1 (TSA1) subunit of the TS complex or by indole synthase (Ouyang *et al.*, 2000).

### 1.3.2 Transport

Transport of auxins can occur over long distance through the phloem or over short distances between nearby cells (Gälweiler *et al.*, 1998; Friml *et al.*, 2003; Blakeslee *et al.*, 2007). Auxin transported by the phloem becomes gradually unloaded to certain sinks, from which it can be redistributed via short distance cell-to-cell transport. For the most part cell-to-cell transport occurs via active polar auxin transport (PAT) or passive diffusion (Rubery & Sheldrake, 1974). As all auxins are weak acids, the pH of the environment affects the penetration ability through the plasma membrane (PM) (Bates & Goldsmith, 1983). Undissociated IAA molecules (IAAH+) enter cells by passive diffusion, as the pH of the apoplast is approximately 5.5. However, influx of dissociated IAA(-) can only occur via transporters of the AUXIN-RESISTANT1/LIKE AUX1 AUX1/LAX family (Swarup *et al.*, 2001, 2008) as well as the nitrate transporter NRT1.1 (Krouk *et al.*, 2010). Inside the cell, where the pH is approximately 7, the majority of the IAA molecules become dissociated IAA, and thus require active transport to leave the cell. There at least two types of auxin efflux carriers actively exports IAA, ATP-BINDING CASSETTE B/P-glycoproteins (ABCB/PGP) and PIN-FORMED proteins (PIN) (Noh *et al.*, 2001; Friml *et al.*, 2003). In addition, certain members of the PIN family (Ding *et al.*, 2012; Bender *et al.*, 2013), as well as members of the PIN-LIKES (PILS) family (Barbez *et al.*, 2012), control intracellular auxin trafficking. Utilizing these and probably more auxin transporters, asymmetric distribution of auxin contributes to auxin maxima or minima governing many different developmental processes.

The four *A. thaliana* AUX1/LAX family members, AUX1 and LAX1-3 are all functional influx carriers, which regulate auxin developmental processes in different parts of the plant (Péret *et al.*, 2012). Homologues of AUX/LAX have been found in both single celled and colony forming Chlorophyta species (De Smet *et al.*, 2011) as well as in all land plants studied (de Billy *et al.*, 2001; Schnabel & Frugoli, 2004; Rensing *et al.*, 2008; Pattison & Catalá, 2012). Even though auxin can diffuse into a cell without the aid of a carrier protein, certain processes require a high and rapid increase of auxin influx which simple diffusion cannot handle. Binding of IAA and several of its analogs to AtAUX1 have been shown under certain pH-dependent conditions (Carrier *et al.*, 2008). Furthermore, the *aux1* mutant cannot be rescued by auxins requiring active influx, while application of NAA can restore certain defects such as the *aux1* agravitropic phenotype (Yamamoto & Yamamoto, 1998; Marchant *et al.*, 1999). Although each AUX1/LAX family member appears to regulate specific developmental processes based on their distinct expression domains, some functional overlap have also been observed (Péret *et al.*, 2012). All

AUX1/LAX members perform a role in leaf phyllotaxy where sequential mutations result in increased disruption in phyllotactic organisation (Bainbridge et al. 2008). However, although AUX1 and LAX3 cooperatively regulate lateral root (LR) development, AUX1 controls the initiation (Marchant *et al.*, 2002) and LAX3 the emergence of LRs (Swarup *et al.*, 2008). Auxin influx facilitated by NRT1.1 in response to nitrogen starvation also controls LR formation (Krouk *et al.*, 2010). NRT1.1 activity represses LR growth by facilitating auxin uptake promoting basipetal auxin transport (Krouk *et al.*, 2010).

The PIN proteins are the most well studied auxin exporters, which comprise a small family of 8 members in *A. thaliana,* 5 of which are PM localised (PIN1, 2, 3, 4 and 7) while 3 are localised to the ER membrane (PIN5, 6 and 8) (Friml *et al.*, 2003; Mravec *et al.*, 2009; Ding *et al.*, 2012; Bender *et al.*, 2013). All PIN proteins have a similar structure which seems to be conserved throughout evolution, however the PINs that localise to the PM have a long central hydrophilic loop, while the ER localised PINs have a short loop (Viaene *et al.*, 2013). Direct evidence that PIN proteins can transport auxin came when they were expressed in human and yeast cells and there could transport exogenously added auxin across membranes (Petrášek *et al.*, 2006; Wiśniewska *et al.*, 2006). Further, altered auxin distribution in *pin* mutants result in severe phenotypes such as failure of lateral organ initiation. For example the *pin1* single mutant develops needle-like inflorescences that lack lateral organs (Gälweiler *et al.*, 1998). In addition, the PIN proteins that localise to the PM are important for organ development, as well as for vascularization and tropic responses (Reviewed by Petrášek & Friml, 2009). Further research has shown that to function properly the PM PIN proteins require several layers of posttranslational modifications and intercellular trafficking. Polar recycling of PINs to and from the PM allows for fast turnover for mobilisation in response to environmental and developmental stimuli. Endocytotic polar recycling of PIN proteins between the PM and endosomal compartments is regulated by ARF-GEF GNOM which is required for vesicle transport of PINs to establish and maintain PAT (Geldner *et al.*, 2001; Kleine-Vehn *et al.*, 2008; Luschnig & Vert, 2014). In addition, apical-basal targeting of polar recycling of PIN proteins is regulated by the phosphorylation of the vesicles which allows for cargo targeting to either the apical or basal side of the cell (Michniewicz *et al.*, 2007). Auxin appears to positively affect *PIN* gene expression (Vieten *et al.*, 2005), whereas the longevity of PIN proteins is negatively regulated by auxin (Baster *et al.*, 2013). Lastly, the short PINs, which mainly localize to the ER are important in intra-cellular auxin homeostasis (Dal Bosco *et al.*, 2012; Ding *et al.*, 2012; Ganguly *et al.*, 2014).

Further a new family of auxin transport proteins, PILS, has been identified which specifically regulate intracellular auxin accumulation in the ER (Barbez *et al.*, 2012). In knock-out mutant lines the amount of free IAA is increased, and the mutants show developmental defects such as increased density of lateral roots (Barbez *et al.*, 2012).

Several substances are known to block auxin efflux such as 1-N-naphthylphthalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA), which strongly inhibit auxin efflux and cause typical auxin depletion phenotypes (Keitt & Baker, 1966; Parry *et al.*, 2001; Rojas-Pierce *et al.*, 2007). These compounds have long been used to define sites of auxin efflux regulation by the long PINs. For example NPA can block root gravitropism, and as a result roots are unable to reorientate properly (Rashotte *et al.*, 2000; Rojas-Pierce *et al.*, 2007). As a result many mutations found in the *pin1* mutant are phenocopied when NPA is added (Geldner *et al.*, 2001).

The members of the plant ATP-binding cassette family of transporters have diverse functions associated with movement of a wide variety of molecules, nutrients, and zenobiotics (Reviewed by Verrier *et al.*, 2008). The ATP-binding cassette of these transporters is composed of two nucleotide-binding domains (NBD), which bind and hydrolyse ATP to allow transport (Hobson *et al.*, 1984; Higgins *et al.*, 1985). Of the 21 ATP-binding cassette transporters in *A. thaliana*, five show auxin transport capacity. These are the ABC-B/multidrug resistance/P-glycoproteins (ABCB/MDR/PGP) 1, 4, 14, 15 and 19 (Titapiwatanakun *et al.*, 2009; Kaneda *et al.*, 2011). The *abcb1* and *abcb19* single mutants show similar, but much less severe defects compared to *pin* single mutants (Noh *et al.*, 2001), whereas the *abcb1 abcb19* double mutant exhibits a strong decrease in PAT (Blakeslee *et al.*, 2007). The ABCB auxin transporters have been shown to localise at the PM mainly in a non-polar fashion, although they can be polarly localized in certain tissues, or conditions (Reviewed by Cho *et al.*, 2012). Independently of their localization, they contribute to long-range auxin transport (Geisler *et al.*, 2003, 2005; Blakeslee *et al.*, 2007; Bailly *et al.*, 2008). It has been suggested that these ABCBs act to minimize auxin reflux from the apoplast (Bailly *et al.*, 2012, 2014). As mentioned above, interaction between PIN's and ABCB's has also been proposed at the subcellular level to increase substrate specificity (Blakeslee *et al.*, 2007; Titapiwatanakun *et al.*, 2009). It has also been shown that ABCB4 is able to switch from importing to exporting auxin when auxin levels become high (Yang & Murphy, 2009).

Activity of the vacuolar ZINC INDUCED FACILITATOR-LIKE1.1 (ZIFL1.1) transporter is required for fine-tuning of polar auxin transport rates (Remy *et al.*, 2013). The ZIFL1.1 transporter is active during strong auxin

flows, where it actively promotes PIN1 abundance at the PM and thus is a positive modulator of PAT (Remy *et al.*, 2013).

To date WALLS ARE THIN1 (WAT1) is the only identified vacuolar auxin transporter, which has been found to transport IAA and other metabolites into vacuoles (Ranocha *et al.*, 2013). WAT1 is thought to be a $H^+$ -$IAA^-$ symporter, which helps to regulate auxin homeostasis in the cell (Ranocha *et al.*, 2013). Both auxin and tryptophan are decreased in the *wat1* mutant, which also shows up- and downregulated genes involved in secondary wall fibre production and vacuolar transport (Ranocha *et al.*, 2010, 2013).

The auxin precursor IBA use a specific set of transporters, separate from those used by IAA, which actively transports IBA across the PM. The IBA efflux carriers are members of the PLEIOTROPIC DRUG RESISTANCE (PDR) subgroup of the ABCG family of ABC transporters known as ABCG36 and 37. In accordance, IBA hyper-accumulates in root tips of the *abcg36* and *abcg37* mutants (Strader *et al.*, 2008; Strader & Bartel, 2011). To date nothing is known about the entry of IBA into cells.

### 1.3.3 Modifications for storage and degradation

Auxin-related molecules with different signalling and transport capacities are being produced in plants. This gives the plant the possibility to store or transport auxin in less active forms. These forms could either be converted back to active auxin again, or used as mildly active forms of auxin, or even result in permanent inactivation of auxin and subsequent degradation. The major forms of auxin destined for degradation include 2-oxoindole-3-acetic acid (oxIAA) and oxIAA-glucoside (oxIAA-Glc) (Zhao *et al.*, 2013; Tanaka *et al.*, 2014). Catabolism of IAA to oxIAA in rice, occurs through the dioxygenase for auxin oxidation (DAO) gene, encoding a putative 2-oxoglutarate-dependent-Fe (II) dioxygenase (Zhao *et al.*, 2013). The subsequent conversion of oxIAA to oxIAA-Glc can occur through two separate UDP-glycosyltransferases UGT74B1 (Jackson *et al.*, 2002) and UGT74D1 (Jin *et al.*, 2013; Tanaka *et al.*, 2014).

There are basically three forms of auxin conjugates used for temporary storage of IAA; conjugates to esters or acyl alkyl acetals (Tam *et al.*, 2000; Normanly, 2010), amides (Tam *et al.*, 2000) and amino acids (Seidel *et al.*, 2006). However, little is known about the ester and amide conjugates, only that some exist and may play a role in plant development (Tam *et al.*, 2000). In contrast, considerable amount of information is known about amino acid conjugation. The GRETCHEN HAGEN 3 (GH3) enzymes catalyze the formation of the following most commonly found IAA-amino acid conjugates; IAA-Asp, IAA-Glu, IAA-Leu and IAA-Ala (Tam *et al.*, 2000; LeClere *et al.*,

2002; Staswick, 2009). The reversion of IAA-amide conjugates to free IAA is catalyzed by amino hydrolases such as IAA-LECINE RESISTANT1 (ILR1), ILR1-LIKE1-5 (ILL1-5) and IAA-ALANINE RESISTANT3 (IAR3). These hydrolases display a high affinity for IAA-Leu and IAA-Ala but less affinity for other conjugates (LeClere *et al.*, 2002; Rampey *et al.*, 2004). Thus, hydrolysis of IAA-Leu and IAA-Ala will contribute to free IAA while hydrolysis of other conjugates has not been discovered yet.

### 1.3.4 Perception and signalling

Once auxin is synthesized it can be bound directly to an auxin receptor that elicits a biological response. Two receptor systems are known, the membrane localized AUXIN BINDING PROTEIN1 (ABP1) system and the nuclear SKP1/CULLIN/F-BOX PROTEIN (SCF) ubiquitin E3 ligase complex of which the latter, existing in two variants, has been investigated in more detail. The first variant of the SCF complex includes the F-box protein subunit TRANSPORT INHIBITOR RESPONSE 1 (TIR1), or one of its homologous proteins named AUXIN-RELATED F-BOX1-5 (AFB1-5), all of which bind to auxin with different affinities (Villalobos *et al.*, 2012). The complex $SCF^{TIR1/AFB1-5}$ is formed after one of the TIR1/AFB proteins bind auxin (Gray *et al.*, 1999; Farras *et al.*, 2001; Dharmasiri *et al.*, 2005). The phenotype of *tir1* is very weak due to the ability of the other AFB proteins to compensate for the loss of TIR1 (Li *et al.*, 2011). Mutations in several *AFB* genes result in severe phenotypical defects, similar to those caused by a loss in auxin response (Dharmasiri *et al.*, 2005). Upon TIR/AFB auxin-binding, the $SCF^{TIR1/AFB1-5}$ ubiquitin protein ligase complex is able to bind and ubiquitinate proteins from the AUXIN/INDOLE ACETIC ACID (Aux/IAA) family causing their degradation. Cells with low auxin levels retain the Aux/IAAs, which by forming heterodimers with members of the AUXIN RESPONSE FACTORS (ARF), a class of transcription factors binding auxin response elements (AuxRE) in the promoters of auxin regulated genes, inhibits the ARF proteins. However, after degradation of the Aux/IAAs the ARFs are released to activate or repress their target genes (Ulmasov *et al.*, 1999). An example of primary auxin response genes includes members of the SMALL AUXIN UP-RNA (SAUR) gene family. It was recently shown that auxin dependent SAUR activation causes increased activity of the PM $H^+$-ATPases, promoting cell-expansion (Spartz *et al.*, 2012, 2014).

Interestingly, the *Aux/IAA*s are also primary auxin responsive genes carrying AuxRE in their promoter regions. Most Aux/IAA proteins found in *A. thaliana* have 4 conserved domains. Domain I binds to co-repressor proteins such as TOPLESS (TPL), a cofactor required for transcriptional repression of

certain ARF's (Tiwari *et al.*, 2004; Szemenyei *et al.*, 2008). Domains III and IV are required to mediate dimerization with Aux/IAAs, ARFs and other proteins (Mockaitis & Estelle, 2008). Lastly, domain II contains an auxin degrading degron motif, which interacts with auxin, the $SCF^{TIR1/AFB1-5}$ complex, and the ARFs (Gray *et al.*, 2001; Ramos *et al.*, 2001). Interactions between different combinations of Aux/IAAs, ARFs, of which there are 29 and 23 in *A. thaliana* respectively, and $SCF^{TIR1/AFB1-5}$ complexes regulate different cellular processes (Finet *et al.*, 2012). Furthermore ARFs can be transcriptional activators or transcriptional repressors, however the activators more easily interact with the Aux/IAAs than do the repressors (Vernoux *et al.*, 2011). Together with the TIR/AFB receptors, the Aux/IAAs can form different combinatorial interactions depending on the binding capacity of the latter (Villalobos *et al.*, 2012). Therefore it is not surprizing that the phenotypes of lines carrying mutations in different Aux/IAAs are quite diverse (Reviewed in Reed, 2001). Despite the complexity of mapping an interactome between the Aux/IAAs and ARFs, a simple distribution and structure of this pathway was identified in the *A. thaliana* SAM (Vernoux *et al.*, 2011).

The potential to create a synthetic auxin response reporter has been exploited several times as a result of the work with the Aux/IAAs. For example the $DR5_{pro}$*::GUS/GFP* auxin response reporter construct, which consists of tandem direct 11 bp repeats including a TGTCTC sequence of the AuxRE, shows greater auxin responsiveness than a natural composite AuxRE or auxin inducible full-length promoters, such as the *GH3* promoter (Ulmasov *et al.*, 1997). The *DR5* promoter has been used to reveal auxin responsiveness across most tissue types. Another synthetic reporter construct is DII-VENUS, which uses a constitutive promoter (CaMV35S) to drive the expression of the fluorescent marker VENUS fused in-frame to the Aux/IAA auxin-interaction DII domain, which is rapidly degraded in response to auxin (Brunoud *et al.*, 2012). Characterization of the DII-VENUS auxin response reporter indicates that fluorescence levels are inversely correlated to endogenous auxin levels (Reviewed by Wells *et al.*, 2013). Furthermore, a control line, mDII-VENUS, containing a mutation in the DII sequence that disrupts the interaction between Aux/IAA, auxin and the TIR1/AFBs, was also constructed which allows the researcher to find out possible biases in the expression domains of the 35S promoter (Brunoud *et al.*, 2012). Recently a ratiometric auxin biosensor was reported that allows sensitive measurements of auxin after transient transformation in cell culture where two fluorescent markers are used in the same construct (Wend *et al.*, 2013).

The second SCF-related auxin receptor is the $SCF^{SKP2A}$ complex. The F-box protein S-Phase Kinase-Associated Protein 2A (SKP2A) can, just as the

TIR/SFBs, bind auxin directly. SKP2A is a cell cycle regulator and interacts with the cell cycle factors DIMERIZATION PARTNERS OF E2Fs b (DPB) to promote their degradation (Jurado *et al.*, 2008, 2010). DPBs are E2F transcription factors regulating gene expression in proliferating and differentiating cells (Pozo *et al.*, 2006). SKP2A is unable to induce degradation of DPB without auxin binding and mutations in *SKP2A* cause additive effects together with the *tir1* mutation, such as enhanced inhibition of root growth (Jurado *et al.*, 2010). At the moment research in this area is in its infancy, and more work is required before we have a clear understanding of the role of SKP2A in plant development.

The membrane associated auxin receptor, ABP1, was postulated to be an auxin receptor 40 years ago, however its biological relevance was unclear for a long time. ABP1 is located at the PM and ER controlling ion fluxes and acting upstream of non-transcriptional auxin responses such as inhibition of the clathrin-dependent endocytosis pathway (Robert *et al.*, 2010) by activating ROP GTPases (Chen *et al.*, 2012). Alone, ABP1 influences the recruitment of clathrins to the membrane, which then enhances the positioning of PIN auxin exporters at the PM by promoting endocytosis leading to reduced internalization (Robert *et al.*, 2010). However in the presence of auxin the recruitment of clathrin to the PM is inhibited, which results in reduced internalization of PIN proteins. This becomes very important in processes such as root gravitropism where unequal distribution of auxin exporters has been shown to guide the root downwards (Chen *et al.*, 2012). Furthermore, recent research has shown that ABP1 acts to negatively regulate the $SCF^{TIR1/AFB1-5}$ complex by stabilizing the Aux/IAA homeostasis and ultimately controlling their recruitment to the complex (Tromas *et al.*, 2013). This is a big step forward in understanding how regulation of auxin at the transcriptional and non-transcriptional levels interacts during plant development.

## 1.4 Auxin regulation of moss growth

The phytohormone auxin, described above with respect to *A. thaliana*, similarly regulates morphological processes in all embryophytes including mosses (Figure 1A). To date several moss species have been used to delve into the evolution of plant growth regulators, such as *P. patens*, *Funaria hygrometrica* and *Polytrichum obioense*, among others (Johri & Desai, 1973; Ashton *et al.*, 1979b; Cove & Ashton, 1984; Johri, 2008; Panigrahi *et al.*, 2009). IAA is synthesized in all three bryophyte groups, the liverworts, hornworts and mosses plus some species of algae (Sztein *et al.*, 2000; Johri, 2008). Further, the main auxin pathway in the moss *P. obioense* is a tryptophan

dependent pathway, similar to *A. thaliana* (Sztein *et al.*, 2000). In comparison to liverworts and hornworts, mosses and vascular plants have a meager amount of free IAA while an ample amount of IAA conjugates (Sztein *et al.*, 1995, 2000). Although mosses and vascular plants are both likely to regulate free IAA with conjugates, they produce different IAA conjugates (Sztein *et al.*, 1995, 1999).

### 1.4.1 Auxin homeostasis related homologues in *P. patens*

Genes encoding most major components of the auxin machinery exist in the *P. patens* genome (Table 1; Rensing *et al.*, 2008). Previous studies have shown that TIR1/AFB receptors and Aux/IAA repressors (Prigge *et al.*, 2010) as well as the GH3 enzymes driving auxin conjugation (Ludwig-Müller, 2011) are present and functionally conserved in *P. patens*. Additionally, gene inventories and phylogenetic studies indicate that homologues of the ARFs (Paponov *et al.*, 2009; Finet *et al.*, 2012), TPL (Causier *et al.*, 2012), PINs (Viaene *et al.*, 2013) and PILs (Feraru *et al.*, 2012) are also present in the *P. patens* genome.

*Table 1.* Copy number comparison of key auxin gene families from *A. thaliana* (A.t) and *P. patens* (P.p).

| Gene family | A.t | P.p | Function |
|---|---|---|---|
| *SHI/STY* | 10 | 2 | Regulation of Biosynthesis |
| *YUC* | 11 | 6-7 | Biosynthesis |
| *TAA1/TAR* | 5 | 6 | |
| *CYP69B* | 2 | ? | |
| *PIN* | 8 | 4 | Transport |
| *AUX1/LAX* | 4 | 4-8 | |
| *ABCB* | 5 | ? | |
| *PILS* | 7 | 5 | |
| *TIR1* | 7 | 4 | Receptors |
| *ABP1* | 1 | 1 | |
| *AUX/IAA* | 29 | 2 | Signalling |
| *ARF* | 24 | 14 | |
| *GH3* | 19 | 2 | Conjugation |
| *ILR* | 7 | *?* | *Re-activation* |

As stated in the previous section, the TIR1 receptor binds auxin allowing for the subsequent release of auxin signalling capabilities to effect downstream targets. As in *A. thaliana*, the PpAux/IAA proteins have been shown to interact

with proteins related to the AFB/TIR1 receptors, PpAFB (Prigge *et al.*, 2010). Further, point mutations in domain II of PpAux/IAA proteins, corresponding to mutations efficiently blocking auxin dependent degradation of the *A. thaliana* Aux/IAA homologues (Tian & Reed, 1999; Uehara *et a*l., 2008), lead to auxin insensitivity phenotypes such as arrest of chloronema to caulonema transition (Ashton *et al.*, 1979a; Prigge *et al.*, 2010). Thus, the mechanisms of auxin perception and signalling seems conserved as the genome encodes the necessary components and these interact with each other and auxin in the expected way (Prigge *et al.*, 2010). Further, a homologue of the tomato cyclophilin *DIAGEOTROPICA* (*DGT*) gene is involved in auxin signalling where mutational studies show a lower ratio of chloronema to caulonema and fewer rhizoids (Lavy *et al.*, 2012). DGT appears to affect a subset of auxin regulated genes including several AUX/IAA transcriptional repressors (Oh *et al.*, 2006), which is conserved throughout land plants (Lavy *et al.*, 2012).

In *A. thaliana* there are 19 GH3 proteins separated into three groups, whereas *P. patens* possess only two. Mutational studies of the two *PpGH3* genes did show an increase of free IAA and a contrasting decrease in conjugates formed (Ludwig-Muller *et al.*, 2009). The double knockout produced a meager amount of IAA conjugates but could still synthesize ester conjugates (Ludwig-Muller *et al.*, 2009). Despite the low amount of IAA conjugation, the GH3 proteins seem to only play a role during gametophore development, as protonemal development was unaffected (Ludwig-Muller *et al.*, 2009).

Many molecular tools, which have already been established in *A. thaliana*, have the potential to be used in *P. patens*. For example the $GmGH3_{pro}$*::GUS* and $DR5_{pro}$*::GUS* reporter constructs have been utilized to monitor auxin responses during *P. patens* development, though the latter proved to be less useful due to low overall expression levels (Bierfreund *et al.*, 2003). Generally, these reporters indicated that auxin responses were limited to young tissues (Bierfreund *et al.*, 2003), as is the case in flowering plants (Ljung *et al.*, 2001).

Lastly, PAT has been shown to exist in several algae species (Boot *et al.*, 2012; Raven, 2013; Zhang & van Duijn, 2014) and therefore evolved before the movement of plants onto land. Indeed during sporophyte formation in *P. patens*, PAT has been shown to participate in the regulation of its growth but not in the gametophore (Fujita *et al.*, 2008). Both *P. obioense* (Poli *et al.*, 2003) and *P. patens* (Fujita *et al.*, 2008) have been shown to transport auxin basipetally during sporophyte development. Similar to *A. thaliana* (Pagnussat *et al.*, 2009), embryogenesis is impaired with the addition of auxin transport inhibitors in *P. patens* (Fujita *et al.*, 2008).

### 1.4.2 Developmental processes regulated by auxin in *P.patens*

Development of protonemal, gametophore and sporophyte tissue requires the action of auxin in mosses. First, an increasing and convincing body of evidence supports that the gradual transition from chloronemal to caulonemal cell identity at the tip of protonemal filaments is stimulated by auxin. This suggestion originally stems from the long standing observation that exogenous auxin will cause early onset of the formation, and an increase in the relative abundance, of caulonema (Ashton *et al.*, 1979a; Cove, 2005). Auxin levels are furthermore suggested to peak in the tip cells of protonemal filaments (Bopp & Atzorn, 1992; Bierfreund *et al.*, 2003) which is the site where this differentiation process occurs. Further support is lent by the more recent observation that the point mutations in PpAux/IAA discussed above, likely to render insensitivity of this protein to auxin dependent degradation, results in the arrest or delay of chloronema to caulonema transition (Prigge *et al.*, 2010). The auxin stimulation of caulonema formation is at least partly mediated by the transcriptional activation by auxin of two ROOT HAIR DEFECTIVE SIX-LIKE1 (RSL) transcription factor genes (Jang & Dolan, 2011). Consequently, the *Pprsl1Pprsl2* double mutant does not respond to auxin and no caulonema is produced, even after the exogenous addition of auxin (Jang & Dolan, 2011). Finally, (Imaizumi *et al.*, 2002) has also shown that chloronema to caulonema transition is affected by light quality through cryptochrome mediated changes in auxin sensitivity.

The initiation of a gametophore shoot relies on changes in cell fate determination occurring in a single-celled side branch initial anchored in a caulonemal mother filament. This transition, resulting in a switch from filamentous tip growth to the three-dimensional growth of the shoot, is known to be triggered by cytokinin (Ashton *et al.*, 1979b; Decker *et al.*, 2006). In addition, bud formation has repeatedly been proposed to also require auxin activity (Lehnert & Bopp, 1983; Ashton *et al.*, 1979b; Sood & Hackenberg, 1979). While this may reflect an actual need for a combination of cytokinin and auxin for the change in cell fate to occur, the effect may also be indirect and reflect a hyperformation of caulonema giving more side branch initials capable of undergoing the transition.

Development of the gametophore shoot and its different organs furthermore appears influenced by auxin as judged from effects caused by exogenous application of the phytohormone. Systemic treatment with auxin is a relatively blunt tool and the physiological significance of some of these effects should be regarded as preliminary until confirmed by independent observations. Exogenous application of auxin first of all causes the upper part of the gametophore stem to elongate (Fujita *et al.*, 2008). The *GmGH3*$_{pro}$*::GUS*

reporter suggests this effect to be accompanied by an elevation and expansion of an auxin response domain normally restricted to a limited sub-apical region of the stem (Fujita *et al.*, 2008). Second, exogenous auxin also causes the elongation of mature leaf-like structures of the gametophore (Decker *et al.*, 2006; Barker, 2011). At higher concentrations the effects are even more devastating as long term treatment will suppress the formation of leaf-like structures altogether (Sakakibara *et al*. 2003) while short term treatment may cause them to wither and die (Barker, 2011). The proposed *GmGH3*$_{pro}$*::GUS* auxin response reporter shows activity in leaf-like structures following prolonged treatment with high concentrations of auxin while no signals above the detection limit are scored in absence of external auxin (Bierfreund *et al*., 2003; Fujita *et al*., 2008). Third, both the basal and mid-stem rhizoids can be induced via exogenously added auxin, albeit the latter also requires another unidentified signal (Ashton *et al.*, 1979b; Sakakibara *et al.*, 2003). In analogy with the auxin induction of caulonema formation, stimulation of rhizoid development is also mediated by the *PpRSL* genes (Jang *et al.*, 2011). The *GmGH3*$_{pro}$*::GUS* reporter indicates auxin response activity in both developing rhizoids and in the basal part of the stem from where basal rhizoids emerge (Bierfreund *et al*., 2003, Fujita *et al*., 2008). Finally, although a connection between axillary hairs and auxin has not been put forward prior to this thesis work, these organs display clear *GmGH3*$_{pro}$*::GUS* signals indicating that they may be sites of auxin response activity (Fujita *et al.*, 2008).

## 1.5 Next generation sequencing

High-throughput sequencing has increased in usage and importance over the recent years (Reviewed by Chu & Corey, 2012). This includes different technologies that allow whole genome sequencing, exome sequencing, targeted resequencing, transciptome analyses and methylation sequencing. Utilizing RNA-sequencing (RNA-seq) an entire transcriptome of any organism can be analysed and differentially expressed genes can be examined over many different conditions and stimuli. Thus, RNA-seq can be thought of as a modern screening process where candidate genes can be found for certain processes. For example, in Brown *et al.*, 2012 they were able to discover several candidate genes to use for the improvement of ricinoleic acid levels in transgenic plants. Due to the success of RNA-seq several pipelines have been proposed for both the wet lab and data analysis depending on the experiment being performed (Brouwer *et al.*, 2012; Lohse *et al.*, 2012; Patel & Jain, 2012; Torri *et al.*, 2012).

Before starting an experiment involving RNA-seq it is important to have a clear biological question as many error sources can appear, resulting in loss of data. Aims can vary from differential gene expression dependent of a condition via discovery of alternative splicing to annotation of miRNAs. Further, the project design will also dictate which platform will be chosen, such as Illumina, Roche454 or SOLiD and which specifications will be applied to each. Here I will briefly discuss the Illumina platform, which at the moment is best suited in most situations due to the low error rates, high sequencing rates and availability of software for data analysis. Several important steps for sample preparation are required before sequencing which are discussed in Brown *et al.* (2012) such as adding poly-adenylated tails to the RNA. Concerning sequencing specifications the amount of reads is an important question that will also vary with the context. For example in Wang *et al.* (2011) 30 million reads which were 75 bp in length was enough to detect all annotated genes in chicken lungs, while 10 million (75 bp) identified about 80%. Lastly, the number of biological replicates is always an important and controversial topic, as different levels of replicates will require different statistical analysis. However, if possible, the experimenter should aim for 3 but 2 is also acceptable (Anders *et al.*, 2013).

After the sequencing is complete the next step is to analyze the data. Several commercial and open-source software options exist, however only a pipeline for the latter will be discussed here. Two programs known as Tophat, a fast splice junction mapper for RNA-seq reads and Cufflinks (Trapnell *et al.*, 2010), used to reconstruct the full transcripts, resolve individual variants, and even quantitate expression analysis are used jointly to align reads to a reference genome. Once aligned differential expression analysis can be completed through either EdgeR or DESeq (Robinson *et al.*, 2010; Anders & Huber, 2012). All programs discussed above are available through the Bioconductor software package. Of course there are many other options, which are discussed throughout the community.

# 2 Aims of the present study

The intent of this work was to make an evolutionary study of the role of different components of the auxin regulatory network in the bryophyte *Physcomitrella patens*. A central part of the work has been on the functional characterization of *P. patens* homologues of the SHI/STY-family during the gametophytic part of the life cycle. As members of this transcription factor family were found to directly regulate the auxin biosynthesis genes *YUC4* and *YUC8* in *A. thaliana*, we aimed to investigate the role of PpSHIs in auxin related processes, and to elucidate if downstream targets of the SHI/STY family are conserved during land plant evolution. We also aimed to compare the role of endogenous auxin in developmental processes between moss and *A. thaliana*. For this we made reverse genetics on the *PpSHI* genes, moss homologues of auxin biosynthesis genes (*PpYUC*'s and *PpTAR*'s) and genes encoding auxin transporters (*PpPIN* and *PpLAX* genes).

# 3 Results and Discussion

## 3.1 The *PpSHI* genes are expressed in auxin response sites and affect auxin biosynthesis rates in *P. patens* (I, II)

The two PpSHI proteins encoded by the *P. patens* genome are very similar and belong to a plant specific transcription factor family, which was present in the plant lineage before the divergence of land plants from common ancestors with charophytae algae. The *SHI/STY* genes, which often have overlapping functions, play important roles in many steps of the angiosperm life cycle (Kuusk *et al.*, 2002, 2006; Sohlberg *et al.*, 2006; Eklund *et al.*, 2010; Zawaski *et al.*, 2011; Ståldal *et al.*, 2012; Yuo *et al.*, 2012; Baylis *et al.*, 2013). The *A. thaliana* SHI/STY protein STY1 directly binds to and activates the transcription of the auxin biosynthesis genes *YUC4* and *YUC8* (Sohlberg *et al.*, 2006; Eklund *et al.*, 2010; Ståldal *et al.*, 2012) suggesting that SHI/STY proteins contribute to the regulation of auxin peaks and gradients.

Here, we aimed to investigate if SHI/STY proteins control auxin biosynthesis also in early diverging land plants, such as the bryophyte *P. patens*, and if so, if the *PpSHI* genes could be utilized to understand the function of the plant hormone auxin during the moss plant life cycle. We therefore made knock-out (KO) mutant lines for each of the two *PpSHI* genes and could show that both the auxin biosynthesis rate and the IAA level are reduced in these lines compared to wild-type. In addition, overexpression (OE) of *PpSHI1* increases the levels of IAA, as well as of the inactive oxIAA conjugate. Together these results suggest that the PpSHI proteins indeed regulate auxin biosynthesis and thus also the levels of active auxin.

We also produced $PpSHI1_{pro.}$*:PpSHI1-GFP*, $PpSHI1_{pro.}$*:PpSHI1-GUS* and $PpSHI2_{pro.}$*::PpSHI2-GUS* translational knock-in (KI) reporter constructs to observe the endogenous expression domains of the *PpSHI* genes during the moss life-cycle. The expression pattern was compared to that of the auxin

response reporter *GmGH3$_{pro}$::GUS,* which previously had been used to detect sites of auxin signalling in moss (Bierfreund *et al.*, 2003; Fujita *et al.*, 2008; Ludwig-Muller *et al.*, 2009). Although the expression of the *PpSHI* genes and *GmGH3$_{pro}$::GUS* did not overlap during the protonemal phase, their expression pattern was almost identical in the adult gametophore. All three reporters showed expression maxima in rhizoids and axillary hairs of the leafy shoot. In addition, their expression strongly overlaps in the female reproductive organs, the archegonia, developing in the apex of the leafy shoot. *PpSHI2* is activated first, and is expressed in the apex and egg cell precursor of very young archegonia. The expression of *PpSHI1* is turned on slightly later, and both *PpSHI* genes are active in the egg cell as well as in the internal cells of the archegonial neck, which eventually will degrade to form an open canal guiding the sperm cells to the egg cell. The *GmGH3$_{pro}$::GUS* is activated after *PpSHI1*, and is expressed in the degrading canal cells, the egg cell, and in the apical cells that will detach to open the canal in the apex. This suggests that in moss, PpSHI-mediated auxin biosynthesis participates in creating the auxin maxima required for signalling and response in certain tissues and developmental stages.

## 3.2 The *PpSHI* genes are required for vegetative and reproductive development or growth in *P. patens* (I, II)

We then utilized the *PpSHI* KO, OE and KI lines to study the function of the two *PpSHI* genes during the haploid phase of the moss life cycle. In protonemata, *PpSHI* gene expression could only be detected in caulonemal filaments, and these filaments showed a reduced proliferation rate in the two *PpSHI* KO lines. By contrast, *PpSHI* OE enhanced caulonemata, and supressed chloronemata proliferation, just as exogenous application of auxin to wild-type protonemal tissue (Ashton *et al.*, 1979a), suggesting that the PpSHIs affect protonemal identity and proliferation at least partially through regulation of auxin biosynthesis. This would require the involvement of cell modifying and intercellular movement proteins known to be regulated by auxin. A reduced number of gametophores were produced in KO lines, most likely as a result of suppressed caulonema formation. Although *PpSHI* expression was not detected in the gametophore stem, the stem internode length was significantly reduced in the KO mutants, suggesting that auxin or some other transportable component synthesized under the control of PpSHIs in other tissues may be transported to the stem. The most likely tissues could be the axillary hairs positioned on the adaxial basal end of the leaves close to the stem, or the rhizoids, which directly connects to the stem. The importance of axillary hairs

in gametophore development is reflected by the increased number of hairs produced on each leaf of the KO lines. These are thought to be units producing mucilage for secretion as well as auxin, suggesting that more hairs may be required when biosynthesis rates are reduced in these structures. Although auxin is known to induce rhizoid production (Sakakibara *et al.*, 2003), the rhizoid initiation was unaffected in KO lines, whereas the position of mid-stem rhizoids was more restricted to the base.

In antheridia and archegonia the *PpSHI* KI expression domains overlapped precisely with sites of phenotypic deviations in the *PpSHI* KO lines. Egg cell development arrested prematurely in the KO lines, and this arrest could be phenocopied by reduction of auxin levels through the activation of the auxin-conjugating enzyme IAAL in the *PpSHI2* expression domain (*PpSHI2*$_{pro}$*:IAAL*). In addition, the degradation of the upper basal cell and the canal cells of the archegonia were also arrested in the *PpSHI* KO and *PpSHI2*$_{pro}$*:IAAL* lines, as were the opening of the apical end of both reproductive organs, prohibiting the expulsion of the sperms and the opening of the canal leading to the egg cavity. Together this suggests that the observed defects in the *PpSHI* KO reproductive organs are caused by a reduction in free auxin levels and that high levels of active auxin in the *PpSHI* expression domains are required for the differentiation of egg, ventral, apical, and canal cells.

## 3.3 PpSHI2 can activate a multitude of genes, several of which are involved in hormonal regulation (III)

In order to identify downstream targets of PpSHI2, we made a heat shock (HS) inducible construct, *GmHS*$_{pro}$*::PpSHI2*, and compared the transcriptomes of wild type and a line expressing this construct at two time points after HS treatment (1h HS + 1h recovery, and 1h HS + 3h recovery). A network of putative direct and indirect downstream target was discovered, including genes involved in auxin, cytokinin and ABA regulation as well as genes encoding enzymes involved in cell wall modifications, cell division and cell death. Although the direct and indirect targets cannot be separated at this point, the data suggest that different hormonal and cellular processes in moss are activated together, or sequentially during moss development.

Arguably the most significant finding is that several genes encoding IAA synthesis enzymes, belonging to the *YUC* and *TAA1/TAR* gene families, are activated by PpSHI2, which suggests a conserved function of SHI/STY members in land plant evolution (Eklund *et al.*, 2010b). SHI2 activity also mobilizes other pathways leading to increased IAA levels. These include the

activation of homologues of genes encoding MES17 and ECH2, which converts MeIAA and IBA to free IAA, respectively and IDD15 which is a direct transcriptional activator of auxin biosynthesis and transport genes (Cui *et al.*, 2013). Genes regulating auxin transport (auxin efflux, influx, and cellular compartmentalization) were also affected by the induction of PpSHI2, suggesting that PpSHI2 directly or indirectly regulates auxin distribution through several processes.

The transition of chloronema to caulonema is positively regulated by auxin (Ashton & Cove, 1990; Jang & Dolan, 2011), whereas ABA is promoting chloronema identity (Takezawa *et al.*, 2011). The downregulation of several ABA signalling elements following PpSHI2 induction may suggest that PpSHI2 directly, or via auxin accumulation, represses ABA responses in protonema to activate caulonema differentiation.

Similarly, bud formation involves the cross talk between ABA, cytokinin and auxin, where ABA inhibits and the latter two induces bud formation on caulonemal filaments (Cove & Ashton, 1984). Auxin and cytokinin are required simultaneously in order to stimulate bud induction, and as PpSHI2 activates cytokinin biosynthesis and receptor genes in protonema as well as auxin biosynthesis genes, PpSHI2 may act as a master regulator of bud formation. Both the transition of chloronema to caulonema and bud induction requires cell expansion and division processes. Several genes, which encode enzymes that promote cell wall modifications required for cell expansion, as well as cell division, are also activated by PpSHI2. Thus, PpSHI2 could be a key element in regulating cellular modifying decisions, or these activities could be mediated through PpSHI2 induced hormonal regulation.

Several components of the cell death pathway were also among the candidates significantly over-represented in a GO term analysis. It has been previously shown that cell death is under a certain level of auxin control (Lim *et al.*, 2007). We can thus speculate, as the majority of the candidates are down regulated or implicated in cell death prevention, that PpSHI2 acts to prevent cell death during cell division, modification and perhaps the transition of chloronema to caulonema.

Lastly, a large portion of differentially expressed genes lacked homologues in *A. thaliana*, which is not surprizing but quite fascinating. This represents a potential evolutionary difference between *A. thaliana* and *P. patens* but must be worked out with further laboratory work.

## 3.4 Genes encoding auxin biosynthesis enzymes are expressed in sites overlapping with PpSHIs (I, II, IV)

The *A. thaliana* TAA1/TAR and YUC enzymes act in the main auxin biosynthesis pathway known as the IPyA pathway (Mashiguchi *et al.*, 2011; Stepanova *et al.*, 2011; Won *et al.*, 2011). The genome of *P. patens* encodes six *TAA1/TAR* and seven *YUC* homologous genes. The capacity of the enzymes encoded by these moss genes to induce auxin responses was tested in a transient transformation assay. A heat shock (HS) inducible promoter was fused to the reading frame of each gene, and introduced into protoplasts of a line carrying the auxin response reporter $GmGH3_{pro}$*::GUS*. Upon HS induction of the transgenes, the auxin response signal was significantly elevated, suggesting that all PpTAR and PpYUC enzymes mediate auxin biosynthesis and that the role of *TAA1/TAR* and *YUC* genes has been conserved during land plant evolution.

To identify the expression domains of various *PpTAR* and *PpYUC* genes, we made transcriptional promoter-reporter constructs of all genes ($PpTAR_{pro}$*::GFP* and $PpYUC_{pro}$*::GFP*) and transformed these into wild-type *P. patens*. We identified stable transformants for two *PpTAR* and four *PpYUC* genes. In accordance with the *PpSHI* reporter lines, none of the auxin biosynthesis reporter lines show any detectable expression in the gametophore stem. However, just as the *PpSHI*s, four auxin biosynthesis genes are active in axillary hairs and the majority in rhizoids, suggesting that if the stem requires auxin for growth, it may be provided from either of these tissues. In addition, all studied auxin biosynthesis genes are, in contrast to the *PpSHI*s, also expressed in the leaves, suggesting that the PpSHIs cannot account for the activation of auxin biosynthesis in these organs. The expression of four of the five auxin biosynthesis genes initiate at the leaf tip of very young leaves, and the expression domain subsequently expands basally to cover around 2/3 of the leaf length. Only one gene, *PpYUCE*, is active in the basal-most part of the leaves.

An almost complete overlap between the expression domains of *PpSHI* and the studied auxin biosynthesis genes was detected during archegonial development. The overlapping domains include the egg precursor cell, the developing egg, the upper basal cell, the canal cells and the tip cells, suggesting that the developmental arrest of these cells observed in the *PpSHI* KO is caused by reduced auxin biosynthesis, which is also supported by the effects of $PpSHI2_{pro}$*::IAAL* expression (see 3.2). This indicates that *PpSHI* regulated auxin biosynthesis is essential for egg cell maturation, and for cell death to commence in the upper basal cell and the canal cells.

During protonemal growth the auxin biosynthesis genes show both distinct and overlapping expression patters with the *PpSHI* genes. *PpYUCE* and *TARF* are expressed in all caulonemal cells, just as the *PpSHI* genes. In addition, a member of the *AUX1*/*LAX* gene family, encoding PM localized auxin influx proteins in *A. thaliana*, *PpLAXB*, is expressed in caulonemal cells (manuscript III of this thesis). Although the role of auxin in the differentiation to caulonemal identity is well established, the role of auxin in already differentiated caulonemal cells is less understood. It could be important for the establishment of side branches and buds, as the initiation of these also requires controlled levels of auxin. In addition, main caulonemal filaments may be regarded as conductive canals supporting the tip cell with minerals or other compounds from the side branches, and auxin may play a role in the pigmentation and aging of these cells. *PpYUCF*, which was strongly activated by PpSHI2 in our HS-inducible system (see 3.3) is only expressed in young caulonemal cells, while we lack information about the expression domain of the second strongly activated *YUC* gene, *PpYUCG*. In addition to this, five of the studied biosynthesis genes show an apical expression maxima in the chloronemal and caulonemal tip cells, where cell expansion, cell division and sometimes also auxin dependent differentiation occur. As the *PpSHI* genes are not expressed in these cells, other upstream regulators must control this expression.

## 3.5 PIN-mediated polar auxin transport works in concert with auxin biosynthesis to create auxin maxima (IV, V)

Previous work has examined the role of the PIN proteins mediating PAT between cells in angiosperms, whereas their role during the evolution of land plants has remained elusive. The *P. patens* genome includes four *PIN* genes, of which three encodes PIN proteins with a long intracellular loop, and one with a short loop. In paper V of this thesis we could show that the long PpPINs localize to the PM and the short PIN to the ER, just as in *A. thaliana*. In protonemal filaments, the long PpPIN genes are expressed in a number of cells in the apical end of the filaments with an increasing strength towards the tip cell, largely overlapping with the expression pattern of the auxin biosynthesis genes (see section 3.4 above). In these cells, the long PpPIN proteins are localized at the apical PM suggesting that auxin is transported to the tip cell. Surprisingly, the long PpPIN also accumulate at the apical membrane of the tip cell, and we could demonstrate that auxin export from the protonemal filaments to the surrounding environment is dependent on the activity of the long *PpPIN* genes. The transition from the photosynthetically more active

chloronemal to the colony-spreading caulonemal identity of protonemal filaments, which occurs only in tip cells, is positively regulated by auxin (Ashton & Cove, 1990). Loss of long PpPIN activity, which results in entrapment of auxin in the tip cell, leads to premature transition to caulonemal identity, whereas overexpression of long PpPINs, results in enhanced auxin export and delayed protonemal identity transition. It may seem contradictory that auxin is produced in, transported to, and exported from the tip cells. We suggest a model in which the identity of the tip cell is dependent on all three activities and where these help inform the tip cell about the photosynthetic capacity of the colony, and thus the feasibility in transition to caulonemal identity (Figure 5). As the transition from chloronemal to caulonemal identity occurs in the chloronemal tip cell, it is possible to hypothesise that auxin levels above a certain threshold are required to induce this transition. This level can only be reached when the accumulated transport from sub-apical cells into the tip cell exceeds the efflux from the tip. As auxin is synthesised in several subapical cells with increasing intensity towards the tip, and PpPINs are localized apically in these cells, and if the export capacity out of the tip cell has a maximum capacity, more auxin will accumulate in the tip in longer compared to shorter chloronemal filaments, only allowing differentiation of the longer filaments to occur.

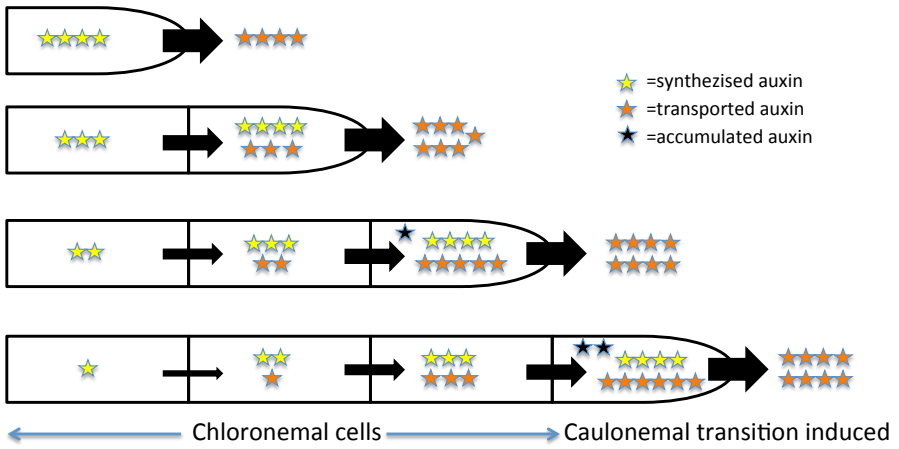


Figure 5. Theoretical model depicting the role of auxin biosynthesis and transport in the transition of chloronema to caulonema during protonemal tip growth. The maximum capacity of auxin transport is reached causing a build of auxin in the tip cell, triggering the transition from chloronema to caulonema. The stars represent an unknown amount of auxin as it is not known at this time how much auxin is transported between cells.

It has been shown that a wave of cell expansion moves basipetally during moss leaf development (Barker, 2011; paper V of this thesis), such that the

largest cells are found in the apex in very young leaves, in the middle in the following stages and most basally in mature leaves. The expression of both the *PpPIN* and the auxin biosynthesis genes follow a similar wave, starting from the apex and subsequently moving basally down the leaf. This suggests that local auxin biosynthesis as well as PAT is involved in regulating cell elongation during leaf development, and that the youngest leaves require an auxin maxima in the tip to induce the apical initial cell elongation. The final elongation of the basal-most cells appear dependent mainly on a flux of auxin from the middle region, as the expression of the majority of auxin biosynthesis genes overlap with that of the *PpPIN* genes in this region, whereas only one auxin biosynthesis gene, *PpYUCE*, is expressed albeit weakly together with *PpPIN*s in the basal region. In accordance with this suggestion, *PpPIN* KO mutant leaf cells in the apical and middle region of the leaf blade, which potentially should be exposed to higher auxin levels, are more elongated, also reported by Bennett et al (2014), whereas the cells of *PpPIN* OE leaves are shorter compared to wild type. The phenotype of the mutant leaves can be phenocopied by growing moss colonies on the auxin efflux inhibitor NPA, linking PIN involvement to its auxin transport activity.

Local auxin maxima play an important role during lateral organ initiation at the flank of the shoot apical meristem in plants (Benková *et al.*, 2003). In contrast, local auxin activities at the leaf initiation site, which consists of a single cell (Harrison *et al.*, 2009), have not been detected in *P. patens*. In accordance with this, we could not detect any activity of the analyzed auxin biosynthesis or *PpPIN* genes in shoot apical cells. This could suggest that auxin is either not important for leaf initiation, or provided by the activity of auxin homeostasis genes not so far studied. In addition, as axillary hairs are produced on the apex of gametophore buds prior to leaf initiation, the hairs may constitute a source of auxin for timing of leaf initiation.

# 4 Conclusions

The role of SHI/STY transcriptional activators in the regulation of auxin biosynthesis rates in seed plants is conserved in moss.

The *PpSHI* transcriptional family members regulate auxin biosynthesis by inducing several auxin biosynthesis genes belonging to the *YUC* and *TAR* families, which in seed plants are active in the main biosynthesis pathway.

The moss PpTAR and PpYUC proteins are functional auxin biosynthesis enzymes.

The role of PIN auxin efflux proteins is conserved during the evolution of land plants, and in moss they regulate PAT to, in concert with auxin biosynthesis, determine the timing of differentiation of protonemal tip cells as well as of cell division and cell expansion during leaf development

PpSHI regulated local auxin biosynthesis is crucial for several steps in reproductive organ development, such as egg cell maturation, clearance of a canal to the egg cavity, as well as apical opening of male and female reproductive organs to allow fertilization.

Regulated distribution of auxin was operational in cell-fate decisions already in the common ancestor of early diverging plant lineages

# 5 Future perspectives

We still do not know which of the genes affected by PpSHI activation that are direct downstream targets. This is important in order to understand the timing of events leading to changes in developmental decisions and could be studied by making a time-series of PpSHI activation and study the activity of downstream targets at earlier time points. In addition, ChIP studies using lines expressing *PpSHI-GFP* fusion proteins and GFP antibodies could help to confirm which genes that represent direct targets.

The roles of selected downstream targets potentially important for fate changes, such as putative auxin transporters belonging to the *LAX* and *WAT* gene families, could be studied by making KO, OE and translational GFP fusions.

It would certainly be interesting to go deeper into studies of the role of auxin during reproductive organ development. The question if auxin can trigger PCD could be answered by changing auxin levels in the cells targeted for degradation as well as in the egg cell. This could be achieved by knocking out *PpTAR* and *PpYUC* genes expressed in these cells, or by increasing the auxin levels using $GmHS_{pro}$*::YUC* or $PpSHI2_{pro}$*::IAAM* constructs. The effect of the changes in auxin levels could be studied on the cellular level using TEM, and changes in the transcriptome could be studied after laser capture of these cells at different developmental stages. This would allow the identification of processes related to auxin induced cell specific differentiation as well as candidates for specific markers or promoters for each cell type which would be useful for spatially restricted manipulation of the levels and/or responsiveness to auxin.

Several interesting patterns have emerged during the study of the auxin biosynthesis family members, which should be looked deeper into. First, the role of auxin during protonemal side-branch initiation, bud formation and chloronema to caulonema transition represent interesting areas, which could be

studied by the analysis of knockouts, overexpressors and reporters for genes involved in auxin synthesis, transport and perception in combination with various compounds such as L-kynurenine (He *et al.,* 2011), NPA (Keitt & Baker 1966) and auxinole (Hayashi *et al.,* 2012) affecting the same processes. To further challenge the model that subapical chloronemal cells may affect the chloronema to caulonema transition of the apical cell (Fig 5), we could attempt experiments where the transition of the apical cell is monitored after subapical cells have been cut off or abolished with a laser.

Further, the position of axillary hairs with auxin biosynthetic capacity in immediate vicinity to the meristematic cell in the shoot apex raises the question if these organs could be important for the initiation and/or early development of new leaves. Laser capture followed by transcriptomics of the axillary hairs could be an option to pinpoint cellular processes active in these cells and to discover hair specific promoters which could be used to test if the expression of various auxin related constructs in the hairs could affect lateral organ emergence at the shoot tip. The development of the blades of leaves has furthermore been shown to be tightly regulated by auxin biosynthesis and transport while possible auxin effects on the development and function of the leaf midrib represents yet another potentially interesting area.

Our results have clearly identified a distinction between the expression domains of auxin biosynthesis and transport and those of the $GmGH3_{pro}$*::GUS*, with latter possessing fewer. Thus, an auxin response reporter that encompasses all sites of auxin action during the lifecycle to be used to map auxin expression domains is an important tool for the future of auxin research in *P .patens*.

Lastly, we have focused on the haploid part of the lifecycle as it is much easier to work with, however a more detailed study into the diploid sporophyte development could now be achieved utilizing the tools in this thesis. However, as the Grandsen *P. patens* line does not produce sporophytes at a high rate another line such as Reute should be used for this purpose.

# References


Anders, S. & Huber, W. (2012). Differential expression of RNA-Seq data at the gene level–the DESeq package. *Heidelberg, Germany: European Molecular Biology Laboratory (EMBL)*.

Anders, S., McCarthy, D. J., Chen, Y., Okoniewski, M., Smyth, G. K., Huber, W. & Robinson, M. D. (2013). Count-based differential expression analysis of RNA sequencing data using R and Bioconductor. *Nature Protocols* 8(9), 1765–1786.

Aoyama, T., Hiwatashi, Y., Shigyo, M., Kofuji, R., Kubo, M., Ito, M. & Hasebe, M. (2012). AP2-type transcription factors determine stem cell identity in the moss Physcomitrella patens. *Development* 3120-3129.

Ashton, N. W. & Cove, D. J. (1990). Mutants as tools for the analytical dissection of cell differentiation in Physcomitrella patens gametophytes. *Bryophyte development: physiology and biochemistry* 17–31.

Ashton, N. W., Cove, D. J. & Featherstone, D. R. (1979a). The isolation and physiological analysis of mutants of the moss, Physcomitrella patens, which over-produce gametophores. *Planta* 144(5), 437–442.

Ashton, N. W., Grimsley, N. H. & Cove, D. J. (1979b). Analysis of gametophytic development in the moss, Physcomitrella patens, using auxin and cytokinin resistant mutants. *Planta* 144(5), 427–435.

Bailly, A., Sovero, V., Vincenzetti, V., Santelia, D., Bartnik, D., Koenig, B. W., Mancuso, S., Martinoia, E. & Geisler, M. (2008). Modulation of P-glycoproteins by Auxin Transport Inhibitors Is Mediated by Interaction with Immunophilins. *Journal of Biological Chemistry* 283(31), 21817–21826.

Bailly, A., Wang, B., Zwiewka, M., Pollmann, S., Schenck, D., Lüthen, H., Schulz, A., Friml, J. & Geisler, M. (2014). Expression of TWISTED DWARF1 lacking its in-plane membrane anchor leads to increased cell elongation and hypermorphic growth. *The Plant Journal* 77(1), 108–118.

Bailly, A., Yang, H., Martinoia, E., Geisler, M. & Murphy, A. S. (2012). Plant Lessons: Exploring ABCB Functionality Through Structural Modeling. *Frontiers in plant science* 108-108.

Banks, J. A., Nishiyama, T., Hasebe, M., Bowman, J. L., Gribskov, M., dePamphilis, C., Albert, V. A., Aono, N., Aoyama, T., Ambrose, B. A., Ashton, N. W., Axtell, M. J., Barker, E., Barker, M. S., Bennetzen, J. L., Bonawitz, N. D., Chapple, C., Cheng, C., Correa, L. G. G.,

Dacre, M., DeBarry, J., Dreyer, I., Elias, M., Engstrom, E. M., Estelle, M., Feng, L., Finet, C., Floyd, S. K., Frommer, W. B., Fujita, T., Gramzow, L., Gutensohn, M., Harholt, J., Hattori, M., Heyl, A., Hirai, T., Hiwatashi, Y., Ishikawa, M., Iwata, M., Karol, K. G., Koehler, B., Kolukisaoglu, U., Kubo, M., Kurata, T., Lalonde, S., Li, K., Li, Y., Litt, A., Lyons, E., Manning, G., Maruyama, T., Michael, T. P., Mikami, K., Miyazaki, S., Morinaga, S., Murata, T., Mueller-Roeber, B., Nelson, D. R., Obara, M., Oguri, Y., Olmstead, R. G., Onodera, N., Petersen, B. L., Pils, B., Prigge, M., Rensing, S. A., Riaño-Pachón, D. M., Roberts, A. W., Sato, Y., Scheller, H. V., Schulz, B., Schulz, C., Shakirov, E. V., Shibagaki, N., Shinohara, N., Shippen, D. E., Sørensen, I., Sotooka, R., Sugimoto, N., Sugita, M., Sumikawa, N., Tanurdzic, M., Theißen, G., Ulvskov, P., Wakazuki, S., Weng, J.-K., Willats, W. W. G. T., Wipf, D., Wolf, P. G., Yang, L., Zimmer, A. D., Zhu, Q., Mitros, T., Hellsten, U., Loqué, D., Otillar, R., Salamov, A., Schmutz, J., Shapiro, H., Lindquist, E., Lucas, S., Rokhsar, D. & Grigoriev, I. V. (2011). The Selaginella Genome Identifies Genetic Changes Associated with the Evolution of Vascular Plants. *Science* 332(6032), 960–963.

Barbez, E., Kubeš, M., Rolčík, J., Béziat, C., Pěnčík, A., Wang, B., Rosquete, M. R., Zhu, J., Dobrev, P. I., Lee, Y., Zažímalovà, E., Petrášek, J., Geisler, M., Friml, J. & Kleine-Vehn, J. (2012). A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. *Nature* 485(7396), 119–122.

Barker, E. I. (2011). *An Examination of Leaf Morphogenesis In The Moss, Physcomitrella Patens*. Diss. Faculty of Graduate Studies and Research, University of Regina. Available from: http://ourspace.uregina.ca:8080/xmlui/handle/10294/3530.

Baskar, V., Gururani, M. A., Yu, J. W. & Park, S. W. (2012). Engineering Glucosinolates in Plants: Current Knowledge and Potential Uses. *Applied Biochemistry and Biotechnology* 168(6), 1694–1717.

Baster, P., Robert, S., Kleine-Vehn, J., Vanneste, S., Kania, U., Grunewald, W., De Rybel, B., Beeckman, T. & Friml, J. (2013). SCFTIR1/AFB-auxin signalling regulates PIN vacuolar trafficking and auxin fluxes during root gravitropism. *The EMBO Journal* 32(2), 260–274.

Bates, G. W. & Goldsmith, M. H. M. (1983). Rapid response of the plasma-membrane potential in oat coleoptiles to auxin and other weak acids. *Planta* 159(3), 231–237.

Baylis, T., Cierlik, I., Sundberg, E. & Mattsson, J. (2013). SHORT INTERNODES/STYLISH genes, regulators of auxin biosynthesis, are involved in leaf vein development in Arabidopsis thaliana. *New Phytologist* 197(3), 737–750.

Bender, R. L., Fekete, M. L., Klinkenberg, P. M., Hampton, M., Bauer, B., Malecha, M., Lindgren, K., A. Maki, J., Perera, M. A. D. N., Nikolau, B. J. & Carter, C. J. (2013). PIN6 is required for nectary auxin response and short stamen development. *The Plant Journal* 74(6), 893–904.

Benková, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertová, D., Jürgens, G. & Friml, J. (2003). Local, Efflux-Dependent Auxin Gradients as a Common Module for Plant Organ Formation. *Cell* 115(5), 591–602.

Bennett, T. A., Liu, M. M., Aoyama, T., Bierfreund, N. M., Braun, M., Coudert, Y., Dennis, R. J., O'Connor, D., Wang, X. Y., White, C. D., Decker, E. L., Reski, R. & Harrison, C. J.Plasma Membrane-Targeted PIN Proteins Drive Shoot Development in a Moss. *Current Biology* DOI: 10.1016/j.cub.2014.09.054.

Bierfreund, N. M., Reski, R. & Decker, E. L. (2003). Use of an inducible reporter gene system for the analysis of auxin distribution in the moss Physcomitrella patens. *PLANT CELL REPORTS* 21(12), 1143–1152.

De Billy, F., Grosjean, C., May, S., Bennett, M. & Cullimore, J. V. (2001). Expression Studies on AUX1-like Genes in *Medicago truncatula* Suggest That Auxin Is Required at Two Steps in Early Nodule Development. *Molecular Plant-Microbe Interactions* 14(3), 267–277.

Blakeslee, J. J., Bandyopadhyay, A., Lee, O. R., Mravec, J., Titapiwatanakun, B., Sauer, M., Makam, S. N., Cheng, Y., Bouchard, R., Adamec, J., Geisler, M., Nagashima, A., Sakai, T., Martinoia, E., Friml, J., Peer, W. A. & Murphy, A. S. (2007). Interactions among PIN-FORMED and P-Glycoprotein Auxin Transporters in Arabidopsis. *The Plant Cell Online* 19(1), 131–147.

Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K. & Scheres, B. (2005). The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. *Nature* 433(7021), 39–44.

Boot, K. J. M., Libbenga, K. R., Hille, S. C., Offringa, R. & van Duijn, B. (2012). Polar auxin transport: an early invention. *Journal of Experimental Botany* 63(11), 4213–4218.

Bopp, M. & Atzorn, R. (1992). morphogenetic system of the moss protonema. *Cryptogamic botany*.

Dal Bosco, C., Dovzhenko, A. & Palme, K. (2012). Intracellular auxin transport in pollen. *Plant Signaling & Behavior* 7(11), 1504–1505.

Bowman, J. L. (2013). Walkabout on the long branches of plant evolution. *Current Opinion in Plant Biology* 16(1), 70–77.

Bowman, J. L., Floyd, S. K. & Sakakibara, K. (2007). Green Genes—Comparative Genomics of the Green Branch of Life. *Cell* 129(2), 229–234.

Brouwer, R. W. W., Hout, M. C. G. N. van den, Grosveld, F. G. & IJcken, W. F. J. van (2012). NARWHAL, a primary analysis pipeline for NGS data. *Bioinformatics* 28(2), 284–285.

Brown, A. P., Kroon, J. T. M., Swarbreck, D., Febrer, M., Larson, T. R., Graham, I. A., Caccamo, M. & Slabas, A. R. (2012). Tissue-Specific Whole Transcriptome Sequencing in Castor, Directed at Understanding Triacylglycerol Lipid Biosynthetic Pathways. *PLoS ONE* 7(2), e30100.

Brunoud, G., Wells, D. M., Oliva, M., Larrieu, A., Mirabet, V., Burrow, A. H., Beeckman, T., Kepinski, S., Traas, J., Bennett, M. J. & Vernoux, T. (2012). A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature* 482(7383), 103–106.

Carrier, D. J., Abu Bakar, N. T., Lawler, K., Dorrian, J. M., Haider, A., Bennett, M. J. & Kerr, I. D. (2009). Heterologous Expression of a Membrane-Spanning Auxin Importer: Implications for Functional Analyses of Auxin Transporters. *International Journal of Plant Genomics* 848145-848145.

Causier, B., Lloyd, J., Stevens, L. & Davies, B. (2012). TOPLESS co-repressor interactions and their evolutionary conservation in plants. *Plant Signaling & Behavior* 7(3), 325–328.

Cheng, Y., Dai, X. & Zhao, Y. (2006a). Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. *Genes & Development* 20(13), 1790–1799.

Cheng, Y., Dai, X. & Zhao, Y. (2006b). Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in Arabidopsis. *Genes & Development* 20(13), 1790–1799.

Cheng, Y., Dai, X. & Zhao, Y. (2007). Auxin Synthesized by the YUCCA Flavin Monooxygenases Is Essential for Embryogenesis and Leaf Formation in Arabidopsis. *Plant Cell* 19(8), 2430–2439.

Chen, Q., Dai, X., De-Paoli, H., Cheng, Y., Takebayashi, Y., Kasahara, H., Kamiya, Y. & Zhao, Y. (2014). Auxin Overproduction in Shoots Cannot Rescue Auxin Deficiencies in Arabidopsis Roots. *Plant and Cell Physiology* 55(6), 1072–1079.

Chen, X., Naramoto, S., Robert, S., Tejos, R., Löfke, C., Lin, D., Yang, Z. & Friml, J. (2012). ABP1 and ROP6 GTPase Signaling Regulate Clathrin-Mediated Endocytosis in Arabidopsis Roots. *Current Biology* 22(14), 1326–1332.

Cho, M., Cho, H.-T. & others (2012). The function of ABCB transporters in auxin transport. *Plant Signal. Behav* 8, e22990.

Chourey, P. S., Li, Q.-B. & Kumar, D. (2010). Sugar–Hormone Cross-Talk in Seed Development: Two Redundant Pathways of IAA Biosynthesis Are Regulated Differentially in the Invertase-Deficient miniature1 (mn1) Seed Mutant in Maize. *Molecular Plant* 3(6), 1026–1036.

Chu, Y. & Corey, D. R. (2012). RNA Sequencing: Platform Selection, Experimental Design, and Data Interpretation. *Nucleic Acid Therapeutics* 22(4), 271–274.

Clarke, J. T., Warnock, R. C. M. & Donoghue, P. C. J. (2011). Establishing a time-scale for plant evolution. *New Phytologist* 192(1), 266–301.

Colins, G. B., Vian, W. E. & Phillips, G. C. (1978). Use of 4-Amino-3,5,6-trichloropicolinic Acid as an Auxin Source in Plant Tissue Cultures1. *Crop Science* 18(2), 286.

Courtice, G. R. M., Ashton, N. W. & Cove, D. J. (1978). Evidence for the restricted passage of metabolites into the sporophyte of the moss *Physcomitrella patens* (Hedw.) Br. Eur. *Journal of Bryology* 10(2), 191–198.

Cove, D. (2000). The Moss, Physcomitrella patens. *Journal of Plant Growth Regulation* 19(3), 275–283.

Cove, D. (2005). The moss Physcomitrella patens. *Annual Review of Genetics* 39, 339–358.

Cove, D. J. & Ashton, N. W. (1984). Hormonal regulation of gametophytic development in bryophytes. *Experimental biology of bryophytes / edited by A.F. Dyer, J.G. Duckett*.

Cove, D. & Knight, C. (1993). The Moss Physcomitrella patens, a Model System with Potential for the Study of Plant Reproduction. *The Plant Cell* 5(10), 1483–1488.

Cui, D., Zhao, J., Jing, Y., Fan, M., Liu, J., Wang, Z., Xin, W. & Hu, Y. (2013). The Arabidopsis IDD14, IDD15, and IDD16 Cooperatively Regulate Lateral Organ Morphogenesis and Gravitropism by Promoting Auxin Biosynthesis and Transport. *PLoS Genetics* e1003759.

Dai, X., Mashiguchi, K., Chen, Q., Kasahara, H., Kamiya, Y., Ojha, S., DuBois, J., Ballou, D. & Zhao, Y. (2013). The Biochemical Mechanism of Auxin Biosynthesis by an Arabidopsis YUCCA Flavin-containing Monooxygenase. *Journal of Biological Chemistry* 288(3), 1448–1457.

Decker, E. L., Frank, W., Sarnighausen, E. & Reski, R. (2006). Moss systems biology en route: Phytohormones in Physcomitrella development. *PLANT BIOLOGY* 8(3), 397–405.

Delbarre, A., Muller, P., Imhoff, V. & Guern, J. (1996). Comparison of mechanisms controlling uptake and accumulation of 2,4-dichlorophenoxy acetic acid, naphthalene-1-acetic acid, and indole-3-acetic acid in suspension-cultured tobacco cells. *Planta* 198(4), 532–541.

Delwiche, C. F., Karol, K. G., Cimino, M. T. & Sytsma, K. J. (2002). PHYLOGENY OF THE GENUS COLEOCHAETE (COLEOCHAETALES, CHAROPHYTA) AND RELATED TAXA INFERRED BY ANALYSIS OF THE CHLOROPLAST GENE rbcL1. *Journal of Phycology* 38(2), 394–403.

Dharmasiri, N., Dharmasiri, S., Weijers, D., Lechner, E., Yamada, M., Hobbie, L., Ehrismann, J. S., Jürgens, G. & Estelle, M. (2005). Plant Development Is Regulated by a Family of Auxin Receptor F Box Proteins. *Developmental Cell* 9(1), 109–119.

Ding, Z., Wang, B., Moreno, I., Dupláková, N., Simon, S., Carraro, N., Reemmer, J., Pěnčík, A., Chen, X., Tejos, R., Skůpa, P., Pollmann, S., Mravec, J., Petrášek, J., Zažímalová, E., Honys, D., Rolčík, J., Murphy, A., Orellana, A., Geisler, M. & Friml, J. (2012). ER-localized auxin transporter PIN8 regulates auxin homeostasis and male gametophyte development in Arabidopsis. *Nature communications* 3, 941.

Eklund, D. M., Cierlik, I., Ståldal, V., Claes, A. R., Vestman, D., Chandler, J. & Sundberg, E. (2011). Expression of Arabidopsis SHORT INTERNODES/STYLISH Family Genes in Auxin Biosynthesis Zones of Aerial Organs Is Dependent on a GCC Box-Like Regulatory Element. *Plant Physiology* 157(4), 2069 –2080.

Eklund, D. M., Ståldal, V., Valsecchi, I., Cierlik, I., Eriksson, C., Hiratsu, K., Ohme-Takagi, M., Sundström, J. F., Thelander, M., Ezcurra, I. & Sundberg, E. (2010). The Arabidopsis thaliana STYLISH1 Protein Acts as a Transcriptional Activator Regulating Auxin Biosynthesis. *The Plant Cell Online* 22(2), 349 –363.

Erwin, D. H. & Davidson, E. H. (2009). The evolution of hierarchical gene regulatory networks. *Nature Reviews Genetics* 10(2), 141–148.

Esmon, C. A., Tinsley, A. G., Ljung, K., Sandberg, G., Hearne, L. B. & Liscum, E. (2006). A gradient of auxin and auxin-dependent transcription precedes tropic growth responses. *Proceedings of the National Academy of Sciences of the United States of America* 103(1), 236–241.

Espiñeira, J. M., Novo Uzal, E., Gómez Ros, L. V., Carrión, J. S., Merino, F., Ros Barceló, A. & Pomar, F. (2011). Distribution of lignin monomers and the evolution of lignification among lower plants. *Plant Biology* 13(1), 59–68.

Expósito-Rodríguez, M., Borges, A. A., Borges-Pérez, A., Hernández, M. & Pérez, J. A. (2007). Cloning and Biochemical Characterization of ToFZY, a Tomato Gene Encoding a Flavin Monooxygenase Involved in a Tryptophan-dependent Auxin Biosynthesis Pathway. *Journal of Plant Growth Regulation* 26(4), 329–340.

Farras, R., Ferrando, A., Jasik, J., Kleinow, T., Okresz, L., Tiburcio, A., Salchert, K., del Pozo, C., Schell, J. & Koncz, C. (2001). SKP1-SnRK protein kinase interactions mediate proteasomal binding of a plant SCF ubiquitin ligase. *The EMBO Journal* 20(11), 2742–2756.

Fauser, F., Roth, N., Pacher, M., Ilg, G., Sanchez-Fernandez, R., Biesgen, C. & Puchta, H. (2012). In planta gene targeting. *Proceedings of the National Academy of Sciences of the United States of America* 109(19), 7535–7540.

Feraru, E., Vosolsobe, S., Feraru, M. I., Petrasek, J. & Kleine-Vehn, J. (2012). Evolution and Structural Diversification of PILS Putative Auxin Carriers in Plants. *Frontiers in Plant Science* [online], 3. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3470039/.

Finet, C., Berne-Dedieu, A., Scutt, C. P. & Marlétaz, F. (2012). Evolution of the ARF Gene Family in Land Plants: Old Domains, New Tricks. *Molecular Biology and Evolution* mss220.

Fiore, S. D., Li, Q., Leech, M. J., Schuster, F., Emans, N., Fischer, R. & Schillberg, S. (2002). Targeting Tryptophan Decarboxylase to Selected Subcellular Compartments of Tobacco Plants Affects Enzyme Stability and in Vivo Function and Leads to a Lesion-Mimic Phenotype. *Plant Physiology* 129(3), 1160–1169.

Frank, W., Decker, E. L. & Reski, R. (2005). Molecular Tools to Study Physcomitrella patens. *Plant Biology* 7(3), 220–227.

Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R. & Jürgens, G. (2003). Efflux-dependent auxin gradients establish the apical–basal axis of Arabidopsis. *Nature* 426(6963), 147–153.

Fujita, T., Sakaguchi, H., Hiwatashi, Y., Wagstaff, S. J., Ito, M., Deguchi, H., Sato, T. & Hasebe, M. (2008). Convergent evolution of shoots in land plants: lack of auxin polar transport in moss shoots. *EVOLUTION & DEVELOPMENT* 10(2), 176–186.

Furt, F., Liu, Y.-C., Bibeau, J. P., Tüzel, E. & Vidali, L. (2013). Apical myosin XI anticipates F-actin during polarized growth of Physcomitrella patens cells. *The Plant Journal* 73(3), 417–428.

Ganguly, A., Park, M., Kesawat, M. S. & Cho, H.-T. (2014). Functional Analysis of the Hydrophilic Loop in Intracellular Trafficking of Arabidopsis PIN-FORMED Proteins. *The Plant Cell* 26(4), 1570–1585.

Geisler, M., Blakeslee, J. J., Bouchard, R., Lee, O. R., Vincenzetti, V., Bandyopadhyay, A., Titapiwatanakun, B., Peer, W. A., Bailly, A., Richards, E. L., Ejendal, K. F. K., Smith, A. P., Baroux, C., Grossniklaus, U., Müller, A., Hrycyna, C. A., Dudler, R., Murphy, A. S. & Martinoia, E. (2005). Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. *The Plant Journal* 44(2), 179–194.

Geisler, M., Kolukisaoglu, H. U., Bouchard, R., Billion, K., Berger, J., Saal, B., Frangne, N., Koncz-Kalman, Z., Koncz, C., Dudler, R., Blakeslee, J. J., Murphy, A. S., Martinoia, E. & Schulz, B. (2003). TWISTED DWARF1, a Unique Plasma Membrane-anchored Immunophilin-like Protein, Interacts with Arabidopsis Multidrug Resistance-like Transporters AtPGP1 and AtPGP19. *Molecular Biology of the Cell* 14(10), 4238–4249.

Geldner, N., Friml, J., Stierhof, Y.-D., Jürgens, G. & Palme, K. (2001). Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. *Nature* 413(6854), 425–428.

Gensel, P. G. (2008). The Earliest Land Plants. *Annual Review of Ecology, Evolution, and Systematics* 39(1), 459–477.

Ghoshdastidar, A. J. & Tong, A. Z. (2013). Treatment of 2,4-D, mecoprop, and dicamba using membrane bioreactor technology. *Environmental Science and Pollution Research* 20(8), 5188–5197.

Glime, J. (2007). Ecophysiology of development: protonema. *Bryophyte ecology* 1.

Graham, L. E. (1985). The Origin of the Life Cycle of Land Plants: A simple modification in the life cycle of an extinct green alga is the likely origin of the first land plants. *American Scientist* 73(2), 178–186.

Graham, L. E., Cook, M. E. & Busse, J. S. (2000). The origin of plants: Body plan changes contributing to a major evolutionary radiation. *Proceedings of the National Academy of Sciences* 97(9), 4535–4540.

Gray, W. M., Kepinski, S., Rouse, D., Leyser, O. & Estelle, M. (2001). Auxin regulates SCFTIR1-dependent degradation of AUX/IAA proteins. *Nature* 414(6861), 271–276.

Gray, W. M., del Pozo, J. C., Walker, L., Hobbie, L., Risseeuw, E., Banks, T., Crosby, W. L., Yang, M., Ma, H. & Estelle, M. (1999). Identification of an SCF ubiquitin-ligase complex required for auxin response in Arabidopsis thaliana. *Genes & Development* 13(13), 1678–1691.

Groth-Malonek, M. & Knoop, V. (2005). Bryophytes and Other Basal Land Plants: The Mitochondrial Perspective. *Taxon* 54(2), 293–297.

Groth-Malonek, M., Pruchner, D., Grewe, F. & Knoop, V. (2005). Ancestors of Trans-Splicing Mitochondrial Introns Support Serial Sister Group Relationships of Hornworts and Mosses with Vascular Plants. *Molecular Biology and Evolution* 22(1), 117–125.

Guo, H., Lee, T.-H., Wang, X. & Paterson, A. H. (2013). Function Relaxation Followed by Diversifying Selection after Whole-Genome Duplication in Flowering Plants. *Plant Physiology* 162(2), 769–778.

Gälweiler, L., Guan, C., Müller, A., Wisman, E., Mendgen, K., Yephremov, A. & Palme, K. (1998). Regulation of Polar Auxin Transport by AtPIN1 in Arabidopsis Vascular Tissue. *Science* 282(5397), 2226–2230.

Haig, D. & Wilczek, A. (2006). Sexual conflict and the alternation of haploid and diploid generations. *Philosophical Transactions of the Royal Society B: Biological Sciences* 361(1466), 335–343.

Hanin, M. & Paszkowski, J. (2003). Plant genome modification by homologous recombination. *Current Opinion in Plant Biology* 6(2), 157–162.

Hardtke, C. S. & Berleth, T. (1998). The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *The EMBO Journal* 17(5), 1405–1411.

Harrison, C. J., Roeder, A. H. K., Meyerowitz, E. M. & Langdale, J. A. (2009). Local Cues and Asymmetric Cell Divisions Underpin Body Plan Transitions in the Moss Physcomitrella patens. *CURRENT BIOLOGY* 19(6), 461–471.

Hayashi, K., Neve, J., Hirose, M., Kuboki, A., Shimada, Y., Kepinski, S. & Nozaki, H. (2012). Rational Design of an Auxin Antagonist of the SCFTIR1 Auxin Receptor Complex. *ACS Chemical Biology* 7(3), 590–598.

He, W., Brumos, J., Li, H., Ji, Y., Ke, M., Gong, X., Zeng, Q., Li, W., Zhang, X., An, F., Wen, X., Li, P., Chu, J., Sun, X., Yan, C., Yan, N., Xie, D.-Y., Raikhel, N., Yang, Z., Stepanova, A. N., Alonso, J. M. & Guo, H. (2011). A Small-Molecule Screen Identifies l-Kynurenine as a Competitive Inhibitor of TAA1/TAR Activity in Ethylene-Directed Auxin Biosynthesis and Root Growth in Arabidopsis[C][W]. *The Plant Cell* 23(11), 3944–3960.

Higgins, C. F., Hiles, I. D., Whalley, K. & Jamieson, D. J. (1985). Nucleotide binding by membrane components of bacterial periplasmic binding protein-dependent transport systems. *The EMBO Journal* 4(4), 1033–1039.

Hobson, A. C., Weatherwax, R. & Ames, G. F. (1984). ATP-binding sites in the membrane components of histidine permease, a periplasmic transport system. *Proceedings of the National Academy of Sciences of the United States of America* 81(23), 7333–7337.

Hübers, M., Kerp, H., Schneider, J. W. & Gaitzsch, B. (2013). Dispersed plant mesofossils from the Middle Mississippian of eastern Germany: Bryophytes, pteridophytes and gymnosperms. *Review of Palaeobotany and Palynology* 193, 38–56.

Imaizumi, T., Kadota, A., Hasebe, M. & Wada, M. (2002). Cryptochrome Light Signals Control Development to Suppress Auxin Sensitivity in the Moss Physcomitrella patens. *The Plant Cell Online* 14(2), 373–386.

Ishizaki, K., Johzuka-Hisatomi, Y., Ishida, S., Iida, S. & Kohchi, T. (2013). Homologous recombination-mediated gene targeting in the liverwort Marchantia polymorpha L. *Scientific Reports* 1532-1532.

Jackson, R. G., Kowalczyk, M., Li, Y., Higgins, G., Ross, J., Sandberg, G. & Bowles, D. J. (2002). Over-expression of an Arabidopsis gene encoding a glucosyltransferase of indole-3-acetic acid: phenotypic characterisation of transgenic lines. *The Plant Journal* 32(4), 573–583.

Jang, G. & Dolan, L. (2011). Auxin promotes the transition from chloronema to caulonema in moss protonema by positively regulating PpRSL1and PpRSL2 in Physcomitrella patens. *New Phytologist* 192(2), 319–327.

Jang, G., Yi, K., Pires, N. D., Menand, B. & Dolan, L. (2011). RSL genes are sufficient for rhizoid system development in early diverging land plants. *Development* 138(11), 2273–2281.

Jin, S.-H., Ma, X.-M., Han, P., Wang, B., Sun, Y.-G., Zhang, G.-Z., Li, Y.-J. & Hou, B.-K. (2013). UGT74D1 Is a Novel Auxin Glycosyltransferase from Arabidopsis thaliana. *PLoS ONE* e61705.

Johri, M. M. (2008). Hormonal regulation in green plant lineage families. *Physiology and Molecular Biology of Plants* 14(1-2), 23–38.

Johri, M. M. & Desai, S. (1973). Auxin Regulation of Caulonema Formation in Moss Protonema. *Nature* 245(146), 223–224.

Jones, V. A. S. & Dolan, L. (2012). The evolution of root hairs and rhizoids. *Annals of Botany* 110(2), 205–212.

Jurado, S., Abraham, Z., Manzano, C., Lopez-Torrejon, G., Pacios, L. F. & Del Pozo, J. C. (2010). The Arabidopsis Cell Cycle F-Box Protein SKP2A Binds to Auxin[C][W]. *The Plant Cell* 22(12), 3891–3904.

Jurado, S., Trivino, S. D., Abraham, Z., Manzano, C., Gutierrez, C. & del Pozo, C. (2008). SKP2A protein, an F-box that regulates cell division, is degraded via the ubiquitin pathway. *Plant Signaling & Behavior* 3(10), 810–812.

Kamisugi, Y., Cuming, A. C. & Cove, D. J. (2005). Parameters determining the efficiency of gene targeting in the moss Physcomitrella patens. *Nucleic Acids Research* 33(19), e173–e173.

Kamisugi, Y., Schlink, K., Rensing, S. A., Schween, G., von Stackelberg, M., Cuming, A. C., Reski, R. & Cove, D. J. (2006). The mechanism of gene targeting in Physcomitrella patens:

homologous recombination, concatenation and multiple integration. *Nucleic Acids Research* 34(21), 6205–6214.

Kaneda, M., Schuetz, M., Lin, B. S. P., Chanis, C., Hamberger, B., Western, T. L., Ehlting, J. & Samuels, A. L. (2011). ABC transporters coordinately expressed during lignification of Arabidopsis stems include a set of ABCBs associated with auxin transport. *Journal of Experimental Botany* 62(6), 2063–2077.

Karol, K. G., McCourt, R. M., Cimino, M. T. & Delwiche, C. F. (2001). The Closest Living Relatives of Land Plants. *Science* 294(5550), 2351–2353.

Keitt, G. W. & Baker, R. A. (1966). Auxin Activity of Substituted Benzoic Acids and Their Effect on Polar Auxin Transport. *Plant Physiology* 41(10), 1561–1569.

Kim, J. I., Sharkhuu, A., Jin, J. B., Li, P., Jeong, J. C., Baek, D., Lee, S. Y., Blakeslee, J. J., Murphy, A. S., Bohnert, H. J., Hasegawa, P. M., Yun, D.-J. & Bressan, R. A. (2007). yucca6, a Dominant Mutation in Arabidopsis, Affects Auxin Accumulation and Auxin-Related Phenotypes. *Plant Physiology* 145(3), 722–735.

Kleine-Vehn, J., Dhonukshe, P., Sauer, M., Brewer, P. B., Wiśniewska, J., Paciorek, T., Benková, E. & Friml, J. (2008). ARF GEF-Dependent Transcytosis and Polar Delivery of PIN Auxin Carriers in Arabidopsis. *Current Biology* 18(7), 526–531.

Krouk, G., Lacombe, B., Bielach, A., Perrine-Walker, F., Malinska, K., Mounier, E., Hoyerova, K., Tillard, P., Leon, S., Ljung, K., Zazimalova, E., Benkova, E., Nacry, P. & Gojon, A. (2010). Nitrate-Regulated Auxin Transport by NRT1.1 Defines a Mechanism for Nutrient Sensing in Plants. *Developmental Cell* 18(6), 927–937.

Kubo, M., Imai, A., Nishiyama, T., Ishikawa, M., Sato, Y., Kurata, T., Hiwatashi, Y., Reski, R. & Hasebe, M. (2013). System for Stable β-Estradiol-Inducible Gene Expression in the Moss Physcomitrella patens. *PLoS ONE* 8(9), e77356.

Kuusk, S., Sohlberg, J. J., Eklund, D. M. & Sundberg, E. (2006). Functionally redundant SHI family genes regulate Arabidopsis gynoecium development in a dose-dependent manner. *PLANT JOURNAL* 47(1), 99–111.

Kuusk, S., Sohlberg, J. J., Long, J. A., Fridborg, I. & Sundberg, E. (2002). STY1 and STY2 promote the formation of apical tissues during Arabidopsis gynoecium development. *DEVELOPMENT* 129(20), 4707–4717.

Lang, D., Eisinger, J., Reski, R. & Rensing, S. A. (2005). Representation and High-Quality Annotation of the Physcomitrella patens Transcriptome Demonstrates a High Proportion of Proteins Involved in Metabolism in Mosses. *Plant Biology* 7(3), 238–250.

Lavy, M., Prigge, M. J., Tigyi, K. & Estelle, M. (2012). The cyclophilin DIAGEOTROPICA has a conserved role in auxin signaling. *Development* 139(6), 1115–1124.

LeClere, S., Schmelz, E. A. & Chourey, P. S. (2010). Sugar Levels Regulate Tryptophan-Dependent Auxin Biosynthesis in Developing Maize Kernels. *Plant Physiology* 153(1), 306–318.

LeClere, S., Tellez, R., Rampey, R. A., Matsuda, S. P. T. & Bartel, B. (2002). Characterization of a Family of IAA-Amino Acid Conjugate Hydrolases from Arabidopsis. *Journal of Biological Chemistry* 277(23), 20446–20452.

Lehmann, T., Hoffmann, M., Hentrich, M. & Pollmann, S. (2010). Indole-3-acetamide-dependent auxin biosynthesis: A widely distributed way of indole-3-acetic acid production? *European Journal of Cell Biology* 89(12), 895 – 905.

Lehnert, B. & Bopp, M. (1983). The Hormonal Regulation of Protonema Development in Mosses I. Auxin-Cytokinin Interaction. *Zeitschrift für Pflanzenphysiologie* 110(5), 379–391.

Leliaert, F., Smith, D. R., Moreau, H., Herron, M. D., Verbruggen, H., Delwiche, C. F. & De Clerck, O. (2012). Phylogeny and Molecular Evolution of the Green Algae. *Critical Reviews in Plant Sciences* 31(1), 1–46.

Lewis, L. A. & McCourt, R. M. (2004). Green algae and the origin of land plants. *American Journal of Botany* 91(10), 1535–1556.

Li, H., Tiwari, S. B., Hagen, G. & Guilfoyle, T. J. (2011). Identical Amino Acid Substitutions in the Repression Domain of Auxin/Indole-3-Acetic Acid Proteins Have Contrasting Effects on Auxin Signaling1. *Plant Physiology* 155(3), 1252–1263.

Li, L.-C., Qin, G.-J., Tsuge, T., Hou, X.-H., Ding, M.-Y., Aoyama, T., Oka, A., Chen, Z., Gu, H., Zhao, Y. & Qu, L.-J. (2008). SPOROCYTELESS modulates YUCCA expression to regulate the development of lateral organs in Arabidopsis. *New Phytologist* 179(3), 751–764.

Li, L., Ljung, K., Breton, G., Schmitz, R. J., Pruneda-Paz, J., Cowing-Zitron, C., Cole, B. J., Ivans, L. J., Pedmale, U. V., Jung, H.-S., Ecker, J. R., Kay, S. A. & Chory, J. (2012). Linking photoreceptor excitation to changes in plant architecture. *Genes & Development* 26(8), 785–790.

Lim, P. O., Kim, H. J. & Gil Nam, H. (2007). Leaf Senescence. *Annual Review of Plant Biology* 58(1), 115–136.

Ljung, K., Bhalerao, R. P. & Sandberg, G. (2001). Sites and homeostatic control of auxin biosynthesis in Arabidopsis during vegetative growth. *The Plant Journal* 28(4), 465–474.

Ljung, K., Hull, A. K., Celenza, J., Yamada, M., Estelle, M., Normanly, J. & Sandberg, G. (2005). Sites and Regulation of Auxin Biosynthesis in Arabidopsis Roots. *The Plant Cell Online* 17(4), 1090–1104.

Lohse, M., Bolger, A., Nagel, A., Fernie, A. R., Lunn, J. E., Stitt, M. & Usadel, B. (2012). RobiNA: a user-friendly, integrated software solution for RNA-Seq-based transcriptomics. *Nucleic Acids Research* gks540.

Lucas, W. J., Groover, A., Lichtenberger, R., Furuta, K., Yadav, S.-R., Helariutta, Y., He, X.-Q., Fukuda, H., Kang, J., Brady, S. M., Patrick, J. W., Sperry, J., Yoshida, A., López-Millán, A.-F., Grusak, M. A. & Kachroo, P. (2013). The Plant Vascular System: Evolution, Development and FunctionsF. *Journal of Integrative Plant Biology* 55(4), 294–388.

Ludwig-Muller, J., Julke, S., Bierfreund, N. M., Decker, E. L. & Reski, R. (2009). Moss (Physcomitrella patens) GH3 proteins act in auxin homeostasis. *NEW PHYTOLOGIST* 181(2), 323–338.

Ludwig-Müller, J. (2011). Auxin conjugates: their role for plant development and in the evolution of land plants. *Journal of Experimental Botany* 62(6), 1757–1773.

Luschnig, C. & Vert, G. (2014). The dynamics of plant plasma membrane proteins: PINs and beyond. *Development* 141(15), 2924–2938.

Mano, Y., Nemoto, K., Suzuki, M., Seki, H., Fujii, I. & Muranaka, T. (2010). The AMI1 gene family: indole-3-acetamide hydrolase functions in auxin biosynthesis in plants. *Journal of Experimental Botany* 61(1), 25–32.

Marchant, A., Bhalerao, R., Casimiro, I., Eklöf, J., Casero, P. J., Bennett, M. & Sandberg, G. (2002). AUX1 Promotes Lateral Root Formation by Facilitating Indole-3-Acetic Acid Distribution between Sink and Source Tissues in the Arabidopsis Seedling. *The Plant Cell* 14(3), 589–597.

Marchant, A., Kargul, J., May, S. T., Muller, P., Delbarre, A., Perrot-Rechenmann, C. & Bennett, M. J. (1999). AUX1 regulates root gravitropism in Arabidopsis by facilitating auxin uptake within root apical tissues. *The EMBO Journal* 18(8), 2066–2073.

Marín-Rodríguez, M. C., Orchard, J. & Seymour, G. B. (2002). Pectate lyases, cell wall degradation and fruit softening. *Journal of Experimental Botany* 53(377), 2115–2119.

Mashiguchi, K., Tanaka, K., Sakai, T., Sugawara, S., Kawaide, H., Natsume, M., Hanada, A., Yaeno, T., Shirasu, K., Yao, H., McSteen, P., Zhao, Y., Hayashi, K., Kamiya, Y. & Kasahara, H. (2011). The main auxin biosynthesis pathway in Arabidopsis. *Proceedings of the National Academy of Sciences* 108(45), 18512–18517.

Masucci, J. D. & Schiefelbein, J. W. (1994). The rhd6 Mutation of Arabidopsis thaliana Alters Root-Hair Initiation through an Auxin- and Ethylene-Associated Process. *Plant Physiology* 106(4), 1335–1346.

McDaniel, S. F. & Shaw, A. J. (2003). Phylogeographic Structure and Cryptic Speciation in the Trans-Antarctic Moss Pyrrhobryum Mnioides. *Evolution* 57(2), 205–215.

Menand, B., Yi, K., Jouannic, S., Hoffmann, L., Ryan, E., Linstead, P., Schaefer, D. G. & Dolan, L. (2007). An ancient mechanism controls the development of cells with a rooting function in land plants. *SCIENCE* 316(5830), 1477–1480.

Michniewicz, M., Zago, M. K., Abas, L., Weijers, D., Schweighofer, A., Meskiene, I., Heisler, M. G., Ohno, C., Zhang, J., Huang, F., Schwab, R., Weigel, D., Meyerowitz, E. M., Luschnig, C., Offringa, R. & Friml, J. (2007). Antagonistic Regulation of PIN Phosphorylation by PP2A and PINOID Directs Auxin Flux. *Cell* 130(6), 1044–1056.

Mikkelsen, M. D., Naur, P. & Halkier, B. A. (2004). Arabidopsis mutants in the C–S lyase of glucosinolate biosynthesis establish a critical role for indole-3-acetaldoxime in auxin homeostasis. *The Plant Journal* 37(5), 770–777.

Mishler, B. D. & Churchill, S. P. (1985). Transition to a Land Flora: Phylogenetic Relationships of the Green Algae and Bryophytes. *Cladistics* 1(4), 305–328.

Mockaitis, K. & Estelle, M. (2008). Auxin Receptors and Plant Development: A New Signaling Paradigm. *Annual Review of Cell and Developmental Biology* 24(1), 55–80.

Mravec, J., Skupa, P., Bailly, A., Hoyerova, K., Krecek, P., Bielach, A., Petrasek, J., Zhang, J., Gaykova, V., Stierhof, Y.-D., Dobrev, P. I., Schwarzerova, K., Rolcik, J., Seifertova, D., Luschnig, C., Benkova, E., Zazimalova, E., Geisler, M. & Friml, J. (2009). Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. *Nature* 459(7250), 1136–1140.

Nakaoka, Y., Miki, T., Fujioka, R., Uehara, R., Tomioka, A., Obuse, C., Kubo, M., Hiwatashi, Y. & Goshima, G. (2012). An Inducible RNA Interference System in Physcomitrella patens

Reveals a Dominant Role of Augmin in Phragmoplast Microtubule Generation. *The Plant Cell Online* 24(4), 1478–1493.

Neuteboom, L. W., Veth-Tello, L. M., Clijdesdale, O. R., Hooykaas, P. J. J. & Zaal, B. J. van der (1999). A Novel Subtilisin-like Protease Gene from Arabidopsis thaliana is Expressed at Sites of Lateral Root Emergence. *DNA Research* 6(1), 13–19.

Nishiyama, T. (2007). Evolutionary Developmental Biology of Nonflowering Land Plants. *International Journal of Plant Sciences* 168(1), 37–47.

Nishiyama, T., Hiwatashi, Y., Sakakibara, K., Kato, M. & Hasebe, M. (2000). Tagged Mutagenesis and Gene-trap in the Moss, Physcomitrella patens by Shuttle Mutagenesis. *DNA Research* 7(1), 9–17.

Nishiyama, T., Wolf, P. G., Kugita, M., Sinclair, R. B., Sugita, M., Sugiura, C., Wakasugi, T., Yamada, K., Yoshinaga, K., Yamaguchi, K., Ueda, K. & Hasebe, M. (2004). Chloroplast Phylogeny Indicates that Bryophytes Are Monophyletic. *Molecular Biology and Evolution* 21(10), 1813–1819.

Noh, B., Murphy, A. S. & Spalding, E. P. (2001). Multidrug Resistance–like Genes of Arabidopsis Required for Auxin Transport and Auxin-Mediated Development. *The Plant Cell Online* 13(11), 2441–2454.

Normanly, J. (2010). Approaching Cellular and Molecular Resolution of Auxin Biosynthesis and Metabolism. *Cold Spring Harbor Perspectives in Biology* a001594.

Normanly, J., Cohen, J. D. & Fink, G. R. (1993). Arabidopsis thaliana auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. *Proceedings of the National Academy of Sciences* 90(21), 10355–10359.

Normanly, J., Grisafi, P., Fink, G. R. & Bartel, B. (1997). Arabidopsis mutants resistant to the auxin effects of indole-3-acetonitrile are defective in the nitrilase encoded by the NIT1 gene. *The Plant Cell Online* 9(10), 1781–1790.

O'Donoghue, M.-T., Chater, C., Wallace, S., Gray, J. E., Beerling, D. J. & Fleming, A. J. (2013). Genome-wide transcriptomic analysis of the sporophyte of the moss Physcomitrella patens. *Journal of Experimental Botany* 64(12), 3567–3581.

Oh, K., Ivanchenko, M. G., White, T. J. & Lomax, T. L. (2006). The diageotropica gene of tomato encodes a cyclophilin: a novel player in auxin signaling. *Planta* 224(1), 133–144.

Ouyang, J., Shao, X. & Li, J. (2000). Indole-3-glycerol phosphate, a branchpoint of indole-3-acetic acid biosynthesis from the tryptophan biosynthetic pathway in Arabidopsis thaliana. *The Plant Journal* 24(3), 327–334.

Pagnussat, G. C., Alandete-Saez, M., Bowman, J. L. & Sundaresan, V. (2009). Auxin-Dependent Patterning and Gamete Specification in the Arabidopsis Female Gametophyte. *Science* 324(5935), 1684–1689.

Panigrahi, K. C. S., Panigrahy, M., Vervliet-Scheebaum, M., Lang, D., Reski, R. & Johri, M. M. (2009). Auxin-binding proteins without KDEL sequence in the moss Funaria hygrometrica. *Plant Cell Reports* 28(11), 1747–1758.

Paponov, I. A., Teale, W., Lang, D., Paponov, M., Reski, R., Rensing, S. A. & Palme, K. (2009). The evolution of nuclear auxin signalling. *BMC EVOLUTIONARY BIOLOGY* 9.

Parry, G., Delbarre, A., Marchant, A., Swarup, R., Napier, R., Perrot-Rechenmann, C. & Bennett, M. J. (2001). Novel auxin transport inhibitors phenocopy the auxin influx carrier mutation aux1. *The Plant Journal* 25(4), 399–406.

Patel, R. K. & Jain, M. (2012). NGS QC Toolkit: A Toolkit for Quality Control of Next Generation Sequencing Data. *PLoS ONE* 7(2), e30619.

Pattison, R. J. & Catalá, C. (2012). Evaluating auxin distribution in tomato (Solanum lycopersicum) through an analysis of the PIN and AUX/LAX gene families. *The Plant Journal* 70(4), 585–598.

Péret, B., Swarup, K., Ferguson, A., Seth, M., Yang, Y., Dhondt, S., James, N., Casimiro, I., Perry, P., Syed, A., Yang, H., Reemmer, J., Venison, E., Howells, C., Perez-Amador, M. A., Yun, J., Alonso, J., Beemster, G. T. S., Laplaze, L., Murphy, A., Bennett, M. J., Nielsen, E. & Swarup, R. (2012). AUX/LAX Genes Encode a Family of Auxin Influx Transporters That Perform Distinct Functions during Arabidopsis Development. *The Plant Cell Online* 24(7), 2874–2885.

Petrášek, J. & Friml, J. (2009). Auxin transport routes in plant. *Development* 136(16), 2675–2688.

Petrášek, J., Mravec, J., Bouchard, R., Blakeslee, J. J., Abas, M., Seifertová, D., Wiśniewska, J., Tadele, Z., Kubeš, M., Čovanová, M., Dhonukshe, P., Skůpa, P., Benková, E., Perry, L., Křeček, P., Lee, O. R., Fink, G. R., Geisler, M., Murphy, A. S., Luschnig, C., Zažímalová, E. & Friml, J. (2006). PIN Proteins Perform a Rate-Limiting Function in Cellular Auxin Efflux. *Science* 312(5775), 914–918.

Phillips, K. A., Skirpan, A. L., Liu, X., Christensen, A., Slewinski, T. L., Hudson, C., Barazesh, S., Cohen, J. D., Malcomber, S. & McSteen, P. (2011). vanishing tassel2 Encodes a Grass-Specific Tryptophan Aminotransferase Required for Vegetative and Reproductive Development in Maize. *The Plant Cell Online* 23(2), 550 –566.

Pickett-Heaps, J. (1969). The evolution of the mitotic apparatus: an attempt at comparative ultrastructural cytology in dividing plant cells. *Cytobios* 3, 257–280.

Pickett-Heaps, J. D. & Marchant, H. J. (1972). Phylogeny of the green algae: a new proposal. *Cytobios*.

Pires, N. D. & Dolan, L. (2012). Morphological evolution in land plants: new designs with old genes. *Philosophical Transactions of the Royal Society B: Biological Sciences* 367(1588), 508–518.

Poli, D., Jacobs, M. & Cooke, T. J. (2003). Auxin regulation of axial growth in bryophyte sporophytes: its potential significance for the evolution of early land plants. *American Journal of Botany* 90(10), 1405–1415.

Pollmann, S., Neu, D. & Weiler, E. W. (2003). Molecular cloning and characterization of an amidase from Arabidopsis thaliana capable of converting indole-3-acetamide into the plant growth hormone, indole-3-acetic acid. *Phytochemistry* 62(3), 293–300.

Pozo, J. C. del, Diaz-Trivino, S., Cisneros, N. & Gutierrez, C. (2006). The Balance between Cell Division and Endoreplication Depends on E2FC-DPB, Transcription Factors Regulated by the Ubiquitin-SCFSKP2A Pathway in Arabidopsis. *The Plant Cell Online* 18(9), 2224–2235.

Pressel, S., Ligrone, R. & Duckett, J. G. (2008). Cellular Differentiation in Moss Protonemata: A Morphological and Experimental Study. *Annals of Botany* 102(2), 227–245.

Prigge, M. J., Lavy, M., Ashton, N. W. & Estelle, M. (2010). Physcomitrella patens Auxin-Resistant Mutants Affect Conserved Elements of an Auxin-Signaling Pathway. *Current Biology* 20(21), 1907–1912.

Qiu, Y., Li, L., Wang, B., Chen, Z., Dombrovska, O., Lee, J., Kent, L., Li, R., Jobson, R. W., Hendry, T. A., Taylor, D. W., Testa, C. M. & Ambros, M. (2007). A Nonflowering Land Plant Phylogeny Inferred from Nucleotide Sequences of Seven Chloroplast, Mitochondrial, and Nuclear Genes. *International Journal of Plant Sciences* 168(5), pp. 691–708.

Qudeimat, E., Faltusz, A. M. C., Wheeler, G., Lang, D., Holtorf, H., Brownlee, C., Reski, R. & Frank, W. (2008). A PIIB-type Ca2+-ATPase is essential for stress adaptation in Physcomitrella patens. *Proceedings of the National Academy of Sciences* 105(49), 19555–19560.

Quittenden, L. J., Davies, N. W., Smith, J. A., Molesworth, P. P., Tivendale, N. D. & Ross, J. J. (2009). Auxin Biosynthesis in Pea: Characterization of the Tryptamine Pathway. *Plant Physiology* 151(3), 1130–1138.

Ramos, J. A., Zenser, N., Leyser, O. & Callis, J. (2001). Rapid Degradation of Auxin/Indoleacetic Acid Proteins Requires Conserved Amino Acids of Domain II and Is Proteasome Dependent. *The Plant Cell* 13(10), 2349–2360.

Rampey, R. A., LeClere, S., Kowalczyk, M., Ljung, K., Sandberg, G. & Bartel, B. (2004). A Family of Auxin-Conjugate Hydrolases That Contributes to Free Indole-3-Acetic Acid Levels during Arabidopsis Germination. *Plant Physiology* 135(2), 978–988.

Ranocha, P., Denancé, N., Vanholme, R., Freydier, A., Martinez, Y., Hoffmann, L., Köhler, L., Pouzet, C., Renou, J.-P., Sundberg, B., Boerjan, W. & Goffner, D. (2010). Walls are thin 1 (WAT1), an Arabidopsis homolog of Medicago truncatula NODULIN21, is a tonoplast-localized protein required for secondary wall formation in fibers. *The Plant Journal* 63(3), 469–483.

Ranocha, P., Dima, O., Nagy, R., Felten, J., Corratgé-Faillie, C., Novák, O., Morreel, K., Lacombe, B., Martinez, Y., Pfrunder, S., Jin, X., Renou, J.-P., Thibaud, J.-B., Ljung, K., Fischer, U., Martinoia, E., Boerjan, W. & Goffner, D. (2013). Arabidopsis WAT1 is a vacuolar auxin transport facilitator required for auxin homoeostasis. *Nature Communications* 4.

Rashotte, A. M., Brady, S. R., Reed, R. C., Ante, S. J. & Muday, G. K. (2000). Basipetal Auxin Transport Is Required for Gravitropism in Roots of Arabidopsis. *Plant Physiology* 122(2), 481–490.

Raven, J. A. (2013). Polar auxin transport in relation to long-distance transport of nutrients in the Charales. *Journal of Experimental Botany* 64(1), 1–9.

Raven, P. H., Evert, R. F. & Eichhorn, S. E. (2005). *Biology of Plants*. W. H. Freeman. ISBN 9780716710073.

Reed, J. W. (2001). Roles and activities of Aux/IAA proteins in Arabidopsis. *Trends in Plant Science* 6(9), 420–425.

Remy, E., Baster, P., Friml, J. & Duque, P. (2013). ZIFL1.1 transporter modulates polar auxin transport by stabilizing membrane abundance of multiple PINs in Arabidopsis root tip. *Plant Signaling & Behavior* 8(10), e25688.

Rensing, S. A., Lang, D., Zimmer, A. D., Terry, A., Salamov, A., Shapiro, H., Nishiyama, T., Perroud, P.-F., Lindquist, E. A., Kamisugi, Y., Tanahashi, T., Sakakibara, K., Fujita, T., Oishi, K., Shin-I, T., Kuroki, Y., Toyoda, A., Suzuki, Y., Hashimoto, S., Yamaguchi, K., Sugano, S., Kohara, Y., Fujiyama, A., Anterola, A., Aoki, S., Ashton, N., Barbazuk, W. B., Barker, E., Bennetzen, J. L., Blankenship, R., Cho, S. H., Dutcher, S. K., Estelle, M., Fawcett, J. A., Gundlach, H., Hanada, K., Heyl, A., Hicks, K. A., Hughes, J., Lohr, M., Mayer, K., Melkozernov, A., Murata, T., Nelson, D. R., Pils, B., Prigge, M., Reiss, B., Renner, T., Rombauts, S., Rushton, P. J., Sanderfoot, A., Schween, G., Shiu, S.-H., Stueber, K., Theodoulou, F. L., Tu, H., Van de Peer, Y., Verrier, P. J., Waters, E., Wood, A., Yang, L., Cove, D., Cuming, A. C., Hasebe, M., Lucas, S., Mishler, B. D., Reski, R., Grigoriev, I. V., Quatrano, R. S. & Boore, J. L. (2008). The Physcomitrella genome reveals evolutionary insights into the conquest of land by plants. *SCIENCE* 319(5859), 64–69.

Reski, R. (1998). Development, genetics and molecular biology of mosses. *Botanica Acta* 111(1), 1–15.

Robert, H. S., Grones, P., Stepanova, A. N., Robles, L. M., Lokerse, A. S., Alonso, J. M., Weijers, D. & Friml, J. (2013). Local Auxin Sources Orient the Apical-Basal Axis in Arabidopsis Embryos. *Current Biology* 23(24), 2506–2512.

Roberts, A. W., Dimos, C. S., Budziszek, M. J., Jr, Goss, C. A. & Lai, V. (2011). Knocking out the wall: protocols for gene targeting in Physcomitrella patens. *Methods in molecular biology (Clifton, N.J.)* 715, 273–290.

Robert, S., Kleine-Vehn, J., Barbez, E., Sauer, M., Paciorek, T., Baster, P., Vanneste, S., Zhang, J., Simon, S., Čovanová, M., Hayashi, K., Dhonukshe, P., Yang, Z., Bednarek, S. Y., Jones, A. M., Luschnig, C., Aniento, F., Zažímalová, E. & Friml, J. (2010). ABP1 Mediates Auxin Inhibition of Clathrin-Dependent Endocytosis in Arabidopsis. *Cell* 143(1), 111–121.

Robinson, M. D., McCarthy, D. J. & Smyth, G. K. (2010). edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26(1), 139–140.

Rojas-Pierce, M., Titapiwatanakun, B., Sohn, E. J., Fang, F., Larive, C. K., Blakeslee, J., Cheng, Y., Cuttler, S., Peer, W. A., Murphy, A. S. & Raikhel, N. V. (2007). Arabidopsis P-Glycoprotein19 Participates in the Inhibition of Gravitropism by Gravacin. *Chemistry & Biology* 14(12), 1366–1376.

Ross, J. J., Tivendale, N. D., Reid, J. B., Davies, N. W., Molesworth, P. P., Lowe, E. K., Smith, J. A. & Davidson, S. E. (2011). Reassessing the role of YUCCAs in auxin biosynthesis. *Plant Signaling & Behavior* 6(3), 437–439.

Rubery, P. H. & Sheldrake, A. R. (1974). Carrier-mediated auxin transport. *Planta* 118(2), 101–121.

Saidi, Y., Finka, A., Chakhporanian, M., Zrÿd, J.-P., Schaefer, D. G. & Goloubinoff, P. (2005). Controlled Expression of Recombinant Proteins in Physcomitrella patens by a Conditional Heat-shock Promoter: a Tool for Plant Research and Biotechnology. *Plant Molecular Biology* 59(5), 697–711.

Sakakibara, K., Ando, S., Yip, H. K., Tamada, Y., Hiwatashi, Y., Murata, T., Deguchi, H., Hasebe, M. & Bowman, J. L. (2013). KNOX2 Genes Regulate the Haploid-to-Diploid Morphological Transition in Land Plants. *Science* 339(6123), 1067–1070.

Sakakibara, K., Nishiyama, T., Deguchi, H. & Hasebe, M. (2008). Class 1 KNOX genes are not involved in shoot development in the moss *Physcomitrella patens* but do function in sporophyte development. *Evolution & Development* 10(5), 555–566.

Sakakibara, K., Nishiyama, T., Sumikawa, N., Kofuji, R., Murata, T. & Hasebe, M. (2003). Involvement of auxin and a homeodomain-leucine zipper I gene in rhizoid development of the moss Physcomitrella patens. *Development* 130(20), 4835–4846.

Savka, M. A., Black, R. C. & Binns, A. N. (2001). Tobacco etch virus leader sequence enhances inducible indoleacetic acid-lysine synthetase activity in transgenic plants. *Plant Physiology and Biochemistry* 39(7-8), 631–641.

Schaefer, D. G. & Zryd, J. P. (1997). Efficient gene targeting in the moss Physcomitrella patens. *PLANT JOURNAL* 11(6), 1195–1206.

Schlink, K. & Reski, R. (2002). Preparing high-quality DNA from moss (Physcomitrella patens). *Plant Molecular Biology Reporter* 20(4), 423–423.

Schnabel, E. L. & Frugoli, J. (2004). The PIN and LAX families of auxin transport genes in Medicago truncatula. *Molecular Genetics and Genomics* 272(4), 420–432.

Seidel, C., Walz, A., Park, S., Cohen, J. D. & Ludwig-Müller, J. (2006). Indole-3-Acetic Acid Protein Conjugates: Novel Players in Auxin Homeostasis. *Plant Biology* 8(3), 340–345.

Shaw, A. J., Szövényi, P. & Shaw, B. (2011). Bryophyte diversity and evolution: Windows into the early evolution of land plants. *American Journal of Botany* 98(3), 352–369.

De Smet, I., Voss, U., Lau, S., Wilson, M., Shao, N., Timme, R. E., Swarup, R., Kerr, I., Hodgman, C., Bock, R., Bennett, M., Jürgens, G. & Beeckman, T. (2011). Unraveling the evolution of auxin signaling. *Plant Physiology* 155(1), 209–221.

Sohlberg, J. J., Myrenas, M., Kuusk, S., Lagercrantz, U., Kowalczyk, M., Sandberg, G. & Sundberg, E. (2006). STY1 regulates auxin homeostasis and affects apical-basal patterning of the Arabidopsis gynoecium. *PLANT JOURNAL* 47(1), 112–123.

Spartz, A. K., Lee, S. H., Wenger, J. P., Gonzalez, N., Itoh, H., Inze, D., Peer, W. A., Murphy, A. S., Overvoorde, P. J. & Gray, W. M. (2012). The SAUR19 subfamily of SMALL AUXIN UP RNA genes promote cell expansion. *The Plant journal : for cell and molecular biology* 70(6), 978–990.

Spartz, A. K., Ren, H., Park, M. Y., Grandt, K. N., Lee, S. H., Murphy, A. S., Sussman, M. R., Overvoorde, P. J. & Gray, W. M. (2014). SAUR Inhibition of PP2C-D Phosphatases Activates Plasma Membrane H+-ATPases to Promote Cell Expansion in Arabidopsis. *The Plant Cell Online* tpc.114.126037.

Sperry, J. S. (2003). Evolution of Water Transport and Xylem Structure. *International Journal of Plant Sciences* 164(S3), S115–S127.

Staswick, P. E. (2009). The tryptophan conjugates of jasmonic and indole-3-acetic acids are endogenous auxin inhibitors. *Plant physiology* 150(3), 1310–1321.

Stepanova, A. N., Robertson-Hoyt, J., Yun, J., Benavente, L. M., Xie, D.-Y., Dolezal, K., Schlereth, A., Jürgens, G. & Alonso, J. M. (2008). TAA1-Mediated Auxin Biosynthesis Is Essential for Hormone Crosstalk and Plant Development. *Cell* 133(1), 177–191.

Stepanova, A. N., Yun, J., Robles, L. M., Novak, O., He, W., Guo, H., Ljung, K. & Alonso, J. M. (2011). The Arabidopsis YUCCA1 Flavin Monooxygenase Functions in the Indole-3-Pyruvic Acid Branch of Auxin Biosynthesis[W]. *The Plant Cell* 23(11), 3961–3973.

Stewart, K. D. & Mattox, K. R. (1975). Comparative Cytology, Evolution and Classification of the Green Algae with Some Consideration of the Origin of Other Organisms with Chlorophylls A and B. *Botanical Review* 41(1), pp. 104–135.

Strader, L. C. & Bartel, B. (2011). Transport and Metabolism of the Endogenous Auxin Precursor Indole-3-Butyric Acid. *Molecular Plant* 4(3), 477–486.

Strader, L. C., Culler, A. H., Cohen, J. D. & Bartel, B. (2010). Conversion of Endogenous Indole-3-Butyric Acid to Indole-3-Acetic Acid Drives Cell Expansion in Arabidopsis Seedlings1[C][W][OA]. *Plant Physiology* 153(4), 1577–1586.

Strader, L. C., Monroe-Augustus, M. & Bartel, B. (2008). The IBR5 phosphatase promotes Arabidopsis auxin responses through a novel mechanism distinct from TIR1-mediated repressor degradation. *BMC Plant Biology* 8, 41.

Strader, L. C., Wheeler, D. L., Christensen, S. E., Berens, J. C., Cohen, J. D., Rampey, R. A. & Bartel, B. (2011). Multiple Facets of Arabidopsis Seedling Development Require ?Indole-3-Butyric Acid-Derived Auxin[W]. *The Plant Cell* 23(3), 984–999.

Ståldal, V., Cierlik, I., Chen, S., Landberg, K., Baylis, T., Myrenås, M., Sundström, J. F., Eklund, D. M., Ljung, K. & Sundberg, E. (2012). The Arabidopsis thaliana transcriptional activator STYLISH1 regulates genes affecting stamen development, cell expansion and timing of flowering. *Plant Molecular Biology* 78(6), 545–559.

Sugawara, S., Hishiyama, S., Jikumaru, Y., Hanada, A., Nishimura, T., Koshiba, T., Zhao, Y., Kamiya, Y. & Kasahara, H. (2009). Biochemical analyses of indole-3-acetaldoxime-dependent auxin biosynthesis in Arabidopsis. *Proceedings of the National Academy of Sciences* 106(13), 5430–5435.

Sun, J., Qi, L., Li, Y., Chu, J. & Li, C. (2012). PIF4-Mediated Activation of YUCCA8 Expression Integrates Temperature into the Auxin Pathway in Regulating Arabidopsis Hypocotyl Growth. *PLoS Genetics* [online], 8(3). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3315464/.

Sun, J., Qi, L., Li, Y., Zhai, Q. & Li, C. (2013). PIF4 and PIF5 Transcription Factors Link Blue Light and Auxin to Regulate the Phototropic Response in Arabidopsis. *The Plant Cell Online* tpc.113.112417.

Swarup, K., Benková, E., Swarup, R., Casimiro, I., Péret, B., Yang, Y., Parry, G., Nielsen, E., De Smet, I., Vanneste, S., Levesque, M. P., Carrier, D., James, N., Calvo, V., Ljung, K., Kramer, E., Roberts, R., Graham, N., Marillonnet, S., Patel, K., Jones, J. D. G., Taylor, C. G., Schachtman, D. P., May, S., Sandberg, G., Benfey, P., Friml, J., Kerr, I., Beeckman, T., Laplaze, L. & Bennett, M. J. (2008). The auxin influx carrier LAX3 promotes lateral root emergence. *Nature Cell Biology* 10(8), 946–954.

Swarup, R., Friml, J., Marchant, A., Ljung, K., Sandberg, G., Palme, K. & Bennett, M. (2001). Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. *Genes & Development* 15(20), 2648–2653.

Szemenyei, H., Hannon, M. & Long, J. A. (2008). TOPLESS Mediates Auxin-Dependent Transcriptional Repression During Arabidopsis Embryogenesis. *Science* 319(5868), 1384–1386.

Sztein, A. E., Cohen & Cooke, T. J. (2000). Evolutionary Patterns in the Auxin Metabolism of Green Plants. *International Journal of Plant Sciences* 161(6), 849–859.

Sztein, A. E., Cohen, J. D., Fuente, I. G. de la & Cooke, T. J. (1999). Auxin metabolism in mosses and liverworts. *American Journal of Botany* 86(11), 1544–1555.

Sztein, A. E., Cohen, J. D., Slovin, J. P. & Cooke, T. J. (1995). Auxin Metabolism in Representative Land Plants. *American Journal of Botany* 82(12), 1514–1521.

Takezawa, D., Komatsu, K. & Sakata, Y. (2011). ABA in bryophytes: how a universal growth regulator in life became a plant hormone? *Journal of Plant Research* 124(4), 437–453.

Tam, Y. Y., Epstein, E. & Normanly, J. (2000). Characterization of Auxin Conjugates in Arabidopsis. Low Steady-State Levels of Indole-3-Acetyl-Aspartate, Indole-3-Acetyl-Glutamate, and Indole-3-Acetyl-Glucose. *Plant Physiology* 123(2), 589–596.

Tanaka, K., Hayashi, K., Natsume, M., Kamiya, Y., Sakakibara, H., Kawaide, H. & Kasahara, H. (2014). UGT74D1 Catalyzes the Glucosylation of 2-Oxindole-3-Acetic Acid in the Auxin Metabolic Pathway in Arabidopsis. *Plant and Cell Physiology* 55(1), 218–228.

Tao, Y., Ferrer, J.-L., Ljung, K., Pojer, F., Hong, F., Long, J. A., Li, L., Moreno, J. E., Bowman, M. E., Ivans, L. J., Cheng, Y., Lim, J., Zhao, Y., Ballaré, C. L., Sandberg, G., Noel, J. P. & Chory, J. (2008). Rapid Synthesis of Auxin via a New Tryptophan-Dependent Pathway Is Required for Shade Avoidance in Plants. *Cell* 133(1), 164–176.

Tian, Q. & Reed, J. W. (1999). Control of auxin-regulated root development by the Arabidopsis thaliana SHY2/IAA3 gene. *Development* 126(4), 711–721.

Titapiwatanakun, B., Blakeslee, J. J., Bandyopadhyay, A., Yang, H., Mravec, J., Sauer, M., Cheng, Y., Adamec, J., Nagashima, A., Geisler, M., Sakai, T., Friml, J., Peer, W. A. & Murphy, A. S. (2009). ABCB19/PGP19 stabilises PIN1 in membrane microdomains in Arabidopsis. *The Plant Journal* 57(1), 27–44.

Tivendale, N. D., Davidson, S. E., Davies, N. W., Smith, J. A., Dalmais, M., Bendahmane, A. I., Quittenden, L. J., Sutton, L., Bala, R. K., Le Signor, C., Thompson, R., Horne, J., Reid, J. B. & Ross, J. J. (2012). Biosynthesis of the Halogenated Auxin, 4-Chloroindole-3-Acetic Acid1. *Plant Physiology* 159(3), 1055–1063.

Tivendale, N. D., Davies, N. W., Molesworth, P. P., Davidson, S. E., Smith, J. A., Lowe, E. K., Reid, J. B. & Ross, J. J. (2010). Reassessing the Role of N-Hydroxytryptamine in Auxin Biosynthesis. *Plant Physiology* 154(4), 1957–1965.

Tivendale, N. D., Ross, J. J. & Cohen, J. D. (2014). The shifting paradigms of auxin biosynthesis. *Trends in Plant Science* 19(1), 44–51.

Tiwari, S. B., Hagen, G. & Guilfoyle, T. J. (2004). Aux/IAA Proteins Contain a Potent Transcriptional Repression Domain. *The Plant Cell Online* 16(2), 533–543.

Torri, F., Dinov, I. D., Zamanyan, A., Hobel, S., Genco, A., Petrosyan, P., Clark, A. P., Liu, Z., Eggert, P., Pierce, J., Knowles, J. A., Ames, J., Kesselman, C., Toga, A. W., Potkin, S. G., Vawter, M. P. & Macciardi, F. (2012). Next Generation Sequence Analysis and Computational Genomics Using Graphical Pipeline Workflows. *Genes* 3(3), 545–575.

Trapnell, C., Williams, B. A., Pertea, G., Mortazavi, A., Kwan, G., Baren, M. J. van, Salzberg, S. L., Wold, B. J. & Pachter, L. (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nature Biotechnology* 28(5), 511–515.

Tromas, A., Paque, S., Stierlé, V., Quettier, A.-L., Muller, P., Lechner, E., Genschik, P. & Perrot-Rechenmann, C. (2013). Auxin-Binding Protein 1 is a negative regulator of the SCFTIR1/AFB pathway. *Nature Communications* [online], 4. Available from: http://www.nature.com/ncomms/2013/130920/ncomms3496/abs/ncomms3496.html.

Trouiller, B., Charlot, F., Choinard, S., Schaefer, D. G. & Nogué, F. (2007). Comparison of gene targeting efficiencies in two mosses suggests that it is a conserved feature of Bryophyte transformation. *Biotechnology Letters* 29(10), 1591–1598.

Uehara, T., Okushima, Y., Mimura, T., Tasaka, M. & Fukaki, H. (2008). Domain II Mutations in CRANE/IAA18 Suppress Lateral Root Formation and Affect Shoot Development in Arabidopsis thaliana. *Plant and Cell Physiology* 49(7), 1025–1038.

Ulmasov, T., Hagen, G. & Guilfoyle, T. J. (1999). Activation and repression of transcription by auxin-response factors. *Proceedings of the National Academy of Sciences of the United States of America* 96(10), 5844–5849.

Ulmasov, T., Murfett, J., Hagen, G. & Guilfoyle, T. J. (1997). Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *The Plant Cell Online* 9(11), 1963–1971.

Vernoux, T., Brunoud, G., Farcot, E., Morin, V., Van den Daele, H., Legrand, J., Oliva, M., Das, P., Larrieu, A., Wells, D., Guédon, Y., Armitage, L., Picard, F., Guyomarc'h, S., Cellier, C., Parry, G., Koumproglou, R., Doonan, J. H., Estelle, M., Godin, C., Kepinski, S., Bennett, M., De Veylder, L. & Traas, J. (2011). The auxin signalling network translates dynamic input into robust patterning at the shoot apex. *Molecular Systems Biology 7*, 508.

Verrier, P. J., Bird, D., Burla, B., Dassa, E., Forestier, C., Geisler, M., Klein, M., Kolukisaoglu, Ü., Lee, Y., Martinoia, E., Murphy, A., Rea, P. A., Samuels, L., Schulz, B., Spalding, E. P., Yazaki, K. & Theodoulou, F. L. (2008). Plant ABC proteins – a unified nomenclature and updated inventory. *Trends in Plant Science* 13(4), 151–159.

Viaene, T., Delwiche, C. F., Rensing, S. A. & Friml, J. (2013). Origin and evolution of PIN auxin transporters in the green lineage. *Trends in Plant Science* 5-10.

Vieten, A., Vanneste, S., Wiśniewska, J., Benková, E., Benjamins, R., Beeckman, T., Luschnig, C. & Friml, J. (2005). Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. *Development* 132(20), 4521–4531.

Villalobos, L. I. A. C., Lee, S., De Oliveira, C., Ivetac, A., Brandt, W., Armitage, L., Sheard, L. B., Tan, X., Parry, G., Mao, H., Zheng, N., Napier, R., Kepinski, S. & Estelle, M. (2012). A combinatorial TIR1/AFB-Aux/IAA co-receptor system for differential sensing of auxin. *Nature Chemical Biology* 8(5), 477–485.

Vorwerk, S., Biernacki, S., Hillebrand, H., Janzik, I., Müller, A., Weiler, E. W. & Piotrowski, M. (2001). Enzymatic characterization of the recombinant Arabidopsis thaliana nitrilase subfamily encoded by the NIT2/NIT1/NIT3-gene cluster. *Planta* 212(4), 508–516.

Wang, Y., Ghaffari, N., Johnson, C. D., Braga-Neto, U. M., Wang, H., Chen, R. & Zhou, H. (2011). Evaluation of the coverage and depth of transcriptome by RNA-Seq in chickens. *BMC Bioinformatics* 12(Suppl 10), S5.

Weaver, L. M. & Herrmann, K. M. (1997). Dynamics of the shikimate pathway in plants. *Trends in Plant Science* 2(9), 346–351.

Weijers, D., Schlereth, A., Ehrismann, J. S., Schwank, G., Kientz, M. & Jürgens, G. (2006). Auxin Triggers Transient Local Signaling for Cell Specification in Arabidopsis Embryogenesis. *Developmental Cell* 10(2), 265–270.

Wellman, C. H., Osterloff, P. L. & Mohiuddin, U. (2003). Fragments of the earliest land plants. *Nature* 425(6955), 282–285.

Wells, D. M., Laplaze, L., Bennett, M. J. & Vernoux, T. (2013). Biosensors for phytohormone quantification: challenges, solutions, and opportunities. *Trends in Plant Science* 18(5), 244–249.

Wend, S., Bosco, C. D., Kämpf, M. M., Ren, F., Palme, K., Weber, W., Dovzhenko, A. & Zurbriggen, M. D. (2013). A quantitative ratiometric sensor for time-resolved analysis of auxin dynamics. *Scientific Reports* 3: 2052.

Wiśniewska, J., Xu, J., Seifertová, D., Brewer, P. B., Růžička, K., Blilou, I., Rouquié, D., Benková, E., Scheres, B. & Friml, J. (2006). Polar PIN Localization Directs Auxin Flow in Plants. *Science* 312(5775), 883.

Won, C., Shen, X., Mashiguchi, K., Zheng, Z., Dai, X., Cheng, Y., Kasahara, H., Kamiya, Y., Chory, J. & Zhao, Y. (2011). Conversion of tryptophan to indole-3-acetic acid by TRYPTOPHAN AMINOTRANSFERASES OF ARABIDOPSIS and YUCCAs in Arabidopsis. *Proceedings of the National Academy of Sciences* 108(45), 18518–18523.

Wright, A. D., Sampson, M. B., Neuffer, M. G., Michalczuk, L., Slovin, J. P. & Cohen, J. D. (1991). Indole-3-Acetic Acid Biosynthesis in the Mutant Maize orange pericarp, a Tryptophan Auxotroph. *Science* 254(5034), 998–1000.

Wyatt, H. D. M., Ashton, N. W. & Dahms, T. E. S. (2008). Cell wall architecture of Physcomitrella patens is revealed by atomic force microscopy. *Botany* 86(4), 385–397.

Xiao, L., Zhang, L., Yang, G., Zhu, H. & He, Y. (2012). Transcriptome of Protoplasts Reprogrammed into Stem Cells in Physcomitrella patens. *PLoS ONE* 7(4), e35961.

Yamamoto, M. & Yamamoto, K. T. (1998). Differential Effects of 1-Naphthaleneacetic Acid, Indole-3-Acetic Acid and 2,4-Dichlorophenoxyacetic Acid on the Gravitropic Response of Roots in an Auxin-Resistant Mutant of Arabidopsis, auxl. *Plant and Cell Physiology* 39(6), 660–664.

Yang, H. & Murphy, A. S. (2009). Functional expression and characterization of Arabidopsis ABCB, AUX 1 and PIN auxin transporters in Schizosaccharomyces pombe. *The Plant Journal* 59(1), 179–191.

Yuo, T., Yamashita, Y., Kanamori, H., Matsumoto, T., Lundqvist, U., Sato, K., Ichii, M., Jobling, S. A. & Taketa, S. (2012). A SHORT INTERNODES (SHI) family transcription factor gene regulates awn elongation and pistil morphology in barley. *Journal of Experimental Botany* 63(14), 5223–5232.

Zawaski, C., Kadmiel, M., Ma, C., Gai, Y., Jiang, X., Strauss, S. H. & Busov, V. B. (2011). SHORT INTERNODES-like genes regulate shoot growth and xylem proliferation in Populus. *New Phytologist* 191(3), 678–691.

Zhang, S. & van Duijn, B. (2014). Cellular Auxin Transport in Algae. *Plants* 3(1), 58–69.

Zhao, Y., Christensen, S. K., Fankhauser, C., Cashman, J. R., Cohen, J. D., Weigel, D. & Chory, J. (2001). A Role for Flavin Monooxygenase-Like Enzymes in Auxin Biosynthesis. *Science* 291(5502), 306–309.

Zhao, Y., Hull, A. K., Gupta, N. R., Goss, K. A., Alonso, J., Ecker, J. R., Normanly, J., Chory, J. & Celenza, J. L. (2002). Trp-dependent auxin biosynthesis in Arabidopsis: involvement of cytochrome P450s CYP79B2 and CYP79B3. *Genes & Development* 16(23), 3100–3112.

Zhao, Z., Zhang, Y., Liu, X., Zhang, X., Liu, S., Yu, X., Ren, Y., Zheng, X., Zhou, K., Jiang, L., Guo, X., Gai, Y., Wu, C., Zhai, H., Wang, H. & Wan, J. (2013). A Role for a Dioxygenase in Auxin Metabolism and Reproductive Development in Rice. *Developmental Cell* 27(1), 113–122.

Zimmer, A. D., Lang, D., Buchta, K., Rombauts, S., Nishiyama, T., Hasebe, M., Peer, Y. V. de, Rensing, S. A. & Reski, R. (2013). Reannotation and extended community resources for the genome of the non-seed plant Physcomitrella patens provide insights into the evolution of plant gene structures and functions. *BMC Genomics* 14(1), 498.

Zolman, B. K., Martinez, N., Millius, A., Adham, A. R. & Bartel, B. (2008). Identification and Characterization of Arabidopsis Indole-3-Butyric Acid Response Mutants Defective in Novel Peroxisomal Enzymes. *Genetics* 180(1), 237–251.

Zolman, B. K., Nyberg, M. & Bartel, B. (2007). IBR3, a novel peroxisomal acyl-CoA dehydrogenase-like protein required for indole-3-butyric acid response. *Plant Molecular Biology* 64(1-2), 59–72.

# Acknowledgements

First a general thanks to everyone that was a part of my life during my time as a PhD student who prevented me from burning my own bridge and pushed me to become a better person and researcher.

I would like to thank my supervisors; Eva, Mattias and Katarina all of whom are really amazingly nice people and quite clever as well. Each of them possess different strengths that allowed our group to be incredibly functional. I would not have made it to the end without them.

Also to all past and present group members; Andrea, Veronika, Bela, Jia, Magnus, Tom, Stefan, Poorva, Viki, Christina, Emma, Marisa, Song, Jim and Tammy. Each of you was instrumental in both getting me to the end of my PhD and keeping my morale up. I would also like to mention Tom Viaene who is a very passionate researcher and a good example of how hard work combined with efficiency will eventually pay off.

Thanks to the Uppsala University groups of Peter and Annelie for all the important feedback and interesting talks during our group meetings as well as helpful lab tips.

Teaching was something I looked forward to every year with Björn who always had confidence in my ability to get through the course without any problems, which was a nice feeling. I also enjoyed ranting about how terrible microsoft and mac are with him.

I have to also thank the administrative staff who were always really patient with me. Specifically to Mona, good luck with your retirement. Also Lasse who was constantly fixing things that I was breaking.

Some admiral mentions include people that have been instrumental for getting me through the day and sometimes after working hours as well: Hanneke, Jia, Carolin, Adriana, Maaike, Bela, Veronika, Andrea, Christina, Arne, Niki, Jordi, Ulrike, Phillip, Nina, Rocky, Duarte, Rita, Ramesh, Matt, Daniel, Johan, Anna and Shah. I hope my presence was a lift for you as well.

To everybody who I played innebandy with during the first couple of years, it was the perfect distraction from everything outside that building. I still play on but with two vastly different groups than the one I started with.

I would also not have made it without several people that I have met over the years who are now scattered all over the world. Matt, Jenna, Dave, Derek, Jessica, Jason and Sara, thanks for all the good times. Also, Mark and Linda, even though you are half-way across the world you have always been there when I needed a distraction and you will always remind me of the good old days in Vancouver. Martin, my first friend in Sweden, will always be my friend no matter our differences. The travels we did together were amazing. Linnéa's friends who were and still are welcoming to this person from a strange country. And Karolina, for reminding me that I am only human exactly when I needed to remember.

Lastly I have to thank my family. My parents who always support me no matter where I go or what I do. Linnéa's parents for being so welcoming and helping out with Robin and making sure we had a fun time at the cabin. Robin who had no idea where I was going everyday, but always had a wave and a smile anyways. And to Linnéa, my love, who has had to suffer quite a bit because of the time frame between our defences. Hopefully now we can have some more time for ourselves.