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The Moss, *Physcomitrella patens*

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Abstract

The tractability of the moss, *Physcomitrella patens,* to genetic analysis and the accessibility of its living tissues to direct observation make this species an extremely attractive system for studying plant development. The gametophyte generation, being haploid, allows direct detection of mutant phenotypes. The protonemal stage of gametophyte development is composed of cell filaments that facilitate detailed study of cell polarity and pattern determination. Techniques for the molecular analysis of gene expression include transformation, using either polyethylene glycol mediated uptake of DNA by protoplasts or biolistic delivery into protonemal tissue. When transforming DNA contains sequences homologous to genomic sequences, recombination can occur with high frequency, providing a way not only for the directed inactivation of genes, but also for precise allele replacement. Further development of the system is required, and priorities include the establishment of a gene tagging system. Other moss species have different advantages and a further priority must be the extension of the techniques devised for *Physcomitrella* to other moss species.

Key words: Moss; *Physcomitrella patens*; *Ceratodon purpureus*; Homologous recombination; Gene targeting

Life Cycle

Mosses, like ferns and seed plants, show an alternation of generations (Figure 1). The haploid, gametophyte generation is, however, dominant. Spores are produced by meiosis from the diploid generation, the sporophyte. Spores germinate to form protonemal tissue, consisting of simple cell filaments of chloronemal cells. Chloronemal filaments made up of cells densely packed with large chloroplasts extend by serial division of their apical cells, with a cell cycle time of about 24 h. The subapical cells of chloronemal filaments undergo a limited number of divisions to form side branches. Chloronemal apical cells can undergo a transition to give rise to caulonemal filaments. This second protonemal filament type has cells containing fewer, less developed chloroplasts. As with chloronemal filaments, caulonemal filaments extend by serial division of their apical cells, but with a cell cycle of only about 6 h. This coupled with their more rapid rate of linear extension (about 40 μm/h for caulonema compared with 2 μm/h for chloronema) allows rapid colonization of the soil or agar surface. Subapical cells of caulonemal filaments divide a limited number of times to produce side branches. Most side branches growing in high levels of light develop into chloronemal filaments, others develop into caulonemal filaments or buds. Bud production involves a transition from two-dimensional filament growth to three-dimensional shoot development. Shoots produce gametangia and gametes, and are therefore termed "gametophores." Fertilization gives rise to the diploid sporophyte, which comprises a seta, the stalk that bears the capsule in which spores are produced. The sporophyte of *Physcomitrella patens* (referred to simply as *Physcomitrella* throughout) is much reduced compared with some moss species and lacks elaborate structures for spore release. The seta is only a few millimeters long, and

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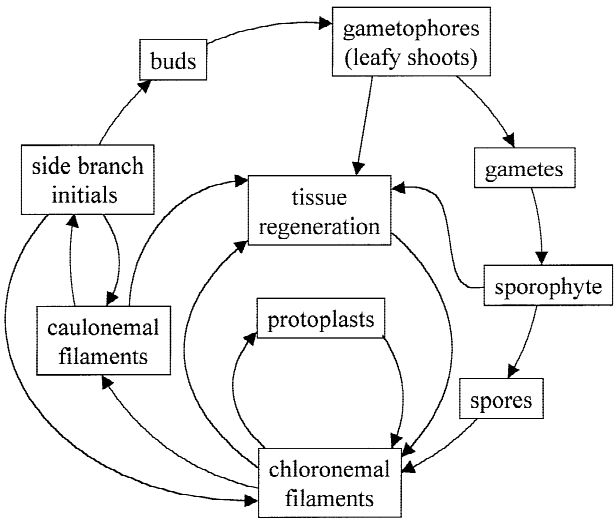

Figure 1. Simplified life cycle of *Physcomitrella patens.* In normal development, all stages shown here, with the exception of the zygote and sporophyte that it gives rise to, are haploid and comprise the gametophyte stage of development. However, sporophytic tissue, on aposporous regeneration, gives rise to diploid gametophytic tissue.

so the spore capsule lies within the cluster of leaves at the apex of the gametophore. About 4,000 spores are produced in each sporophyte. *Physcomitrella* is monoecious, and male and female gametangia are produced on the same gametophore. The life cycle of *Physcomitrella* can be completed in culture in about 8 weeks (Figure 1). For a more detailed description of the development of *Physcomitrella* see Cove (1991); Cove and colleagues (1997); and Reski (1998).

Cells from almost any tissue of *Physcomitrella,* whether gametophytic or sporophytic, regenerate to produce protonemal tissue. Regeneration of sporophytic tissue therefore generates diploid gametophytic tissue, indicating that the morphogenetic differences between sporophyte and gametophyte are not caused by differences in ploidy. Protoplasts are readily isolated from young protonemal tissue. Protoplast regeneration occurs at a high rate, does not require supplemention of medium with hormones, and results directly in the formation of chloronemal filaments. It thus resembles spore germination.

Culture Methods

The nutritional requirements of *Physcomitrella* are simple. It can be cultured in liquid or on solid media containing only inorganic salts. It is routinely grown on agar-gelled medium in Petri dishes or in liquid medium in flasks and can also be grown in a fermenter. For details of media and other culture procedures see Cove (1997). Because of its regenerative capacity, cultures may be started with inocula from any gametophytic tissue but are most quickly established from young protonemal tissue. A simple way of obtaining tissue is to fragment protonemal tissue with a blender and to inoculate the tissue homogenate onto fresh medium. Growth into the agar medium can be prevented by overlaying the medium with cellophane, simplifying the subsequent harvesting of tissue. Tissue obtained in this way and harvested 1 week after inoculation is composed largely of chloronemal cells and is excellent material for the isolation of protoplasts or of DNA, RNA, or proteins for molecular analyses. A standard (90-mm diameter) Petri dish inoculated with protonemal fragments and incubated for 7 days (at 25°C in continuous white light) yields approximately 500 mg fresh weight of tissue. Subsequent digestion with the cellulolytic enzyme, Driselase, yields about 5×10^6 protoplasts.

Most rapid development occurs from 22 to 27°C. Development is slower at 15°C, and this cooler temperature is necessary to induce gamete production. Fluorescent light between 5 and 20 Wm^{-2} is optimal, and lower photon flux densities lead to altered development. *Physcomitrella* grows well in continuous light. Although the effects of different light-dark cycles have not been studied extensively, cell division in young protonemal tissue can be entrained by a 16 h day/8 h night cycle.

Culture storage is possible by cryopreservation (Grimsley and Withers 1983). However, because tissue does not always survive freezing and thawing, multiple replicates need to be stored for safety. If maintained in Petri dishes that are sealed once a mature plant is established, cultures may be kept for at least a year at 15°C without subculture.

Genetics

Phylogenetic Relationships within Bryophytes

Two recent articles present the results of phylogenetic analyses of bryophytes. Beckert and others (1999) use nucleotide sequences of the mitochondrial *nad*5 gene, whereas Newton and others (2000) use sequence data of a number of nuclear genes together with morphologic data. Both studies arrive at essentially similar conclusions, confirming liverworts (*Hepaticopsida*) and mosses (*Bryopsida*) as monophyletic groups that are closely related. Only the former study includes *Physcomitrella patens,* but both studies include its close relative *Funaria hygrometrica.* The two other species referred to in this re-

view, *Ceratodon purpureus* and *Tortula ruralis* are each included in only one of these studies. However, combined, it appears that *Physcomitrella* and *Funaria* are closely related, and that *Ceratodon* and *Tortula* are fairly closely related both to one another and to *Physcomitrella.*

Sexual Crosses

Physcomitrella is a member of the *Funariales* and hybridizes with other members of the genus (von Wettstein 1932). The best estimate of chromosome number is 27 (Reski and others 1994), which is high for mosses. The genome size has been estimated to be about 420 Mb (Reski and others 1994), which is not dissimilar to most moss species that have been investigated (Voglmayr 1998) but is small compared with many flowering plants. Male and female gametes are produced on the same plant. However, crossing using mixed cultures can be achieved at high frequency because some vitamin-requiring mutant strains are self-sterile when cultured at low levels of supplementation. This effect has been attributed to sporophytes having a higher requirement for supplements (Courtice and others 1978). Crosses between different vitamin-requiring mutant strains result in sporophytes that do not require vitamins because of complementation of the two genomes in the resulting hybrid sporophyte. Segregation for vitamin requirement of the "parents" in the resultant haploid progeny shows the simple predicted Mendelian ratios (Cove 1983).

Somatic Hybridization

Many developmental mutants are unable to complete their natural life cycle and are sexually sterile. Such strains can be maintained because tissue regeneration allows their continued propagation. Genetic analysis of sexually sterile strains is possible by way of somatic hybridization. Somatic hybrids have been generated by protoplast fusion of strains having complementary auxotrophies followed by selection (Grimsley and others 1977). More recently somatic hybrids have been obtained by the use of transgenic strains resistant for different antibiotics, with hybrids being selected on media containing both antibiotics (DJ Cove, unpublished). The latter procedure has the advantage that any strain can be fused with any other once the necessary transgenic derivatives have been obtained by transformation. Somatic hybrids allow dominance and complementation testing. They also have the potential to allow the recovery of multiply mutant strains, by way of apogamous or parthenogenetic sporophyte production (Cove and Knight 1993). However, the control of these processes is not understood, and it is not possible to manipulate them experimentally. An example of genetic analysis by way of this pathway is reported in Knight and others (1991).

Transformation

Transformation of *Physcomitrella* can be achieved either by polyethylene glycol (PEG)–mediated uptake of DNA by protoplasts (Schaefer and others 1991) or biolistically (Sawahel and others 1992).

Most successful transformations involving constructs with no homology to moss genomic sequences have used pUC-derived plasmids. Both methods of transformation yield three types of regenerants after selection for plasmid-encoded antibiotic resistance: transient, weak, and strong resistance phenotypes. Depending on when regenerants are subcultured, some lose resistance and were presumably expressing resistance transiently. Of those regenerants that continue to show resistance, most show only weak resistance. This class of transgenic only retains plasmid DNA when selection is maintained continuously, losing resistance after about 14 days of growth on nonselective medium. Such transgenics are therefore termed unstable and are likely to replicate plasmid DNA extrachromosomally (Ashton and others 2000; Knight 1994). The minority of transgenics grow strongly on selective medium, maintain their resistant phenotype in the absence of selection, and show a Mendelian pattern of inheritance (Schaefer and others 1991). This class of transgenic is termed stable, and molecular analysis confirms integration of the plasmid at a genomic locus. Multiple copies of the plasmid DNA are commonly found to be integrated at the same locus and in the same orientation.

Transformation frequencies are difficult to compare between the two procedures. Both methods yield about the same number of transgenics and so are operationally equivalent. The PEG-mediated DNA uptake procedure requires more DNA (routinely 20 µg per transformation) than the biolistic procedure (1 µg per shot, 2 shots per transformation) and therefore efficiencies (calculated as transgenics per µg of DNA) are higher for the biolistic method. Recent experiments have produced up to 20 stable and 200 unstable transgenics from a single PEG transformation, but both techniques still show considerable variability.

Where homology exists between transforming DNA and the *Physcomitrella* genome, there is a high probability that recombination will occur between the homologous sequences. Table 1 summarizes the

Table 1. Results of gene targeting in *Physcomitrella patens*

Target locus	Genomic (g) or cDNA (c)	Transformed with:	Length of Homology		Selection Cassette	No. Transgenics Analyzed	Percent Targeted	Reference
			1	2				
Transgenic locus	g	plasmid	1.8 k	—	nptII	3	100	Kammerer and Cove 1996
Transgenic locus	g	plasmid	3.0 k	—	aphIV	7	86	Kammerer and Cove 1996
Random genomic sequence	g	plasmid	3.6 k	—	nptII	6	100	Schaefer and Zryd 1997
Random genomic sequence	g	plasmid	2.7 k	—	nptII	4	100	Schaefer and Zryd 1997
Random genomic sequence	g	plasmid	2.3 k	—	nptII	6	67	Schaefer and Zryd 1997
fts Z	c	fragment	247	685	nptII	51	14	Strepp and others 1998
des 6	g	fragment	900	900	nptII	5	100	Girke and others 1998
cab	g	plasmid	1 k	—	nptII	9	33	Hoffman and others 1999
mcb	c	fragment	435	420	hph	55	4	Girod and others 1999

Transformations have been performed either with plasmids having the homologous DNA adjacent to the selection cassette (= plasmid), or with DNA fragments in which the selection cassette is inserted within the region of homology (= fragment). In the former case there is therefore only a single region of homology, the length of which is given in the column headed 1, whereas in the latter case, there are two regions of homology, the lengths of which are given in the columns headed 1 and 2.

results of published work involving transformation with constructs containing DNA homologous to genomic sequence. If an intact plasmid, having a selectable marker adjacent to the region of homology, is used, integration occurs by a single crossover, inserting the plasmid into the genome and duplicating the homologous sequence. Multiple inserts usually occur at the homologous locus. If instead, the selectable marker is cloned into the region of homology and transformation is carried out using a linear fragment comprising the homologous sequence containing the selectable marker, then integration occurs by a double crossover, the transforming sequence replacing the genomic sequence. In most, but not all, trangenics produced by this procedure, a single copy of the transforming DNA is integrated.

Strengths

Haploidy Allows Immediate Identification of Mutant Phenotypes

The haploidy of the gametophyte allows the immediate identification of mutant phenotypes. A large range of mutants have been isolated after mutagenic treatment. These include auxotrophic mutants, mutants altered in their response to auxin and cytokinin, and developmentally abnormal mutants. Included in the latter class are mutants in which development is blocked at the chloronema-caulonema transition, mutants unable to produce buds (Ashton and others 1979), mutants having altered gametophore development (Courtice and Cove 1983), and mutants altered in their polar response to light or gravity (Knight and Cove 1989). Successful mutagenic procedures include nitrosoguanidine treatment of spores (Ashton and Cove 1977) or protonemal tissue (Boyd and others 1988a), and ultraviolet irradiation of protonemal tissue (Wagner and others 1997). Protonemal tissue can be protoplasted after mutagenic treatment to establish cell clones derived from single cells.

Regenerative Capacity Allows Vegetative Maintenance of Cultures

The high capacity of moss tissue to regenerate is a considerable technical convenience. Growth tests can be started easily using small protonemal inocula, and blended tissue provides a good source of inoculum for larger cultures. Mutant strains blocked in development are sexually sterile but can be maintained in culture indefinitely, because even chloronemal tissue regenerates strongly.

Microbiologic Culture Techniques Facilitate Study of Metabolism

Physcomitrella is routinely cultured using essentially microbiologic procedures. Growth media may be either liquid or solidified with agar and need contain only inorganic salts. Growth is rapid and doubling times of 24 h at cell densities of in excess of 10 g/l fresh weight, can be achieved using an airlift fermenter (Boyd and others 1988b). Protoplast regeneration allows for the screening of as many as 10^6 cells on a single 90-mm Petri dish.

Development Can Be Studied at the Level of the Individual Living Cell

The accessibility of all cells in living protonemal tissue to direct observation, including the use of time lapse videomicroscopy, allows development to be analyzed in detail at the level of the individual cell. Modification of an established axis of cell polarity can be studied in protonemal apical cells (Knight and Cove 1991), and protonemal development presents excellent opportunities for the study of pattern determination by both control of cell fate and timing of cell division (Russell 1993). A spectacular example of the tractability of moss protonemal cells to developmental study has been recently provided by Brucker and colleagues (2000). Mutants of *Ceratodon purpureus* that are deficient in heme oxygenase are impaired in the synthesis of the phytochrome chromophore and have protonemal apical cells that are unable to align to a directional light source. The wild-type response to directional light was restored by microinjecting a plasmid coding for heme oxygenase into mutant apical cells.

Development Can Be Studied in Both Two-Dimensional Filaments and Three-Dimensional Shoots

Gametophyte development involves growth both of cell filaments, an essentially two-dimensional pattern of development, and of gametophores, more complex three-dimensional structures. Both types of development are sensitive to external environmental inputs including light and gravity, making gametophyte development an important means for studying differences in the two growth patterns. Mutants that have lost the ability to orient caulonemal growth to directional light are also unable to orient gametophore growth to directional light (Cove and others 1978). However mutants that have lost the ability to orient their caulonemal growth to gravity, retain the wild-type gravitropic response of their gametophores (Jenkins and others 1986). The molecular basis behind such mutants has not yet been studied but should provide insights into the contrasting controls involved in two- and three-dimensional patterns of growth.

Transformation Procedures Are Easy to Carry Out and Yield High Numbers of Trangenics

Transformations are carried out using young protonemal tissue that is easily produced. The method using PEG-mediated uptake of DNA by protoplasts allows multiple transformations (up to at least 20) to be carried out in parallel and is particularly amenable to transformation of a single strain by a number of different constructs. Biolistic transformation uses similar tissue and is useful if many different strains need to be transformed. Transformation rates are steadily improving as a result of minor changes to the transformation procedure, and it is unlikely that a supply of transgenics will be the rate-limiting factor in experimental procedures in future. However, at present, transformation rates are not sufficient to allow cloning by direct complementation of mutant phenotypes except for phenotypes observable in unstable transgenics.

Unstable Transgenics Allow Transgenes To Be Expressed Then Lost

The unstable class of transgenics, in which transforming DNA is lost rapidly when selection for a transgenic marker is relaxed, provides for the controlled expression of a transgene, without using special constructs. For example, a transposase expression cassette can be introduced and then eliminated later by relaxing selection for the construct.

Homologous Recombination Allows Gene Surgery

High-frequency recombination between the genome and homologous sequences contained in a transforming plasmid (Schaefer and Zryd 1997) not only allows genes to be inactivated (Strepp and others 1998; Girke and others 1998; Girod and others 1999) but also opens the door for gene surgery, in which control of gene expression can be manipulated and precise allelic changes can be made. Homologous recombination allows allele substitutions involving whole domains or single base pairs.

Weaknesses

Haploidy Prevents Easy Isolation of Lethal Mutations

The downside of gametophyte haploidy is that mutation leading to loss of function of an essential gene will be lethal. There are a number of ways in which this problem might be addressed. The conventional solution for haploid microorganisms is to obtain conditional mutants. It should be possible to isolate temperature-sensitive mutants of *Physcomitrella,* because although maximum growth rates occur in the range of 22° to 27°C significant but slower growth still occurs at temperatures below the maximum range and above about 15°C. However, there are no reports of true temperature-sensitive mutants (but see Futers and others 1986 for a description of a temperature-sensitive phenotype). The establishment of the homologous recombination system opens the way for a more sophisticated means of circumventing this problem. If it is established by gene knockout that loss of function of the gene under investigation is lethal, then transformation with a construct having a deletion of the C-terminal coding sequence, driven by a regulatable promoter, should, after a single recombination event, put the only intact copy of the gene under the control of the regulatable promoter. This technology is being tried in a number of laboratories, but there are no reports yet of its successful application. The final method of dealing with the haploidy of the gametophyte is to avoid it all together by using diploid gametophytes, produced either by protoplast fusion or by aposporous regeneration of sporophytes. The problem in using such material is that it is difficult to recover haploid gametophytes (by apogamy or parthenogenesis, see earlier) from diploid gametophytes.

Lack of Economic Relevance

Mosses, at present, have little or no economic value. Although economic value has not been the primary consideration in the choice and usefulness of animal or microorganism systems for research (for example, *Xenopus laevis, Drosophila melanogaster,* bacteriophage T4), this has always been important for the funding of plant research. Concentration on crop species may well have been tactically worthwhile but may also be why developmental studies of plants lag so far behind those on animals. Research on mosses, in the laboratory of von Wettstein in the period from 1920 to 1944 (see Reski 1998), was at the cutting edge of genetic studies, but it has been difficult to regain this position in the present, more economically oriented climate. However, the wealth of secondary metabolites produced by bryophytes suggest the possibility of untapped economic potential. In addition, about 10,000 moss expressed sequence tags (ESTs) have now been sequenced (for details see http://www.moss.leeds.ac.uk), and it is clear that at the molecular level, moss genes closely resemble those of flowering plants. Consequently, the tractability of *Physcomitrella* for genetic and biochemical studies make it a worthwhile model system for crop plants.

Physcomitrella Lacks some Advantageous Features Possessed by Other Moss Species

Although molecular and genetic technologies have been most successfully developed for *Physcomitrella,* this species is not as suitable as other moss species for certain studies. The regenerative ability of *Ceratodon purpureus* protoplasts and the growth strength of its protonema in darkness has made it the species of choice for cell polarity studies (Cove and others 1996; Lamparter and others 1996; Wagner and others 1997). The extreme desiccation tolerance shown by *Tortula ruralis* makes this the superior species for abiotic stress studies. *Physcomitrella*'s close relative, *Funaria hygrometrica,* is physiologically better characterized (see for example: Christianson and Hornbuckle 1999; D'Souza and Johri 1999) than *Physcomitrella* and has the added advantage of more stereotyped protonemal patterning for developmental studies.

It is not, however, necessarily advantageous to concentrate all studies on a single species especially if the technology is readily transferable. It has already been established that *Ceratodon* can be grown using similar procedures to those used for *Physcomitrella.* The procedures for transformation in *Physcomitrella* yield higher rates of stable transgenics in *Ceratodon.* Although similar constructs work well for both species, the 35S promoter, which has been used extensively in *Physcomitrella,* gives only very low levels of expression in *Ceratodon* (Zeidler and others 1999). A limited numbers of ESTs are now available for both *Ceratodon* and *Tortula* and, where comparisons are possible, it is evident that, at least in exons, nucleotide sequences are similar to those in *Physcomitrella.*

What Is Still Needed

Improvement in Transformation Frequency

Although transformation frequencies in *Physcomitrella* are already high compared with those for plant

systems in general, they have not reached levels obtained in eukaryotic microbes such as the yeasts, and are not high enough to allow cloning by the complementation of a mutant phenotype. The frequency of transformants is rising, and the reliability of the transformation procedure is improving as a result of minor adjustments to protocols and increased expertise of operators, but attempts need to continue to find conditions that will improve both frequency and reliability.

Gene Tagging

Thus far, no system has been devised for the identification of genes by forward selection. Gene tagging systems require the generation of large numbers of transgenics into which identifiable DNA has been inserted into the genome randomly. If a mutant phenotype is then detected, the genomic sequences associated with the inserted DNA can be identified. The *Physcomitrella* homologous recombination system then brings the added benefit that it can be used to confirm that the phenotype is the result of the disruption of a particular open reading flame.

Stable transgenics are themselves a potential source of tagged lines, but it has not yet been determined whether nonhomologous plasmid DNA is inserted into the genome randomly. Assessing the randomness of insertion events should therefore be a priority. Transformation with plasmids containing genomic fragments increases the frequency of stable transgenics (Schaefer and Zryd 1997). If transformation is carried out using a construct into which has been cloned random genomic fragments, the construct will be targeted to random locations. However, only if the genomic fragment contains a coding sequence and lacks both the N terminal and C terminal coding regions, will a single crossover between construct and genome necessarily result in gene knockout.

An alternative strategy now being developed is to insert a selectable marker into random genomic fragments contained in a cloning vector using in vitro transposition. This results in the selectable marker being embedded into random genomic fragments. The genomic fragments can then be excised and used to transform moss. The use of the cloned fragments with the selectable gene inserted within them should reduce nonhomologous insertion, because stable trangenics containing the selectable gene will be generated by double crossovers involving the two homologous sequences flanking the selection case. Success of this technique will depend on the proportion of the genome that comprises gene sequences. However, until more detailed knowledge of genome architecture comes from, for example the sequencing of BAC clones, this is only speculation. If it is assumed that the genome contains about 20,000 genes with an average size of 4 kb, then about one fifth of the *Physcomitrella* genome will comprise gene sequences, and one in five random insertions should lead to gene inactivation. If instead of genomic fragments, a normalized cDNA library is used as the source of homologous sequences, only expressed coding sequences will be targeted. However, cDNAs lack continuous homology with genomic sequences and lead to lower frequencies of homologous recombination (see Table 1). Thus, at present, it is difficult to choose between a strategy of using random genomic fragments and targeting noncoding and coding regions, or using cDNAs and targeting transcribed regions only, but at a lower rate. Both strategies need to be developed.

In vivo transposition is another strategy that needs further development. The Ac/Ds system from *Zea mays* is not active in *Physcomitrella,* although experiments provided the first evidence for the occurrence of recombination between homologous sequences in transforming DNA and the genome (Kammerer and Cove 1996). T DNA has also been tested for insertion in vivo, but without success (C. Knight and B. Hohn, unpublished). However, these procedures were tried some years ago and development of new strains and increased knowledge of promoter activity means that both the Ac/Ds and T DNA systems are worth revisiting. Other heterologous transposition systems also need to be tested.

Development of Conditional Gene Expression System

There is a need for a system that allows the regulation of transformed genes. The wheat *Em* promoter drives ABA-inducible expression of a *gus* reporter gene in *Physcomitrella* (Knight and others 1995). Although expression levels were more than 25 times higher in the presence of ABA than in its absence, uninduced levels were higher than those from the 35S promoter, suggesting that control is insufficiently tight for this the Em system to be useful. A tetracycline-regulatable promoter system operates in *Physcomitrella* (Zeidler and others 1996) and promises to be a more useful tool. The usefulness of other regulatable systems now needs to be assessed, not only in *Physcomitrella* but also in *Ceratodon* and *Tortula.*

Extension of Technology to Other Moss Species

Because other moss species have different advantages (see earlier), it is essential to extend and modify techniques for their use. The procedures for the transformation of *Physcomitrella* are also effective for *Ceratodon,* and even though the 35S promoter drives gene expression only weakly in the latter species, it still functions sufficiently to allow its use for the expression of antibiotic resistance markers. However, antibiotic levels need to be reduced. With lower levels on geneticin (for *npt*II selection) or hygromycin (for *hpt* selection), stable transformants of *Ceratodon* have recently been obtained at five times the frequency of that for *Physcomitrella* (A. Pan, unpublished). A priority for *Ceratodon* must now be to establish whether recombination occurs between transforming DNA and homologous genomic sequences in the same way as it does in *Physcomitrella.* A number of laboratories are investigating this at present, and an answer should be forthcoming soon.

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