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*Review*

# Molecular genetics of *Physcomitrella*

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## Introduction

In the first few decades of this century, scientists working on the genetics of bryophytes – mosses and liverworts – were at the forefront of genetical research: Allen (1917) was the first to describe sex chromosomes in plants, Heitz (1928) demonstrated the continuity of chromosomes during the mitotic cell cycle, and Knapp (1936) employed X-ray mutagenesis for genetical research. Although the beginning of plant molecular genetics was marked by ultraviolet light (UV) mutagenesis of the liverwort *Sphaerocarpos donellii*, which demonstrated DNA as the molecular basis of inheritance (Knapp et al. 1939), bryophytes have only been of marginal interest for modern molecular biologists. The outstanding exception is the liverwort *Marchantia polymorpha*, which was first subjected to genetical analysis with intensive mutation and cross-breeding experiments (Burgeff 1943). It is now well known because complete nucleotide sequences have been determined for its plastid and its mitochondrial DNA (Ohyama et al. 1986; Oda et al. 1992).

Non-mendelian inheritance was first postulated by von Wettstein (1928) who analysed *Funaria hygrometrica* and related mosses. Likewise, von Wettstein (1924) recognised the great potential of the haploid moss protonemata for genetically dissecting differentiation processes. These analyses started with mutant induction in *Physcomitrium piriforme*, *Funaria hygrometrica* and *Physcomitrella patens* (Barthelmes 1940; Oehlkers and Bopp 1957; Engel 1968) and culminated in the synopses on the physiology and genetics of cellular differentiation in *Funaria* and *Physcomitrella* (Bopp 1981, 1990; Cove 1992; Cove and Knight 1993). These classical genetical studies led to the suggestion that the genetic dissection of *Physcomitrella* could make significant contributions to understanding the evolution of plant development processes (Goldberg 1988; Chasan 1992). In recent years, progress has been made in analysing this moss molecular genetically. A major breakthrough was achieved with the demonstration that homologous recombination occurs in its nuclear DNA at frequencies which are orders of magnitudes greater than those known from higher plants (Schaefer and Zryd 1997) making *Physcomitrella* the first land plant with an efficient system for reverse genetics (Strepp et al. 1998). In a preceeding review the primary focus was on physiological aspects of moss development (Reski 1998a). The aim of the present work is to discuss in more detail what is known about the molecular genetics of the most prominent moss species, *Physcomitrella patens* (Hedw.) B.S.G.. Naturally, reviews have to be selective – I apologise to those colleagues whose work could not be cited.

## Plant development

A characteristic of mosses is their heteromorphic *Generationswechsel*, the alternation of two generations which are distinct from each other in terms of nuclear DNA amounts and morphology as well as the dominance of the haploid gametophyte (for a recent review, see Reski 1998a). *Physcomitrella* development starts with a germinating meiospore that gives rise to a filamentous structure, termed a protonema, which represents the juvenile gametophyte (Fig. 1a). The adult gametophyte is the leafy moss plant (Fig. 1b), termed a gametophore as each leafy stem is capable of generating the sex organs. Fertilisation and embryo development take place in the female sex organ, the archegonium, giving rise to the diploid generation, the sporophyte (Fig. 1c). The sporophyte is green in its youth but loses its capability for photosynthesis during development.

Abbreviations: EST = expressed sequence tag; ptDNA = plastid DNA; RFLP = restriction fragment length polymorphism

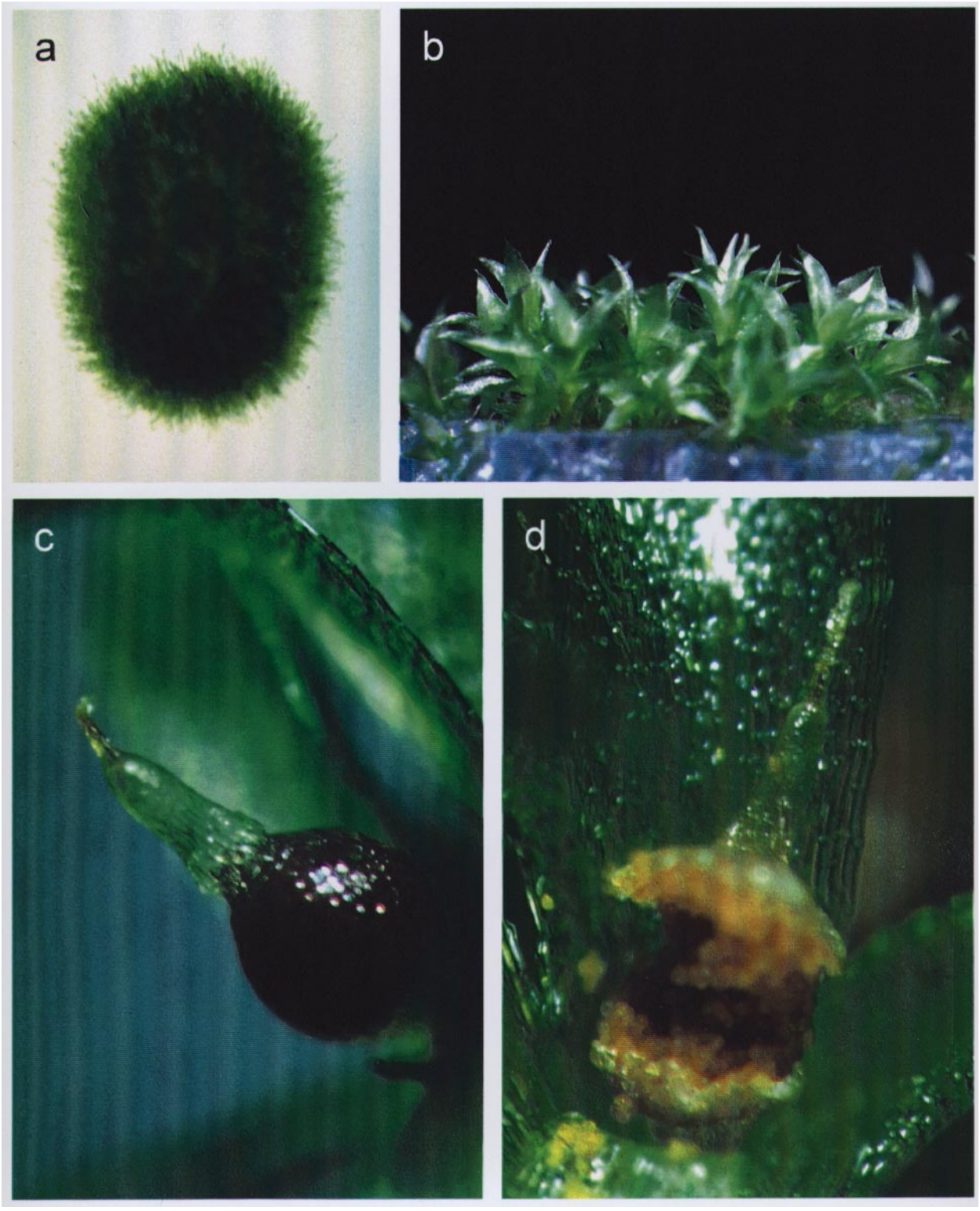


**Fig. 1a–d.** Habitus of *Physcomitrella* at different stages of the life cycle as seen under a stereomicroscope. **a** A *bud*-mutant is defective in the building of three-faced apical cells (buds) and therefore grows solely as protonema (=juvenile gametophyte). **b** Leafy moss shoots (=adult gametophytes) arise from protonemal buds. **c** The diploid generation of *Physcomitrella*, the sporophyte, develops from a fertilised egg in a female sex organ, generated by the adult gametophyte. The sporophyte becomes brown upon maturation and carries the remainder of the female sex organ at its top. This green remainder is called the calyptra. **d** A ripe *Physcomitrella* sporophyte has no specialised structure for dehiscence but disintegrates to release the haploid meiospores. **b–d** courtesy of Rasmus Lüthje

During its entire cycle its nutrition supply is dependent on the gametophyte. Meiosis takes place within the sporophyte, generating about 5000 meiospores. In contrast to other mosses, *Physcomitrella* has no specialised structures for dehiscence and releases its spores simply by disintegration of the sporophyte (Fig. 1d).

Unlike morphogenesis in higher plants, morphogenesis in the moss protonema can be pinpointed to one single-cell event in each case (Bopp 1968). The best known such step is the transition from the juvenile to the adult gametophyte, which is characterised by the transition from apical cell division to division via a three-faced apical cell. This meristem initial of the gametophore is termed a bud. From various reports it is clear that cytokinins induce bud formation in mosses (reviewed by Reski 1998a). However, active phytochrome is needed for bud formation. Cytokinins as well as blue light induce macrochloroplast division in the *Physcomitrella* mutant PC22, whereas blue light is no longer active in bud formation (Abel et al. 1989; Reski et al. 1991). On the other hand, in the mutant, active phytochrome counteracts the cytokinin-stimulus on plastid division (Kasten et al. 1997). Recently, certain developmental *Physcomitrella* mutants were cured by successful functional expression of the bacterial *ipt* gene, conferring enhanced levels of cytokinin (Reutter et al. 1998). Mutants with defects in *budding* (*bud*) and in *plastid division* (*pdi*) but not in *gametophore development* (*gad*) could be cured by this bacterial gene, identifying *gad* as a developmental gene downstream of *bud* but not directly activated by cytokinin. Once activated, *gad* seems to downregulate *bud*, preventing protonemal cells adjacent to gametophores from producing new gametophores. As this phenomenon can be overcome by application of cytokinin, the effect mimicks the classical phenomenon of apical dominance in seed plants. From more than 200 PC22*ipt* transgenics, Reutter et al. (1998) found just one plant (tPC22*ipt*111) which overproduced cytokinins and produced numerous buds but was not able to divide its mutant macrochloroplast. On the basis of these data, a tentative model for a bifurcated cytokinin signal-transduction pathway in mosses is proposed with the two branches interacting differently with the photoreceptors. Additionally, it was suggested that in tPC22*ipt*111 a member of the cytokinin- and blue-light-mediated signal-transduction pathway to plastid division was inactivated (Fig. 2).

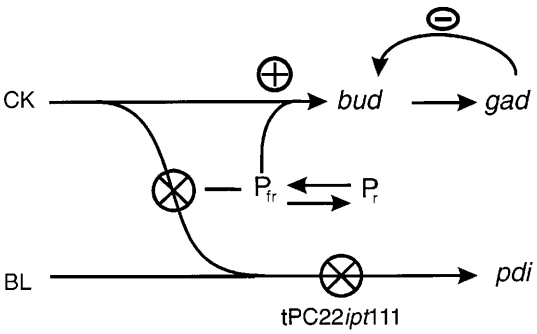


**Fig. 2.** Tentative model for a bifurcated cytokinin signal-transduction pathway in *Physcomitrella*. Cytokinins (*CK*) induce the genes *budding* (*bud*) and *plastid division* (*pdi*). Downstream of *bud* is a gene *gametophore development* (*gad*), which is not directly triggered by CK but downregulates the expression of *bud*. This downregulation can be overcome by addition of cytokinin. Thus this system mimicks classical apical dominance as known from seed plants. Blue light (*BL*) can induce *pdi* but not *bud*. Active phytochrome is needed for the CK stimulus on *bud*, but counteracts the CK stimulus on *pdi*. Amongst some 200 CK-overproducing transgenic *Physcomitrella* mutants, one single strain was isolated in which extra CK activated *bud* but no longer *pdi*. As neither BL nor CK could activate *pdi* in this specific strain, we propose that this transgenic is blocked in a common signal transduction pathway leading to *pdi*

## Large-scale cultures and cell fractionation

Although mosses have been standard objects in classical genetics, only recently have the modern techniques of biochemistry and molecular genetics been applied to them. One of the most obvious reasons for this delay was the limited availability of the plant material: mosses are small- and slow-growing plants even compared with *Arabidopsis*. It was not until ten years ago that an easy-to-handle batch culture in liquid medium was described and efficient protocols for cell fractionation and subsequent isolation of pure nuclear, chloroplast and mitochondrial DNA from *Physcomitrella* were developed (Marienfeld et al. 1989). As *Physcomitrella* can be grown in agitated liquid medium, it is also feasible to obtain large amounts of plant material from bioreactor cultures of this moss (Boyd et al. 1988; Reutter and Reski 1996; Cove et al. 1997).

## Plastid DNA

The first report on the organellar DNA of a moss was a physical map of the plastid (pt) DNA from *Physcomitrella* (Calie and Hughes 1987). This confirmed a conserved synteny, showing that the entire consensus land-plant chloroplast gene order, with two small alterations, is also present in moss ptDNA. The moss ptDNA is a circular molecule of ca. 123 kb (Calie and Hughes 1987) with a guanine plus cytosine content of ca. 38.6% (Reski et al. 1994). Intensive restriction fragment length polymorphism (RFLP) analyses comparing the ptDNA of wild-type *Physcomitrella* with two of its developmental mutants and their somatic hybrid did not detect mutations in these different DNA species but revealed methylation of the ptDNA in a chloroplast mutant. This DNA modification seemed to be restricted to the area of the *rbc*L gene (Reski et al. 1991). Such RFLP analyses, as well as cloning and partial sequencing of ptDNA fragments, resulted in a refined physical map of *Physcomitrella* ptDNA (Fig. 3).

The sequence analysis of eight different plastid genes revealed a nucleotide homology in the coding region of more than 85% to the respective genes from the liverwort *Marchantia polymorpha* and 65–82% to those from tobacco (Reutter et al. 1992). One tRNA gene (*trnR*-CCG) was found to be specifically present in bryophyte ptDNA, but not in the ptDNAs of angiosperms

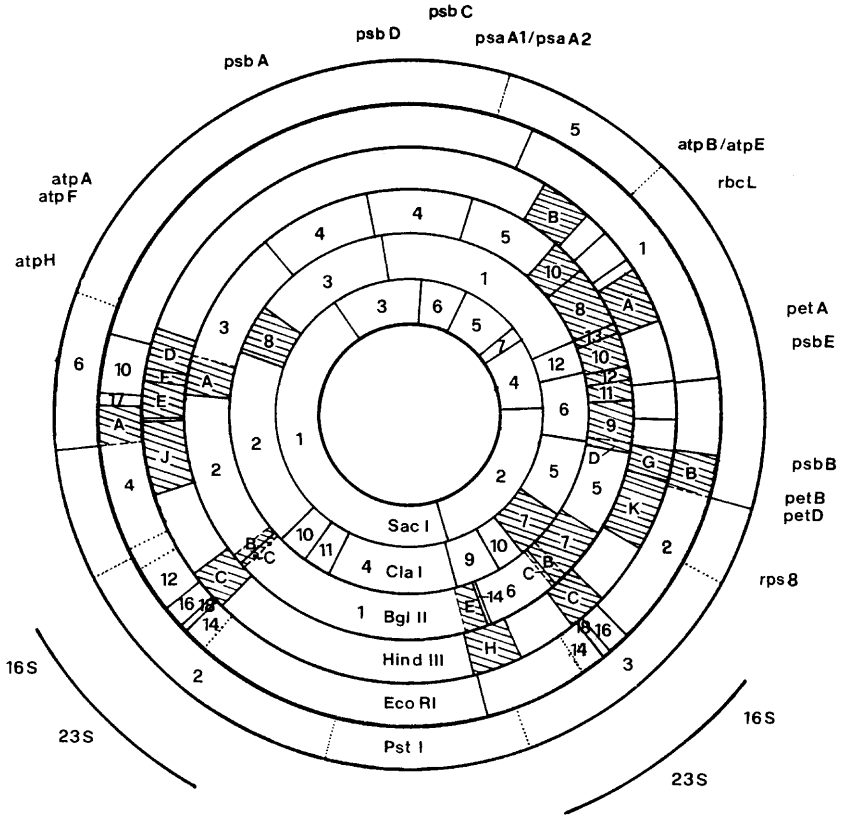


**Fig. 3.** Partial physical map of *Physcomitrella* plastid DNA with identified genes and restriction fragments as deduced from Southern experiments, as well as partial cloning and sequencing (reprinted from Reutter et al. 1992). The ptDNA of *Physcomitrella* is a circular molecule of about 123 kb. Digestion with six different restriction endonucleases (*six circles*) generated fragments of different sizes. Within one digestion the resulting fragments are numbered according to sizes if they could be identified. *Dotted lines* indicate putative *Pst*I recognition sites. *Hatched regions* represent cloned and identified ptDNA restriction fragments. *Letters* identify fragments which could not be classified according to sizes. Outside of the circles the position of identified genes is indicated as determined by Southern analyses and partial sequencing. The *two lines outside of the circles* mark the positions of the inverted repeats

(Kasten et al. 1991). Subsequently, a gene was identified (*zfp*A) which had procaryotic as well as eucaryotic promoter consensus sequences. The mRNA abundance for this gene is regulated by cytokinin (Kasten et al. 1992). A more detailed analysis revealed that not only cytokinin but also illumination and the experimental daytime influenced transcript stabilities of this gene and three other plastome-encoded genes (*atp*A, *psb*A, *psb*B). This effect was especially evident in two different cytokinin-sensitive developmental mutants (Kruse et al. 1995a). From mutant analysis it was concluded that altered mRNA stability in these mutants was dependent on the mutation in *budding*, a nuclear gene governing cytokinin-induced cell differentiation (Kruse et al. 1995a). In a complementary approach, plastid proteins were identified whose levels increased or decreased upon cytokinin treatment. Microsequencing of these proteins identified amongst others three different polypeptides homologous to the enhancer protein OEE2 of the oxygen-evolving complex, four isoforms of phosphoglycerate kinase and a β-chain of chloroplast ATPase. Further sequence information was obtained for both subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase, including two different proteins for the small subunit. These results indicated a coaction of nuclear- and plastid-encoded genes in cytokinin-stimulated differentiation (Kasten et al. 1997).

Heterologous expression of *zfp*A (which had been renamed as *ycf*11) in *Escherichia coli* indicated that the protein synthesised might be associated with membranes and aggregates to tetramers in vivo, supporting the hypothesis that *ycf*11 is *acc*D, a gene encoding the β-subunit of a novel acyl-coenzyme A carboxylase (Kruse and Reski 1996). Interestingly, this gene is evolutionarily conserved from cyanobacteria to the plastid DNAs of archegoniates (ferns, mosses and liverworts) and gymnosperms, is modified in the ptDNAs of dicots, and is not plastome-encoded in monocots (Kruse et al. 1995b). To study molecular evolution of plants, the plastid-encoded *rbc*L sequences are widely used. The *rbc*L gene was sequenced from *Physcomitrella* and it was found that it is far more conserved than *ycf*11/*acc*D, making it a useful molecular marker in the study of global plant evolution (Kruse et al. 1995b).

## Mitochondrial DNA

The first mitochondrial DNA sequence from an archegoniate was obtained from *Physcomitrella*. The coding region of the *cox*3 gene had a homology at the nucleotide level of about 81% to known sequences from angiosperms and the codon usage appeared to be similiar between both plant groups (Marienfeld et al. 1991). Unlike its higher-plant counterparts, the *Physcomitrella cox*3 has an intron, identical to the situation in *Marchantia* (Oda et al. 1992). Intensive RFLP analyses of the mitochondrial DNAs of wild-type *Physcomitrella*, two of its developmental mutants, and their somatic hybrid did not detect any mutations in these different DNA species (Marienfeld et al. 1992).

## Chromosome number, genome size and the cell cycle

There are dioecious mosses which have male and female individuals, as well as monoecious species in which one plant bears both types of sex organ. Correns (1899) analysed 915 species of European mosses and found 54.6% to be dioecious, the remainder, including *Physcomitrella*, being monoecious.

Compiling 3863 reports on chromosome counts in mosses, Newton (1984) argued that species with $n = 6$ or 7 chromosomes are basically haploid and those with about 10 to 14 chromosomes in the gametophytic development may be basically diploid. She suggested that autopolyploidy, allopolyploidy and amphidiploidy may have contributed to the evolution of bryophytes. This view was supported by Wyatt et al. (1988), who presented biochemical data on allopolyploidy and multiple origins of *Plagiomnium medium*. Newton (1984) further suggested that cells of the gametophytic tissue of *Physcomitrella* may have 26 chromosomes and thus be tetraploid. In contrast, the few reports on sexual crosses of the self-fertile species *Physcomitrella* describe segregation ratios of a true haploid species (Grimsley et al. 1977, Cove 1983). Chromosomes of mosses, especially of the *Funariaceae*, are very small and difficult to analyse (Newton 1984). Consequently, different chromosome numbers of *Physcomitrella* have been reported over several decades: von Wettstein (1925) analysed a German isolate and suggested $n = 16$ (15–18), Bryan (1957) drew 27 mitotic chromosomes from an isolate from Michigan, while Engel (1968) showed with photographs of diakinesis $n = 14$ in an English isolate. Finally, based on mitotic as well as on meiotic chromosome counts, Reski et al. (1994) supported Bryan's (1957) observation of $n = 27$ chromosomes.

Discussing the discrepancies between von Wettstein's results and her own, Bryan (1957) suggested two possibilities: (i) the existence of two chromosomal races, or (ii) clumped chromosomes. Frequently, clumped chromosomes were detected, resulting in fewer but bigger chromosomal blocks (Reski et al. 1994). However, in the photograph published by Engel (1968) no such chromosomal blocks can be seen, thus favouring the existence of two chromosomal races. Nevertheless, the Hamburg strain analysed by Reski et al. (1994) originates from the culture which Engel (1968) used and is different from the isolate Bryan (1957) has used. Therefore, it seems unlikely that the observed differences in chromosome numbers are due to the use of different chromosomal races in *Physcomitrella*. However, transient changes upon culturing cannot be excluded.

Based on flow-cytometric quantification of 4,6-diamidino-2-phenylindole (DAPI)-stained nuclei, a genome size for *Physcomitrella* of about 480 Mbp is suggested (unpublished results), comparing to *Arabidopsis* and to *Phaseolus vulgaris* as standards. The moss genome is about three times larger than that of *Arabidopsis*. However, this may be an overestimate since DAPI is an adenine and thymine-specific dye and as measured by thermal denaturation kinetics *Physcomitrella* total DNA is rather rich in adenine and thymine, with a guanine plus cytosire content of 34.6%, whereas this value is 41.4% for *Arabidopsis* (Reski et al. 1994).

These flow-cytometric analyses revealed that *Physcomitrella* protonemata grown in liquid culture under a 16 h light/8 h dark regime with illumination starting at 6 a.m. are highly synchronised and exhibit extraordinary features, making them ideal candidates for cell cycle analyses: chloronema cells are arrested in G2 for most of the day with mitosis occurring mostly between 2 and 4 a.m. Young caulonema cells, in contrast, remain in G1, while older caulonema cells become polyploid. It is as yet unknown whether these profound differences in the mitotic cycle determine or reflect the physiological differences between chloronema and caulonema.

## Sequence homologies and codon usage for nuclear genes

The first nucleotide sequence of a protein-encoding moss gene was published by Long et al. (1989) who cloned and sequenced a genomic clone of a gene encoding the major chlorophyll-binding protein (*cab* gene). Since then, nucleotide data from this species have accumulated (glycerine aldehyde phosphate dehydrogenase: Martin et al. 1993; *myb*-related genes: Leech et al. 1993; *phy*B: Kolukisaoglu et al. 1993; *rbc*S, *cab* and 25 S rDNA: Reski et al. 1994; 18 S rDNA: Capesius 1995) as well as amino acid information for chloroplast proteins (the enhancer protein OEE2 of the oxygen-evolving complex, phosphoglycerate kinase, and plastid ATPase: Kasten et al. 1997). By functional complementation of an *E. coli* mutant, von Schwartzenberg et al. (1998) isolated a gene so far unknown in plants (*adk*: encoding an active adenosine kinase) and demonstrated that the protein is involved in cytokinin metabolism.

Different cDNA libraries have been established from *Physcomitrella* (Martin et al. 1993; Reski et al. 1994; Machuka et al. 1999). Molecular subtraction of 2 libraries from tissues subjected to different cytokinin-treatments yielded about 80 expressed sequence tags (ESTs), which comprise 39 genes so far unknown for plants; amongst these was a cDNA with significant homology at the nucleotide level to a *Neurospora crassa* NADH-ubiquinone oxidoreductase 40-kDa subunit. For those sequences for which a significant homology to a protein of a seed plant species was obtained, the best fit was to a dicotyledon for 11 ESTs, to a monocotyledon for 9 and to a gymnosperm for 3. Amongst these 23 sequences are 16 chloroplast-related ESTs, of which 8 represent distinct *cab* genes of *Physcomitrella* with similarities to genes encoding chlorophyll *a*/*b*-binding proteins from a variety of seed plants. In the classification system for *cab* sequences of higher plants, 10 classes are defined, whereas such information was not available from seedless plants. Additionally, 14 ESTs had significant homologies to the same number of different genes coding for ribosomal proteins, whereas 9 were homologous to known plant genes, coding for histone H2A, cyclophilin and nonsymbiotic hemoglobin (Reski et al. 1998).

Comparison of each entire EST to the seed plant sequence giving the highest score following searches with the BLASTN programme revealed the degree of conservation at the nucleotide level to be between 60% and 75%, regardless of whether the seed plant source was a dicotyledon, a monocotyledon or a gymnosperm. This showed for the first time a high degree of conservation between several expressed genes of seed and seedless plants. Using the information from 22 sequences in which the reading frame could be defined (8 genes encoding *cab*, 9 encoding ribosomal proteins and 5 others), codon usage in *Physcomitrella* was assessed and compared to that in similar genes in dicots and monocots. Upon inspection of the data generated, it became clear that codon preference in *Physcomitrella* was very similar to that in dicots, exhibiting just eight major differences, these being in the codons for cysteine, phenylalanine, glutamine and histidine. For these eight codons, the preference is more similar to that seen in monocots, exhibiting the asymmetric pattern of bias towards cytosine or guanine in the third position, a pattern found in previous codon-usage studies in monocots (Reski et al. 1998).

In a complementary approach, Machuka et al. (1999) constructed a cDNA library from *Physcomitrella* protonemata treated with abscisic acid (ABA), randomly chose cDNAs to be sequenced and obtained 171 ESTs. About 45% of these had homology to known sequences, including genes encoding a voltage-dependent anion channel, initiation factors and several different ribosomal proteins. Several sequences were identified which had similarities to genes which are implicated in plant stress responses, including responses which may involve ABA. Among these were homologues to genes encoding heat-shock proteins, Zn/Cu-superoxide dismutase, NADPH protochlorophyllide oxidoreductase and glutathione S transferase. Codon-usage analysis of the data for 14 *Physcomitrella* sequences with defined open reading frames (ORFs) essentially confirmed the findings of Reski et al. (1998), again demonstrating no significant difference at the level of codon usage between *Physcomitrella* and seed plants (Machuka et al. 1999).

Taken together, these two EST-studies reveal that *Physcomitrella* and seed plants have a high degree of shared codon usage and gene conservation, as well as the already-mentioned synteny. Of even greater relevance it was directly demonstrated that the moss is a rich source for novel sequences previously unknown in plants, implying that the generation of large amounts of *Physcomitrella* sequence information may help to gain new insights into the regulation of plant biochemistry and development.

## Transient and stable transformants

*Physcomitrella* can be stably transformed by polyethyleneglycol-mediated DNA transfer to protoplasts (Schaefer et al. 1991) or by using the particle gun (Sawahel et al. 1992), but so far not by *Agrobacterium*-mediated DNA transfer (Reutter 1994). It is, however, accessible to microinjection (Abel et al. 1989). This moss has been transformed in order to study the role of ABA and osmotic stress using promoter elements from the wheat *Em* gene (Knight et al. 1995), as well as the role of endogenous calcium (Russell et al. 1996, 1998), to cure developmental mutants by the bacterial *ipt* gene (Reutter et al. 1998) and for biotechnological purposes (Reutter and Reski 1996). In these approaches, constructs commonly used in higher plants proved to work successfully in *Physcomitrella*. As in higher plants, regulated expression in *Physcomitrella* can be achieved via the tetracycline-regulated promoter system (Zeidler et al. 1996).

An interesting phenomenon is associated with the transformation process in *Physcomitrella* (reviewed by Knight 1994). When transforming with commonly used plasmid-derived vectors, only about 5% of the initial antibiotic-resistant regenerants are stable transformants, i.e. have integrated the transgene into the genome and inherit it meiotically. Interestingly, most of these stable transgenics had integrated multiple copies of the transgene at one locus, whereas only a few had integrated single copies at two different loci (Schaefer et al. 1991; Reutter and Reski 1996; Reutter et al. 1998).

About 95% of the initial antibiotic-resistant *Physcomitrella* plants fall into two (equally frequent) classes, those that express the reporter gene transiently and a heterogeneous group of unstable transformants in which the plasmid is maintained under selection pressure and is believed to be replicated extrachromosomally. Such unstable transformants appear to be chimaeras: tissue which is in contact with the antibiotic can be regenerated to antibiotic-resistant plants even after months of subculture, whereas tissues from the same plant which are not in direct contact with the medium, like leaves, can only be regenerated to plants on antibiotic-free media (Reutter 1994). While transient transformants will die anyway, the method of choice to discriminate stable from unstable transformants is removal of antibiotic followed by a renewed selection period (reviewed by Knight 1994). Such unstable transformants contain extrachromosomal bacterial plasmids. These plasmids can be recovered from the plants by recloning them in *E. coli* (unpublished results). This characteristic of *Physcomitrella* transformation should help make mutant curing very efficient in this plant.

## An "upstart" for reverse genetics in plants

While working on the polyethyleneglycol-mediated transformation of *Physcomitrella*, Schaefer (1994) noticed that re-transformation of transgenic lines was significantly enhanced when transforming plasmids were used that share, beside different selection markers, extensive sequence homology. Segregation analysis of the double-resistant transgenic plants gave genetical evidence that the two selection markers were tightly linked in about 86% of the clones tested. Confirmatory evidence for highly efficient homologous recombination was obtained by Kammerer and Cove (1996) who

extended the genetical analysis and demonstrated linkage between two transgenes but not to a marker mutation leading to auxotrophy. Subsequently, Schaefer and Zryd (1997) presented molecular evidence for the occurrence of highly efficient homologous recombination in the genomic DNA of *Physcomitrella*. As these data were restricted to the targeting of unidentified genomic DNA, it may be argued that evidence for gene knock-out of a functional gene was still missing. This was provided for two novel genes.

*The first organellar division protein – an ancestral tubulin*. In the laboratory, the moss homologue of the bacterial gene *fts*Z, encoding the cell division protein FtsZ, has recenctly been isolated. Bacterial FtsZ is considered to be an ancestral tubulin. Compared to bacterial proteins, the nuclear-encoded moss protein PpFtsZ had an N-terminal extension that might serve as a leader peptide in organellar import, but computational analysis revealed no localisation signals or clues pointing to where the protein might be targeted intracellularly. In order to disrupt this gene by homologous recombination, the selection marker was inserted into the coding region and a vector-free linear DNA construct consisting of the *npt*II cassette flanked by 247 and 658 bp of the cDNA *Ppfts*Z sequence was used to transform *Physcomitrella* protoplasts, and following regeneration and selection, 51 independent stably transformed plants were isolated. Molecular analysis demonstrated that about 14% of the transgenics were generated by homologous recombination. The defects in these knock-out plants seen by microscopic examination revealed that the disrupted gene encodes a protein essential for chloroplast division, thus identifying PpFtsZ as the first organellar division protein from any eukaryote (Strepp et al. 1998).

*A novel Δ6-acyl-group desaturase*. In collaboration with Ernst Heinz (Institut für Allgemeine Botanik, Universität Hamburg, Germany) and his group a polymerase-chain-reaction approach employing primers deduced from widely conserved histidine boxes of acyl-lipid desaturases was used to isolate a homologous sequence from *Physcomitrella*. The corresponding full-length cDNA shared less than 27% sequence identity at the protein level with known acyl-lipid desaturases. The cDNA sequence was used to identify the genomic locus of this novel gene. To disrupt this gene by homologous recombination, the first histidine box was replaced by a positive selection marker, a 35S-promoter-driven *npt*II gene conferring resistance to kanamycin. The vector-free, linear DNA construct consisting of this selection marker flanked by 923 bp and 1159 bp of genomic putative desaturase-encoding sequence was then used to transform *Physcomitrella* protoplasts. Following protoplast regeneration and growth on selective medium, 56 independent and stably transformed plants were isolated from 5 independent transformation experiments. Molecular analysis of randomly chosen transformants showed that more than 90% of them had been generated by homologous recombination. Analysis by HPLC and feeding-experiments demonstrated that the disrupted gene encodes a novel Δ6-acyl-lipid desaturase (Girke et al. 1998).

Although even 14% knock-out plants amongst transgenics is a convenient rate to screen for, the questions arises as to what influences the rates of homologous recombination in *Physcomitrella*. Based on the two published reports it became obvious that the length of homology is one important parameter: 2082 bp of homology were enough to result in knock-out frequencies of more than 90% (Girke et al. 1998), whereas the use of 905 bp of cDNA was sufficient for knock-out frequencies of about 14% amongst transgenics (Strepp et al. 1998). However, the actual stretches of homology may have been even smaller in the latter case, as the presence of introns in the corresponding genomic region was not excluded by Strepp et al. (1998).

In any case, the homologous recombination was successfully used to analyse the biological function of yet unknown genes in *Physcomitrella* by targeted gene disruption. This approach, termed reverse genetics, avoids the problems with "position effects", cosuppression or unstable phenotypes in anti-sense plants, all known for so long in plant molecular biology (Puchta and Hohn 1996). Although, worldwide, several excellent groups work on homologous recombination in higher plants, especially in *Arabidopsis*, the highest claimed homologous recombination rate, in the latter species, is one event in 1000 transgenic lines (Liljegren and Yanofsky 1998). This best estimation is based only on 3 single events amongst 9000 screened transgenic *Arabidopsis* lines and is therefore not widely accepted by the *Arabidopsis* community (Puchta 1998). Moreover, it is noteworthy that none of these events had produced a phenotypically altered plant. Since, for two genes, 14% (Strepp et al. 1998) and 90% (Girke et al. 1998) knock-out plants have been produced amongst transgenics, *Physcomitrella* apparently is the only plant where the approach of reverse genetics is feasible, at least at present (Reski 1998b).

## Conclusion

From the physiological reports it is obvious that as many basic biological aspects can be studied in the moss as in higher plants, although sometimes more easily. Several genes have now been cloned from *Physcomitrella* and they turn out to be remarkably homologous to their cognate higher-plant genes. All transformation experiments so far have demonstrated that there are no significant differences in promoter function or in codon usage between *Physcomitrella* and dicotyledonous angiosperms such as *Arabidopsis* or *Nicotiana*. Furthermore, the simplicity of the system allows developmental analysis at the cellular level to be carried out, combining the methods of plant physiology and molecular genetics with those of modern cell biology all in one organism.

The success story of yeast as a widely used model system is based on the possibility of using reverse

genetics in a unicellular eukaryote. *Physcomitrella* is not a microorganism and therefore cannot compete with yeast in terms of growth rate and facility of handling. But as a multicellular land plant, *Physcomitrella* obviously has added value for the plant science community, appealing to many different interests.

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