Moss Systems Biology en Route: Phytohormones in *Physcomitrella* Development

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Abstract: **The moss *Physcomitrella patens* has become a powerful model system in modern plant biology. Highly standardized cell culture techniques, as well as the necessary tools for computational biology, functional genomics and proteomics have been established. Large EST collections are available and the complete moss genome will be released soon. A simple body plan and the small number of different cell types in *Physcomitrella* facilitate the study of developmental processes. In the filamentous juvenile moss tissue, developmental decisions rely on the differentiation of single cells. Developmental steps are controlled by distinct phytohormones and integration of environmental signals. Especially the phytohormones auxin, cytokinin, and abscisic acid have distinct effects on early moss development. In this article, we review current knowledge about phytohormone influences on early moss development in an attempt to fully unravel the complex regulatory signal transduction networks underlying the developmental decisions of single plant cells in a holistic systems biology approach.**

Key words: **Bryophyte, cell cycle, homologous recombination, cell differentiation, auxin, cytokinin, ABA.**

Abbreviations:
ABA: abscisic acid
EST: expressed sequence tag

Introduction

The non-vascular, multicellular terrestrial bryophyte, *Physcomitrella patens,* has become increasingly interesting as a model system for functional genomics approaches. *Physcomitrella* combines many advantages over other model systems. Bryophytes were one of the earliest land plants in evolutionary terms, and have several common characteristics with seed plants. The simple morphology, with only a few differentiation steps, makes mosses suitable for the elucidation of developmental processes (Cove and Knight, 1993; Reski, 1998; Lorenz et al., 2003; Sakakibara et al., 2003; Repp et al., 2004). *Physcomitrella* is amenable to *in vitro* tissue culture techniques and all stages of its life cycle can be grown axenically and photoautotrophically in a simple medium consisting of inorganic salts and water. Moreover, moss cells offer an exceptionally high regeneration capacity under hormone-free conditions (Hohe and Reski, 2005). In contrast to cultivation of seed plants, moss suspension cultures resemble the humid environment in which early moss development takes place. Here, the moss cells are in direct contact with the surrounding medium, allowing hormone application simultaneously to all cells.

The standardization of cell culture conditions is an important prerequisite to generate quantitative data for modelling in systems biology approaches. All relevant methods for genetic modification of *Physcomitrella* have been established (Frank et al., 2005 a). Moreover, large cDNA collections and EST databases are available (Rensing et al., 2002; Nishiyama et al., 2003) and sequencing of the whole moss genome is nearly completed. The draft genome is expected to be released in early 2007.

Physcomitrella displays an exceptionally high frequency of homologous recombination in its nuclear DNA, this enables gene/function correlations to be made using reverse genetics via allele replacement (Strepp et al., 1998; Schaefer, 2002; Hohe and Reski, 2003). As the haploid gametophyte dominates moss development, genetic modifications are stable and immediately effective without the need for laborious crossing steps. An unexpected spectrum of phenotypes in a tagged *Physcomitrella* mutant collection (Schween et al., 2005) may be caused by the fact that knockout constructs have been created based on cDNA, thus favouring the targeting of actively transcribed genome regions and by the lower risk of functional redundancy of genes because of the small average gene family size in the moss compared to *Arabidopsis* (Rensing et al., 2002). Together with the possibility of standardization and the simplicity of the system, ideal premises for modelling are available. Apart from obtaining important results within basic research, these advantages have given rise to the biotechnological exploitation of moss, a search for novel gene functions that could be applied to genetic engineering of crop plants (Egener et al., 2002; Wu et al., 2005), as well as use as a bioreactor for the production of complex biopharmaceuticals (Decker and Reski, 2004; Koprivova et al., 2004; Huether et al., 2005).

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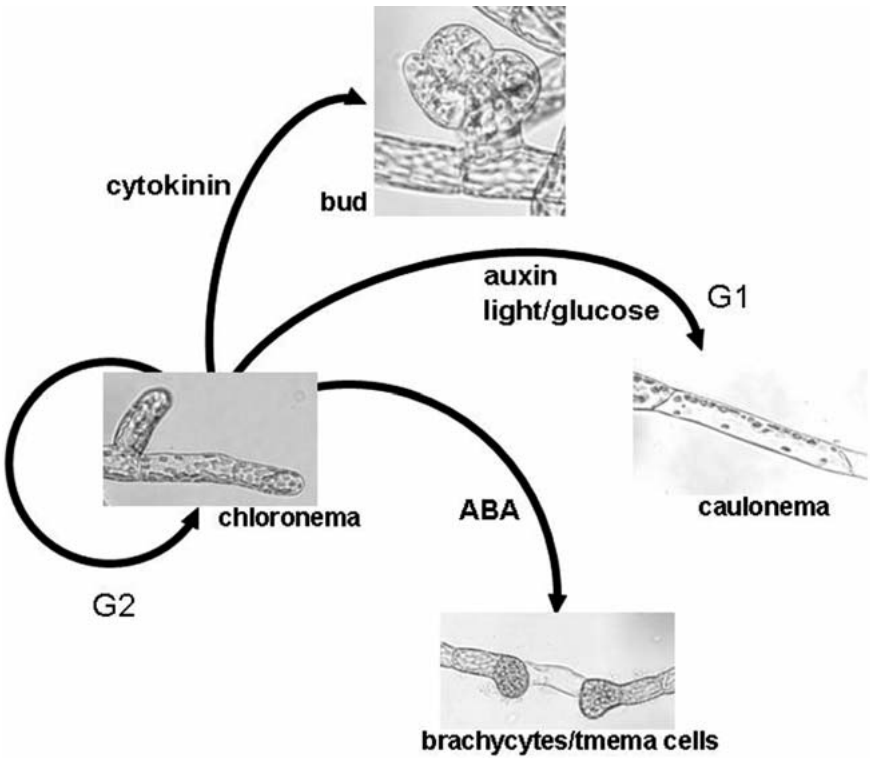

Fig. 1 Developmental fates of *Physcomitrella* chloronema cells. A chloronema cell can proliferate by apical division to form either a new chloronema cell, a caulonema cell (induced by auxin and bright light or glucose), or a bud initial (induced by cytokinin). Under the influence of ABA, subapical cells differentiate into round brachycytes or nearly cytoplasm-free tmema cells. The prevalent phase of the cell cycle for chloronema and caulonema cells (G1 and G2, respectively) is indicated. (Images courtesy of Stefanie Tintelnot.)

The moss life cycle starts with the germination of the haploid spore and the subsequent outgrowth of the protonema, uniseriate branched filaments, which proliferate by apical cell divisions. Spore germination is influenced by water and light availability (Schnepf et al., 1982). Initially formed chloronema cells are characterized by numerous chloroplasts and cell walls perpendicular to the growth axis. Within the protonema filament, a chloronema cell may have four developmental fates (Fig. **1**): 1) it may continue proliferation to form a new chloronema cell, or 2) under auxin influence, it may differentiate into a caulonema cell which is more elongated, contains less chloroplasts and cross walls oblique to the filament growth axis. Caulonema development requires relatively high light intensities (Reski, 1998) and is promoted by glucose (Olsson et al., 2003), indicating a common mechanism in the induction of caulonema growth under high energy conditions, i.e., light or exogenous sugar supply (Thelander et al., 2005). The next step in moss development, induced by cytokinin, is 3) the initiation of a bud from a three-faced apical cell which subsequently gives rise to the leafy gametophore (representing the adult plant). In addition to these differentiation steps directed towards gametophore development, subapical chloronema cells may 4) form brachycytes and tmema cells, respectively, by intercalary cell division (Figs. **2 A – C**). Brachycytes are short, thick-walled, rounded "brood" cells, often formed in chains or flanked by tmema cells, which are short-lived, nearly cytoplasm-free abscission cells (Schnepf and Reinhard, 1997). Both cell types develop under unfavourable environmental conditions and can be induced by application of abscisic acid (ABA).

Thus, auxin, cytokinin, and ABA induce specific cell differentiation events in *Physcomitrella*. Far less is known about other phytohormones in moss development: Components of the jasmonic acid biosynthesis pathway were shown to be present in *Physcomitrella,* as well as brassinosteroid biosynthesis and ethylene receptor gene homologues (Frank et al., unpublished). In the following paragraphs, we will focus on the roles of auxin, cytokinin, and ABA in cellular differentiation processes in *Physcomitrella* and will summarize the molecular and bioinformatics tools which will help to unravel the underlying regulatory networks in a systemic approach.

Auxin

The first step in juvenile (i.e., protonema) moss development, the transition from chloronema to caulonema cells, is induced by auxin (Johri and Desai, 1973; Ashton et al., 1979). Auxin measurements in protonema cultures have revealed that most of the auxin (more than 90%) is found extracellularly, in the medium (Reutter et al., 1998). Hormone uptake from the medium was observed (Rose et al., 1983) and demonstrated to be important for moss development by continuous medium exchange, which abolished further differentiation of protonema filaments (Ashton et al., 1979; Schween et al., 2003). An auxin gradient within protonema filaments, from the tip cell towards subapical cells, was postulated from physiological studies (Bopp and Atzorn, 1992). However, polar auxin transport in mosses is poorly characterized. Important components mediating polar auxin transport in seed plants are the PIN auxin efflux facilitators, which are thought to be crucial for correct cellular coordination (Friml et al., 2002; Blilou et al., 2005; Paponov et al., 2005). There are eight PIN proteins in *Arabidopsis,* and two *PIN* homologues have been identified in *Physcomitrella,* and these have been shown to be distantly related to *PIN* genes from seed plants (Paponov et al., 2005). The Pp*PIN* genes have highest identities to *PIN5* genes of seed plants, which are

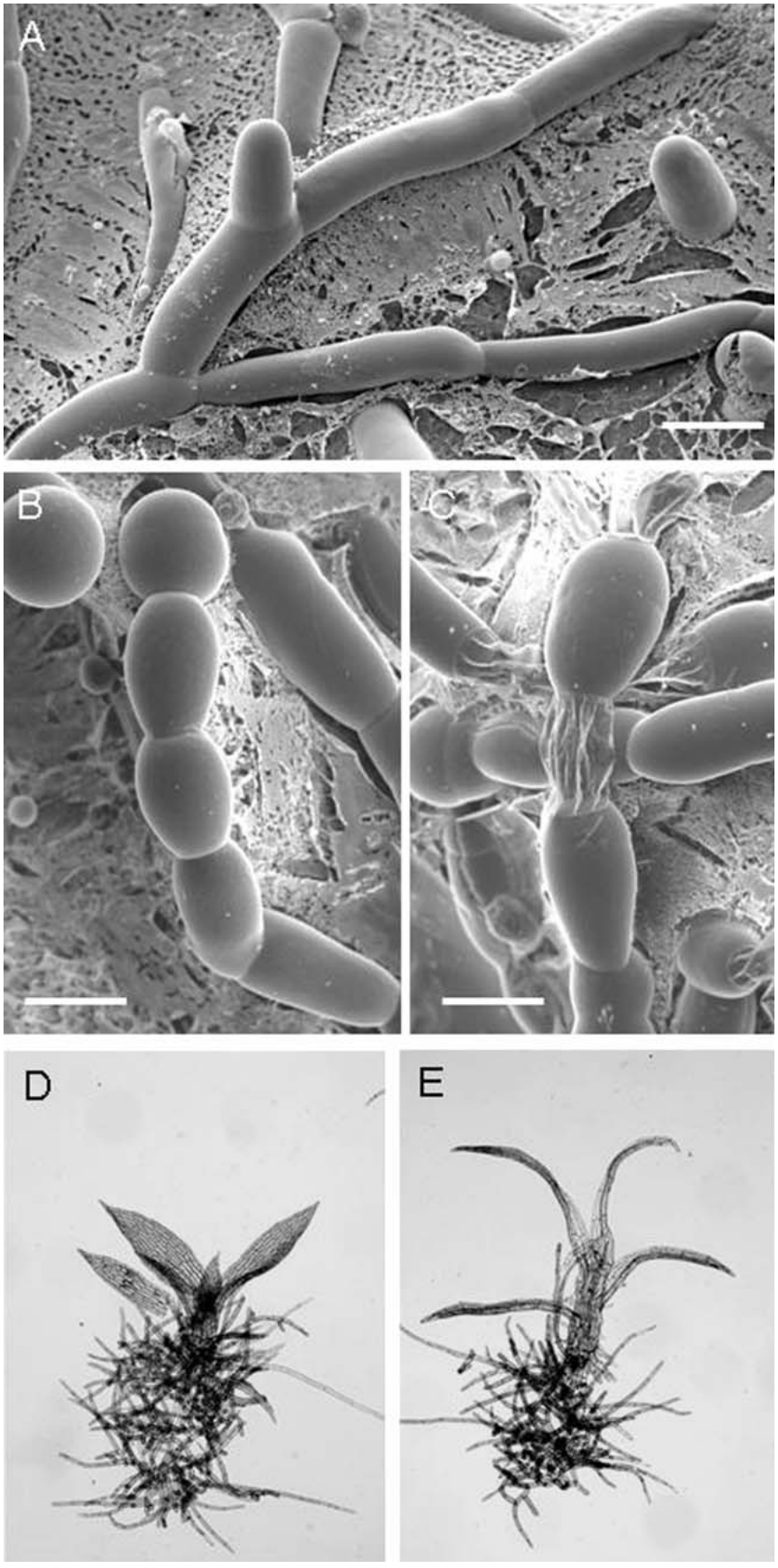

Fig. 2 Phytohormones induce morphological changes in different moss tissues. *Physcomitrella* protonema filaments untreated (**A**) and after treatment with ABA (**B, C**). ABA-treated moss cells differentiate into chains of brachycytes (**B**) or brachycytes flanked by tmema cells (**C**). *Physcomitrella* gametophores untreated (**D**) or after auxin application (**E**). Leaflets of gametophores elongate in response to auxin. Bars, 30 µm. (Images courtesy of Stefanie Tintelnot [**A – C**] and Otmar Lienhart [**D, E**].)

suggested to have diverged from the ancestral *PIN* gene early in plant evolution (Paponov et al., 2005). This places the *Physcomitrella PIN* genes close to the root of the plant-specific gene tree. Initial results of targeted knockout approaches for either Pp*PIN*1 or Pp*PIN*2 demonstrated only minor phenotypic aberrations in leaflet morphology compared to the wild type. Therefore, the importance of PIN auxin efflux facilitators in the basal land plant *Physcomitrella* is difficult to judge at the moment. Partial redundancy in gene function has to be assumed.

Flow cytometric analyses for the evaluation of DNA content in different moss cell types revealed an increase of cell nuclei staying in the G1 phase of the cell cycle in response to auxin application. This is consistent with the observation that caulonema cells are preferentially arrested before G1/S transition, while chloronema cells accumulate in the G2 phase (Schween et al., 2003). This link between cell cycle arrest and cell differentiation points to differential checkpoints used by the different cell types in *Physcomitrella*.

While the influence of auxin on protonema development has been described in detail, far less is known about auxin concentrations or cellular sensitivity to auxin within adult moss plants, the gametophores. Like protonema cells, the leaflets of gametophores are able to elongate in response to auxin. When gametophores were incubated in auxin-supplemented liquid medium for two days, the youngest leaflets were strongly elongated and appeared to be light green, indicating rapid cell expansion without previous chloroplast division (Figs. **2 D, E**). To monitor auxin responsiveness of different moss tissues, GUS constructs driven by the auxin-responsive GH3 promoter from soybean or the synthetic DR5 promoter, which comprises tandem repeats of an auxin-responsive element (Hagen et al., 1991; Guilfoyle, 1999), were used. GUS staining of GH3::GUS plants was strongest in protonema apical cells, buds, and the stems of gametophores, with maxima in the apex and at the base of the stem. These staining patterns indicate highest auxin concentrations in actively growing cells or ontogenetically young tissues. The DR5 element turned out to be less sensitive than GH3 in the moss. In the absence of exogenous auxin, the DR5 element mediated GUS expression in only a few cells of the stem apex, which are analogous to the shoot meristem of seed plants (Bierfreund et al., 2003). After auxin application, all analyzed moss tissues showed GUS staining, demonstrating a general competence of *Physcomitrella* cells to respond to the phytohormone (Bierfreund et al., 2003). The GH3::GUS and DR5::GUS constructs, both auxin-reactive but differentially responding, will be useful tools to examine auxin gradients during moss development in detail. In addition to morphological changes, auxin rapidly and transiently induces transcript accumulation of at least three gene families in seed plants: *SAUR, Aux/IAA,* and *GH3*-related family members (reviewed in Woodward and Bartel, 2005). *GH3* genes comprise a large gene family of at least 19 members in *Arabidopsis* (Hagen and Guilfoyle, 2002). A function for GH3 proteins in connecting light and auxin signal transduction pathways was proposed after analysis of different *Arabidopsis* mutants (Hsieh et al., 2000; Nakazawa et al., 2001; Tanaka et al., 2002; Takase et al., 2003; Takase et al., 2004). The *Physcomitrella GH3*-like gene family is represented by four members (Bierfreund et al., 2004; Richardt et al., unpublished). Two of these were analyzed in more detail. Both genes are expressed in all tissues, starting very early in moss development. Even one day after protoplast isolation, regenerating moss cells expressed both, *PpGH3*-1 and *PpGH3*-2. In contrast, enhanced expression of the genes after auxin application, which was demonstrated for several of the *Arabidopsis GH3* genes, could not be shown for the moss *GH3* homologues. The gene loci were targeted and the resulting knockout plants were analyzed for changes in light response under white, red, and far-red light. In contrast to results with *Arabidopsis* mutants, there was no obvious deviation between *Physcomitrella* wild type and *GH3* knockout lines in response to the different light conditions: In red light, retarded growth was observed for protonema filaments, while gametophores presented an elongated phenotype with shorter and narrower leaves. Both observations were more pronounced under far-red light conditions (Bierfreund et al., 2004). *In vitro* auxin adenylation activity was demonstrated for different *Arabidopsis* GH3-like proteins (Staswick et al., 2002) and their role as IAA-amido synthetases involved in the maintenance of auxin homeostasis by conjugating excess IAA to amino acids was confirmed (Staswick et al., 2005). In line with these results, the PpGH3-KO plants responded more strongly to the hormone when grown on auxin-supplemented medium, thus indicating altered auxin sensitivity when lacking a GH3 family member (Decker, Reski, Ludwig-Mueller, manuscript in preparation).

Cytokinin

Auxin and cytokinin successively regulate protonema development. While auxin promotes the development of caulonema cells, cytokinins induce bud formation in mosses in a concentration-dependent manner. At low concentrations, only chloronema cells were the targets of cytokinin action, whereas at high cytokinin concentrations bud formation increased only on caulonema, indicating a cell type-specific competence to respond to different hormone concentrations (Reski and Abel, 1985). However, high concentrations of exogenously applied cytokinin commonly provoke callus-like growth of buds which do not further differentiate to leafy gametophores (Reski, 1998). In a kinetic study of internal concentrations, highest levels of isopentenyl-type cytokinins (a major cytokinin in this moss) were detected before the onset of budding (Schulz et al., 2000). An evaluation of intra- and extracellular cytokinin amounts revealed that the majority of cytokinin was in the culture medium (Reutter et al., 1998). In addition, in two *Physcomitrella* mutant lines which are defective in bud development and devoid of any gametophores, isopentenyl-type cytokinins were measured. Surprisingly, the cytokinin amounts were in the same range as in wild-type moss. The mutant lines were transformed with the *Agrobacterium tumefaciens* isopentenyl transferase gene, which catalyzes the first step in cytokinin *de novo* biosynthesis. In the resulting transgenic mutant plants, isopentenyl-type cytokinin concentrations were enhanced compared to the wild type. Moreover, in both mutant lines, bud and gametophore development was partially restored, indicating that the mutant lines were hyposensitive to cytokinin (Reutter et al., 1998). Von Schwartzenberg et al. (1998) reported the presence of an active adenosine kinase in *Physcomitrella* which represented the first functional characterization of a plant adenosine kinase. *In vivo* feeding studies suggested that, in *Physcomitrella,* adenosine kinase rather than adenine phosphoribosyl transferase is important for conversion of cytokinins to their nucleotides. We will not focus further on *Physco-*

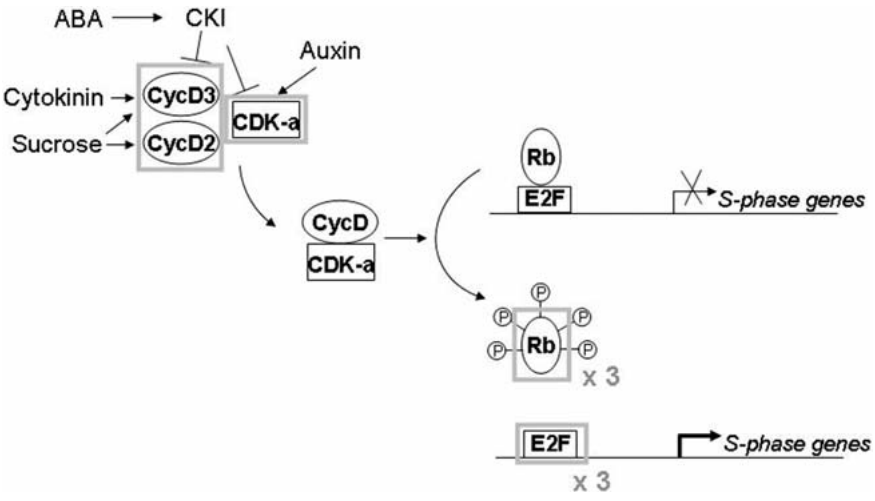

Fig. 3 Components of the plant retinoblastoma pathway. D-type cyclins and CDK-a are regulated by phytohormones and sugar in *Arabidopsis* (reviewed in den Boer and Murray, 2000). Homologs of Rb pathway genes which were identified in *Physcomitrella* are marked with a grey box. CKI: CDK inhibitor. (Modified after den Boer and Murray, 2000.)

mitrella cytokinin metabolism as this is described in detail by von Schwartzenberg elsewhere in this issue.

A coordinated progression through the cell cycle in response to environmental signals is of crucial importance for growth and development of eukaryotic cells. The G1/S transition is an essential checkpoint for developmental decisions within the cell cycle and is controlled by the retinoblastoma (Rb) pathway (Fig. **3**; Gutierrez, 1998; den Boer and Murray, 2000). D-type cyclins are the first components of the Rb pathway and function as integrators of environmental proliferation signals in G1 phase, as their transcription is stimulated by growth factors. Expression of *CycD* genes is stimulated by phytohormones, especially cytokinins, as well as sucrose in *Arabidopsis* (Riou-Khamlichi et al., 1999; Riou-Khamlichi et al., 2000; Oakenfull et al., 2002). The cell cycle-related crosstalk between phytohormones and sugars in seed plants is the focus of the contribution of Hartig and Beck elsewhere in this issue. The complex of a D-type cyclin and a cyclin-dependent kinase (CDK-a) provides the commitment to G1/S transition by hyperphosphorylation of the Rb protein which, in turn, releases E2F transcription factors. By release from Rb interaction, E2F proteins are activated and able to drive the expression of S-phase genes. We analyzed the *Physcomitrella* transcriptome and found all important components of the Rb pathway represented within the existing EST databases. These are a single *cyclin D* gene, one *CDK-a,* three different *Rb* genes, and three putative members of the *E2F* gene family (Lorenz, Heger et al., unpublished). The most surprising result was the existence of a single *Physcomitrella* D-type cyclin, as *CycD* genes comprise a family with ten members in *Arabidopsis* (Vandepoele et al., 2002). Mutant plants of *Physcomitrella* in which the single *CycD* gene was destroyed by targeted knockout demonstrated a role for PpCycD in sensing carbohydrate supply (Lorenz et al., 2003). All plants, knockouts, and controls, developed normally from juvenile to adult tissues under standard growth conditions in medium lacking a carbon source. We also analyzed the plants in glucose-supplemented medium. Glucose is known to enhance proliferation rates but simultaneously retard the developmental progression of wild-type moss. In contrast to the wild-type, Pp*CycD* knockouts proceeded through the developmental steps towards adult plants regardless of the exogenous sugar supply. We hypothesize impairment to sugar sensing, indicating a role for CycD in connecting environmental signals to developmental decisions at the cellular level (Lorenz et al., 2003). The observation of prolonged growth as juvenile tissues instead of proceeding to sexual reproduction under high-energy conditions (such as exogenous carbohydrates or bright light) is supported by other studies. Thelander et al. (2005) observed pronounced caulonema formation under high-energy conditions. On the other hand, "low-energy" conditions (low light, short days, and low temperature) were shown to stimulate formation of gametangia and the development of sporophytes (Hohe et al., 2002).

Rb proteins from all dicotyledonous plants analyzed so far are represented by single copy genes. T-DNA insertion mutants of the *Arabidopsis* gene for retinoblastoma-related protein were found to be gametophytic lethal (Ebel et al., 2004). However, in monocotyledonous plants, as well as in *Physcomitrella, Rb*-related genes are represented by small families (Janos Gyorgyey, personal communication). In contrast to the deviating phenotype displayed by PpCycD KO plants, targeted knockouts of the Pp*Rb1* gene did not result in obvious morphological or physiological deviations. The unaltered phenotype of the moss Rb1 KO plants indicate the capacity of Rb2 or Rb3 proteins to complement the lack of Rb1 (Lorenz et al., unpublished). Double knockouts of different moss *Rb* genes are currently in preparation and will provide an indication of the function of these cell cycle regulators in early land plant evolution.

Abscisic Acid

While division of an apical cell leads to further chloronema or caulonema cells and a three-faced apical cell initiates bud formation, chloronema cells may also divide by intercalary division thereby producing brachycytes or tmema cells. The former may function as vegetative spores to allow the plants to survive unfavourable environmental conditions (Schnepf and Reinhard, 1997), while in tmema cells, protonema filaments may be disrupted into fragments, which support the propagation of the brachycytes. The formation of brachycytes and tmema cells is induced by abscisic acid (ABA; Figs. **2 A – C**). After removal of ABA, brachycytes germinate to build new protonema filaments (Schnepf and Reinhard, 1997).

In seed plants, ABA-responsive Rab or dehydrin gene families are also induced by osmotic stress (Chandler and Robertson, 1994). The presence of common signalling pathways for ABA and osmotic stress was suggested for mosses after analysis of the stress- and ABA-responsive wheat Em promoter in *Physcomitrella* (Knight et al., 1995). However, a promoter region derived from the *Physcomitrella* LEA-type gene *PpLEA-1,* which is sufficient to confer gene expression under ABA and osmotic stress treatments, is not reciprocally active in cereal cells because no promoter activity at all was detected in barley aleurone protoplasts transfected with a *PpLEA-1::GUS* construct (Kamisugi and Cuming, 2005). These studies suggest the existence of additional promoter elements and their interaction with additional trans-acting factors in ABA-mediated gene expression in seed plants. Common pathways were also hypothesized for ABA and cold stress in *Physcomitrella.* Protonema sensitive to cold stress developed freezing tolerance after ABA treatment for 24 h (Minami et al., 2003). The ABA treatment resulted in alterations to organelle morphology and accumulation of free soluble sugars (Nagao et al., 2005). However, ABA-independent cold signalling pathways also seem to exist, as enhancement of the freezing tolerance of protonema cells by incubation at low temperatures was not accompanied by increased endogenous levels of ABA (Minami et al., 2005). ABA-induced genes in *Physcomitrella* are often involved in several stress response pathways, indicating overlapping pathways in the control of stress-responsive genes in this evolutionarily old terrestrial plant, while the respective genes are regulated by distinct pathways in seed plants (Kroemer et al., 2004). A detailed analysis of the impact of different abiotic stress conditions on *Physcomitrella* plants showed high tolerance to dehydration, salt, and osmotic stress (Frank et al., 2005 b), indicating that this plant is a valuable tool for revealing stress adaptation processes. Expression profiles of 45 *Physcomitrella* genes with homology to stress-associated genes were generated in macroarray analyses. With regard to ABA, it was suggested that both ABA-independent and ABA-dependent stress response signalling pathways overlap (Frank et al., 2005 b).

In a recent approach to reveal the *Physcomitrella* secretome (as the entirety of the secreted proteins), several proteins were extracted from moss cell walls (Tintelnot et al., in preparation). After ABA treatment of the plants, an overall reduction in secreted proteins was observed. Ten of the identified extracellular proteins were further analyzed at the level of gene expression after phytohormone application. Two of these were shown to be up-regulated by ABA and six were down-regulated by ABA. Among the latter, homologues of putative compounds of signalling cascades and cell wall-modifying enzymes of the XTH (xyloglucan endotransglycosylase/hydrolase) and PME (pectin methylesterase) families were identified (Tintelnot et al, in preparation). The activities of both XTH and PME are supposed to result in cell wall loosening. In a previous report, one member of the *Physcomitrella* expansin gene family was shown to be up-regulated after ABA treatment (Schipper et al., 2002). Differential gene regulation of cell wall enzymes by ABA is consistent with the specific morphological changes caused by ABA treatment, the formation of thick-walled brachycytes, and fragile tmema cells, which is accompanied by drastic restructuring of cell walls.

Future Developments

The high efficiency of gene targeting enabled us to establish a mutant collection of more than 70 000 *Physcomitrella* plants (Schween et al., 2005). Phenotypic aberrations were observed for nearly 27 % of the mutants. In 10 % of the plants there were indications for physiological mutations as they grew more slowly on standard medium compared to medium supplemented with glucose, micronutrients, membrane lipid compounds, nucleotide precursors, amino acids, and vitamins. Of all the mutants, 3 % displayed retarded growth on both media. The morphological data were listed in the database mossDB (Schween et al., 2005). Linking incoming molecular data of the transformants to this database will provide a valuable biological resource for systems biology. From all EST data available on *Physcomitrella,* 26 123 virtual transcripts were derived, and a high-quality annotation pipeline was developed and used for evaluation of the virtual transcriptome. Consistency in the ratios of the core molecular functions compared to other plants could be demonstrated (Lang et al., 2005). Based on these data, we started work on large-scale expression profiling. *Physcomitrella* transcription factors were identified via homology and domain searches. Expression of immediate early and early transcription factor genes and their responsiveness to phytohormones is currently under investigation.

However, the earliest events in phytohormone signalling, the transduction of the signal from the point of perception to the sites of primary hormone responses, cannot be assayed by monitoring gene expression but relies on the identification of temporary modifications of pre-existing molecules or structures within the cell. As a large number of cellular signalling processes is mediated via sequential protein phosphorylations and dephosphorylations (Ehness et al., 1997; Kwak and Lee, 1997; Grefen and Harter, 2004), temporal resolution of these events is expected to provide valuable insights into the mechanisms of phytohormone action. The standardized *Physcomitrella* growth conditions allow the exact timing of stimulation and sampling necessary to resolve variations in protein phosphorylation status occurring rapidly, within only a few minutes. The total amount of proteins actually undergoing these post-translational modifications in the course of the signal transduction event is expected to be exceedingly low (Yu et al., 2004). A high sensitivity of the detection system is therefore an essential requirement for success. This requirement is met by using a multidimensional liquid separation protocol, combining immobilized metal affinity purification, reverse phase chromatography, capillary zonal electrophoresis, and mass spectrometric analyses (Heintz et al., 2004). The unbiased approach does not restrict analyses to preselected known or predicted routes of signal transduction, but provides insights into the complex messaging network serving the precise coordination of early hormone responses. In order to elucidate early events in cytokinin signal transduction, the first 15 min following the onset of hormone stimulation were monitored using differential phosphoproteomics in *Physcomitrella patens,* and demonstrated the absolute immediate response of several processes to the hormone stimulus (Heintz et al., unpublished).

The potential of moss as a model plant was further extended by exploring the *Physcomitrella* proteome. Experimental conditions were adopted for investigation of the moss. Proteins

were separated by two-dimensional gel electrophoresis and excised spots were analyzed by mass spectrometry (Sarnighausen et al., 2004). The identification of the first 306 *Physcomitrella* protonema proteins led to a reference map which will form the basis for proteomic studies of phytohormone action in the moss.

The results obtained recently, together with the established methods, will help to create a complete image of phytohormone action in moss in which morphological and physiological changes are connected to the responsible molecular events. In a systems biology approach, the regulatory networks underlying the developmental decisions of a single cell will then be unravelled.

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