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Phytochrome of the green alga *Mougeotia*: cDNA sequence, autoregulation and phylogenetic position

Armin Winands and Gottfried Wagner*
*Membran- und Bewegungsphysiologie, Fachbereich Biologie Botanik, Justus-Liebig-Universität, Senckenbergstrasse 17, D-35390 Giessen, Germany (*author for correspondence)*

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Abstract

A cDNA clone encoding phytochrome (apoprotein) of the zygnematophycean green alga *Mougeotia scalaris* has been isolated and sequenced. The clone consisted of 3372 bp, encoded 1124 amino acids, and showed strain-specific nucleotide exchanges for *M. scalaris*, originating from different habitats. No indication was found of multiple phytochrome genes in *Mougeotia*. The 5′ non-coding region of the *Mougeotia* PHY cDNA harbours a striking stem-loop structure. Homologies with higher-plant phytochromes were 52–53% for PHYA and 57–59% for PHYB. Highest homology scores were found with lower-plant phytochromes, for example 67% for *Selaginella* (Lycopodiopsida), 64% for *Physcomitrella* (Bryopsida) and 73% for *Mesotaenium* (Zygnematophyceae). In an unrooted phylogenetic tree, the position of *Mougeotia* PHY appeared most distant to all other known PHYs. The amino acids Gly-Val in the chromophore-binding domain (-Arg-Gly-Val-His-Gly-Cys-) were characteristic of the zygnematophycean PHYs known to date. There was no indication of a transmembrane region in *Mougeotia* phytochrome in particular, but a carboxyl-terminal 16-mer three-fold repeat in both, *Mougeotia* and *Mesotaenium* PHYs may represent a microtubule-binding domain. Unexpected for a non-angiosperm phytochrome, its expression was autoregulated in *Mougeotia* in a red/far-red reversible manner: under P_r conditions, phytochrome mRNA levels were tenfold higher than under P_{fr} conditions.

Introduction

Phytochrome is the ubiquitous red-light sensor found in green and etiolated plants [21]. Phytochrome has evolved from aquatic microorganisms and finally emerged as the dominant sensory pigment in flowering land plants [15]. In the canopy of land plants, but also in germinating seeds in general, phytochrome monitors the basic light versus dark situation, independent of vectorial information [29].

In more structured situations, phytochrome in addition to monitoring the basic light situation, is also enabled to monitor the direction of impinging light, which may or may not be linearly polarized [16]. This dichroic light perception through phytochrome is best demonstrated in plants with no intercellular communication such as in algal trichomes or fern protonemata [32]. Light direction here is perceived within the boundary of individual cells such as in *Mougeotia* trichomes or *Adiantum* protonemata, fully exposed to light with no attenuation and no discerible photoreceptor organelle. Clearly in these cases, phytochrome not only monitors the basic light situation, but provides vectorial information as well to mediate plant photoorientation [33].

To date, the molecular mode of the dichroic action of phytochrome remained obscure. From an elegant series of experiments in the zygnematophycean green alga *Mougeotia*, Haupt and Weisenseel [12] suggested, along the line of Hendricks and Borthwick [13], that

The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X95550. Dedicated to Professor Rüdiger on the occasion of his 60th birthday.

phytochrome possibly shows transmembrane plasmalemma domains which act as a light-regulated Ca^{2+} channel. The hypothesis stimulated a large number of experiments but conclusive evidence is lacking (for review, e.g. [31]).

The deduced *Mougeotia* phytochrome amino acid sequence is analysed here with special emphasis on possible transmembrane domains.

Materials and methods

Trichome culture conditions and light treatments

Mougeotia scalaris Hassall (strain B164.80 of Sammlung von Algenkulturen (SAG) Goettingen [26]) was grown for 4 days in liquid culture [30] at 26 °C under continuous white light (2500 lux), as described [22]. Prior to RNA isolation for cDNA synthesis, trichome cultures were transferred to petri dishes and kept the fifth day in darkness for 24 h.

In some of the experiments, as shown in Fig. 4, the 24 h dark period was followed by monochromatic light treatments: *Mougeotia* trichomes in petri dishes were irradiated with either a single pulse of red light (654 nm, 5 min) or the red light pulse immediately followed by a pulse of far-red light (728 nm or 748 nm, 5 min each). The irradiances were 10 W/m^2 throughout, and the interference filters used (Schott, Darmstadt, Germany) were : DAL 654 (red) or DAL 728 (far-red) or DAL 748 (far-red). After the light treatment and prior to northern blot analysis, the cultures were returned to darkness for 3 h.

Construction and analysis of the cDNA library

Total RNA was isolated as described [4]. Poly(A)$^+$ RNA was isolated from total RNA by the paramagnetic particle technique (Promega/Serva, Heidelberg, Germany).

cDNA synthesis was carried out using 1 μg poly(A)$^+$ RNA of *Mougeotia* in a cDNA synthesis kit (Gibco-BRL, Eggenstein, Germany). The cDNA fragments were size-fractionated by sucrose-density centrifugation and cloned into the *NotI*/*SalI* site of phage vector λgt22a, and a cDNA with 2×10^6 plaque-forming units (pfu) could be established. Plaques were transferred to nitrocellulose membrane according to protocols communicated [24], in double replicates. After baking at 80 °C for 1 h, the membranes were hybridized with the PCR-generated homologous probe (see below) and washed as described [35]. Filters were finally autoradiographed, and corresponding plaques picked from the agar plates.

PCR experiments, including temperature profile and ingredients, were as described [35]. However, 100 ng of *Mougeotia* cDNA were used as template and new primers were developed, based on the 3′ end of the *Mougeotia PHY* fragment (S1266 [35]) and consensus sequences of lower plant phytochromes and *PHYB* (A1699):
Primer 1 (sense orientation = S1266): CCCAACATX-ATGGAXCTYGTGAAATGTGA (X = C/T; Y = A/T)
Primer 2 (anti-sense orientation = A1669): ATAAGCT-GCAXCGAXTGAATAGCATCCATTTC (X = A/G)
The obtained fragment of 403 bp was cloned into the *Sma*I site of the vector pKS+ and sequenced via the dideoxy chain termination method [25]. This *Mougeotia PHY* PCR fragment was [^{32}P]-labelled by random prime labelling [6], and the library was sceened by plaque hybridization (see above).

Nucleotide sequence analysis

Inserts from derived phage vectors were subcloned into the *NotI*/*SalI* site of pKS+. Deletion series were generated by *Exo*III/Nuclease I digestion from the 5′ and 3′ ends by use of the appropriate restriction enzyme and the Erase-a-base System (Promega). Clones were sequenced by the dideoxy chain termination method [25], using T7 DNA Polymerase (Pharmacia, Freiburg, Germany) and ^{35}S-dATP.

Northern blot analysis

Northern blot analysis was performed as described [24] (see also [35]). The [^{32}P]-labelled probe used for hybridization was the 403 bp PCR product obtained with primer combination S1266/A1669 (see above).

Competitive oligonucleotide priming

To enable competitive oligonucleotide priming (COP [8]) of the G/C transition (nt 1025), A/G transition (nt 1142) and C/T transition (nt 1196), respectively, a 564 bp region of *Mougeotia PHY* containing the known allelic sites, was first RT-PCR-amplified from mRNA of *Mougeotia scalaris*, strains Erlangen and Goettingen, respectively, using the primer pairs S705, TATGAX-AGGGTYATGGCTTATAAGTTCCATG (X = C/T; Y = G/T) and A1308, GCYGCTCCATCA-

CATTTCTCYAGXTCCAT (X = A/G; Y = A/T) [35]. Of the initial reaction product 5% each was taken to initiate a further 10 rounds of PCR, with allele-specific COP oligonucleotides and A1308 as the common primer present. Six COP reactions were performed with either the correct match or mismatched primers radiolabelled at the 5′ end and with either the Erlangen or the Goettingen strain S705/A1308 PCR product as DNA template. The PCR products of both strains of *Mougeotia scalaris* were also cloned and sequenced, as described [35].

Sequence comparison

Homology studies and multiple alignments were performed by the GCG programme package (Deutsches Krebsforschungszentrum-DKFZ, Heidelberg) similar as reported [15]. Calculation of distance matrices and evolutionary trees were carried out by PHYLIP (Joseph Felsenstein; Joe@genetics.washington.edu), with distance matrix calculation according to Kimura or maximum likelihood, and tree construction by the Fitch and Margoliash or the neighbour-joining method.

Results and discussion

PHY nucleotide sequence and derived PHY *primary structure*

A screen of a cDNA library from dark-grown *M. scalaris* trichomes by the PCR-generated S1266/A1669 *PHY* fragment yielded five cDNA clones. Size comparison, restriction analysis and partial sequencing of the five clones revealed three identical clones and two smaller ones. One of the identical clones was subjected to full length sequencing and harboured an open reading frame of 3372 bp. The 5′ non-coding region and 3′-untranslated region were 164 bp and 235 bp, respectively; the putative start codon was at nucleotide position 165. The polypeptide encoded by this open reading frame consisted of 1124 amino acids (Fig. 1; MougPHYgo). Besides the chromophore attachment site (Cys-325) and the point charge group (Arg-320), other conserved domains present in MougPHYgo include the amino acid motifs I and II [20] at positions 388–398 and 655–660, respectively, and the conserved Tyr [27] at position 895. The epitope of the monoclonal antibody Pea-25 [3] is at position 761–767 and that of Z-3B1 [1] at position 837–842 (Fig. 1); the identity of PHY peptides [19] from the closely related alga *Mesotaenium caldariorum* is not unexpected as is the 73% homology to the deduced amino acid sequence of MesoPHY1b and MesoPHY1a from this algal species [18].

PHY *domain characterization and comparative sequence analysis*

Although most of the conserved regions are present in *Mougeotia* PHY, some otherwise highly conserved domains cannot be found (Fig. 1). Namely, Gly-Val in the chromophore binding domain (-Arg-Gly-Val-His-Gly-Cys-), consistent with *Mesotaenium* PHY1b and PHY1a, appear specific. The coding nucleotide sequence of the full-length cDNA sequence of *PHY* of *Mougeotia* (Fig. 1; MougPHYgo) versus the genomic fragment of *Mougeotia* phytochrome (Fig. 1; MougPHYer [35]) differs by three encoded amino acids in the region of overlap that was sequenced (Ser-342→Cys; Arg-381→His; Val-399→Ala). The amino acid exchanges are conservative if not homologous throughout and possibly reflect differences between the two strains used of the same species, which were collected from ponds either in the Botanical Garden of Erlangen University [10, 22] or of Goettingen University [26]. To verify possible strain-specific differences, mRNA isolated from *Mougeotia scalaris*, strain Erlangen and strain Goettingen, respectively, was transcribed to cDNA in presence of the primer pair S705/A1308 (see Materials and methods) and used for competitive nucleotide priming (COP [8]) at the three *PHY* nucleotide positions 1025, 1142, and 1196, respectively. For comparison, COP was done for cDNA of both combined strains of *M. scalaris*. Twelvemer oligonucleotides were matched to the triplets TGT, CAT and GCG, respectively [35], and yielded a product radiolabelled always to highest specific activity for the Erlangen strain, while the same was true for the oligonucleotides matching TCT, CGT and GTG and the Goettingen strain (Fig. 1); cDNAs combined from both strains of *M. scalaris* showed radiolabelling independent of the competitive oligonucleotides used (data not shown). The conclusions drawn from these experiments were verified by cloning and sequencing the separate S705/A1308 PCR products. In good agreement, the Erlangen strain of *M. scalaris* was used by Winands et al. (1992), while the Goettingen strain was used in the present paper. Thus, a single phytochrome gene appears to be transcribed in *M. scalaris*, with allelic differences found in strains of different habitats

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MougPHYgo   MSSSKRSQSSGRSSTQTRIQNRVTQASADAKLSTAFEVSSSSGGDSFDYTKSVTASLNPT    60
MesoPHY1b     T RM      E TAK KREV  A  T      N     A AAV  -         G     AG    59

MougPHYgo   -EPLAAKSVTAYLQRMQRGSIIQSFGCMMAVEPGTFRIIAYSENVSEMLGVTPQSVPTGD   119
MesoPHY1b   S AIPSSA            G T T     LM  E S  VR F    AG    DLV  A  SMG   119

MougPHYgo   HQNAIGIGTDVRSLLSPSSVSVVEKAVAANDVSMMNPIAVYSLATQKLFFAILHMNDVGL   179
MesoPHY1b   Q SL AV   I T FTSA    LL    AM T    V    VSLQ R AK P    V  RI      179
                          Mesotaenium-Peptide #33a>>>| P    L  RI

MougPHYgo   VIDLEPISSSSDSAMFSAGAVQSHKLAAKAISRLQSLPGGDICGLCDVVVEEVRELTGYD   239
MesoPHY1b    V    V-RP  PNVSA   M                        GL   A               238
             V    V-RP  |<<<Mesotaenium-Peptide #33a

MougPHYgo   RVMAYKFHDDEHGEVVAEIRRSDLEPYLGLHYPATDIPQASRFLFIKNRIRMICDCTSPQ   299
MougPHYer          |→→start of fragment
MesoPHY1b            E      I                              A    M   V I    SA P   298
             >>>|            I     *           |<<<Mesotaenium-Peptide #33b

                          Chromophore Domain
MougPHYgo   VKVVQDSRIPQEMSLAGSTMRGVHGCHTQYMMNMGSTASLVMSVTIND-----TNEIAGG   354
MougPHYer                                                 C
MesoPHY1b      I  PTMKHPI       L        A   A    V      A I   NSSEEGATA      358

                                                  Motif #1
MougPHYgo   PGMKGRKLWGLIVCHHSTPRHIPFPIRSACEFLMQVFGLQLNMEVELAAQHREKHILRTQ   414
MougPHYer                                H                    A
MesoPHY1b   ILH         V      S  YV   L                        SS L           418

MougPHYgo   TLLCDMLLRDAPMGIVSQSPNVMDLVKCDGAALLFGGRCWLLGISPTQEQVKDIATWLIS   474
MougPHYer     end of fragment→→|
MesoPHY1b                          IT           FYH  A     VT SEA  R    A  LD   478

MougPHYgo   SHTDTTGLSTDSLVDAGYPKARELGVDVCGMAAARITENDFLFWFRGHAQKEVKWAGAKD   534
MesoPHY1b     K S         A      N DS   S           SK        S             Q   538
                      Mesotaenium-Peptide #28>>>|K*    R  S   |<<<

MougPHYgo   GGSE------EDGSRMHPRSSFKAFLEVVKQRSLPWEDVEMDAIHSLQLILRGSFQDIE-   587
MesoPHY1b   EPGDRDREEG E G         Q                                    M G   598
              Mesotaenium-Peptide #47>>>|       *  |<<<

MougPHYgo   -----DKEDRKIVHARLKEMHLQGMEELSSVASEMVRLIETATAPILAVDTAGCVNGWNF   642
MesoPHY1b   EGGGSQQGNKRMIN    NDLK     D    T  N                  SL        A   658

                          Motif #2
MougPHYgo   KISELTGLSIPEVMGKSLVKDLTHPSSKDTVEKLLYMALNGEEEQNVEIRLKTWG--MQQ   700
MesoPHY1b    V       PVS A          VQRE REA  RV                 Q      PQLHS   718

MougPHYgo   GKGPVILMVNACASRDVSEKVVGVCFVAQDVTGEKIVQDKFTRIQGDYTTIVRSHNSLIP   760
MesoPHY1b   HG T   V            S       G        E L   I             R         778
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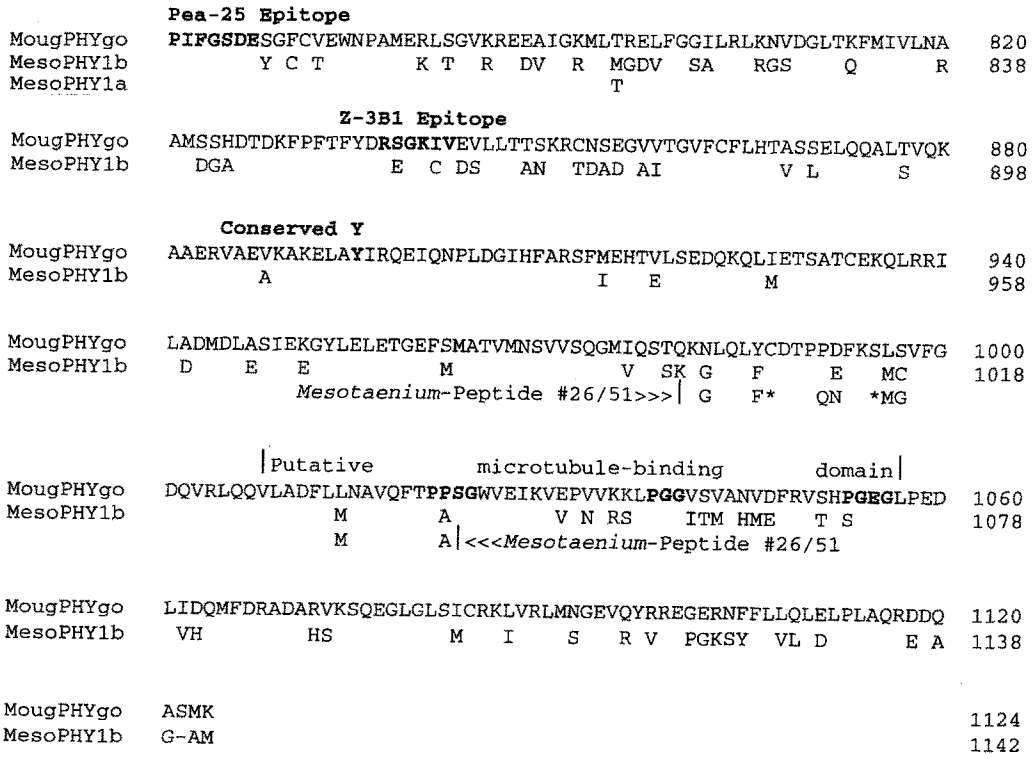

Figure 1. Phytochrome primary structure in *Mougeotia scalaris* (MougPHYgo), derived from its nucleotide sequence. Besides the chromophore attachment site (Cys-325) and the point charge group (Arg-320), conserved domains are present in *Mougeotia* PHY such as motifs I and II [20] at positions 388–398 and 655–660, respectively, and the conserved Tyr at position 895 [27]. The epitope of the monoclonal antibody Pea-25 is at 761–767 [3], and that of Z-3B1 at 837–842 [1]. Derived amino acid sequences of the MougPHYer genomic fragment of *M. scalaris* [35], and of MesoPHY1b, MesoPHY1a [18] and its peptides [19] of the closely related zygnematophycean green alga *Mesotaenium caldariorum* are sequence-compared. Gly-Val in the chromophore-binding domain of *Mougeotia* and *Mesotaenium* PHY (-Arg-Gly-Val-His-Gly-Cys-) are unique. Interconnected repeats of three imperfect copies of a 16-mer amino acid motif in the COOH-terminal part of the PHY molecules, shown here and calculated in secondary structure prediction in Fig. 2, putatively represent a microtubule-binding domain [34]. Gaps to optimize alignment of the aa sequences shown (–), amino acids not detected (*) and borderlines of partial amino acid sequence, peptides and putative domain (|) are indicated.

or cultivars (MougPHYer versus MougPHYgo; Fig. 1).

Exon/intron and intron/exon splice junctions of the genomic fragment of *Mougeotia PHY* [35], within the overlap that was sequenced, are identical to that of introns 2 and 3 of *Mesotaenium PHY1b* and *PHY1a* [18]; next to the 73% homology and the characteristic amino acids Gly-Val in the chromophore-binding domain of PHY, this reflects close relationship of the two zygnematophycean green algal phytochromes. Of interest, in the COOH-terminal part of *Mesotaenium* PHY and *Mougeotia* PHY is an interconnected repeat of three imperfect copies of a 16-mer amino acid motif (homology $\leq$ 36%; Figs 1 and 2), reminiscent of a microtubule-binding domain [34].

The pattern of hydropathy profile [17] of *Mougeotia* PHY is similar to the patterns of PHYA and PHYB of seed plants (data not shown). This shows no hydrophobic characteristics that would point to a transmembrane protein as suggested by earlier experiments with linearly polarized light [11]. The isolation of PHY from the closely related green alga *Mesotaenium* [14] and of its coding sequence [18] had already shown that algal PHYs were hydrophilic like higher-plant PHYs. This finding was corroborated by immunocytologic studies, which localized *Mougeotia* PHY in the cytoplasm [9]. Thus, it appears that *Mougeotia* PHY is a conventional PHY with no exceptional structures or properties, except the characteristics aa sequence change in the chromophore binding domain and the

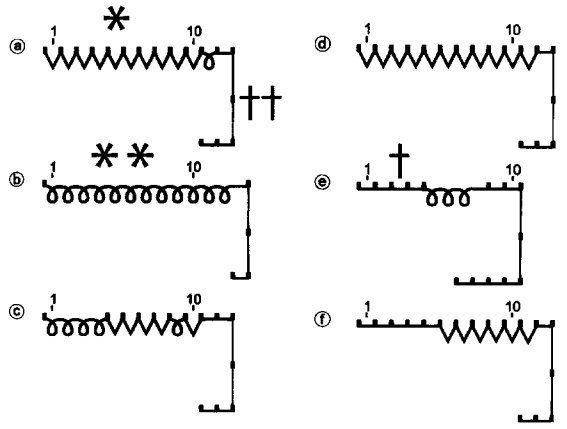

Figure 2. Chou-Fasman secondary structure prediction [2] of putative microtubule-binding repeats in the COOH-terminal part of MougPHY and MesoPHY of *Mougeotia scalaris* and *Mesotaenium caldariorum*, respectively. For comparison, two microtubule-binding repeats characteristic to MAP and tau proteins are shown as well [34]. a, MougPHYgo, repeat 1; b, MougPHYgo, repeat 2; c, MougPHYgo, repeat 3; d, MesoPHY1b, repeat 1; e, murine MAP, repeat 3; f rat tau, repeat 4. No sequence data of plant MAP or tau proteins is available to date. The symbols are: * β-sheet; ** α-helix; † random coil; †† β-turn.

possible microtubule-binding repeats in the COOH-terminal region of the molecule.

The *Mougeotia* PHY sequence was submitted to comparative aa sequence analysis, derived from cDNA sequence analysis (*Solanum*, *Arabidopsis*, *Adiantum*, *Physcomitrella*, *Mougeotia*), genomic DNA sequence analysis (*Mesotaenium*) or both of it (*Nicotiana*, *Psilotum*, *Selaginella*), as published (cf. [15, 28]): *Mougeotia* PHY shows a homology of 52–53% to PHYA, 57–59% to PHYB and 61–73% to PHYs of lower plants on amino acid level, including *Mesotaenium* PHY (Table 1).

PHY mRNA structure and autoregulated gene expression

A structure found in the 5′ non-coding region (164 nt) of *Mougeotia PHY* mRNA may be worth mentioning (Fig. 3). As has been concluded in other systems (e.g. [5, 23]), the ensemble of iterative G-C but also A-T base pairs over a stretch of 36 nucleotides allows the possible formation of a secondary structure that may function in translational activity *in vivo*.

Without knowing the mechanism of *PHY* regulation here, PHY-autoregulated expression in *Mougeotia* may also be a function of the promotor of this gene, or a result of both transcriptional and translational control. We

Table 1. Amino Acid identity (%) of pairs of complete phytochrome sequences, computed by the TREE program [7] of the GCG package provided by the DKFZ database (Heidelberg, Germany). The accession numbers of the sequences used are published (cf. [15, 28]), including *Mesotaenium* (accession number U31284) and *Mougeotia* (accession number X95550) phytochromes. Related families of PHYA and PHYB, respectively, are italicized, and MesoPHY and MougPHY are boxed.

	SolaPHYA	NicoPHYA	ArabPHYA	SolaPHYB	NicoPHYB	ArabPHYB	PsilPHY	AdiaPHY	SelaPHY	PhysPHY	MesoPHY	MougPHY
SolaPHYA	–	*93*	*78*	53	55	53	56	54	59	57	53	53
NicoPHYA	*93*	–	*78*	54	54	52	57	54	59	58	54	52
ArabPHYA	*78*	*78*	–	53	54	53	59	56	59	58	56	53
SolaPHYB	54	53	53	–	*93*	77	61	58	65	62	60	58
NicoPHYB	55	54	54	*93*	–	*79*	62	59	66	63	61	59
ArabPHYB	53	53	53	77	*79*	–	58	55	62	59	59	57
PsilPHY	56	57	56	61	62	58	–	65	73	69	64	62
AdiaPHY	54	54	56	58	59	55	65	–	70	66	64	61
SelaPHY	59	59	59	65	66	62	74	70	–	76	72	67
PhysPHY	57	58	58	62	63	59	69	66	78	–	69	64
MesoPHY	53	54	56	59	61	59	64	64	72	69	–	73
MougPHY	53	53	53	59	59	57	62	61	67	64	73	–

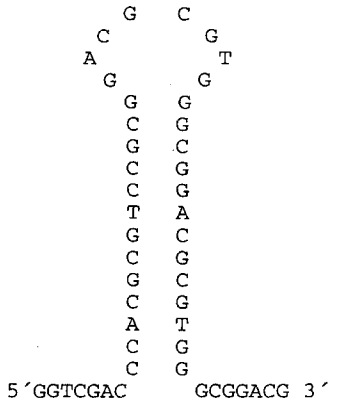

Figure 3. RNA secondary structure prediction of the 5′ non-coding region of phytochrome cDNA (*MougPHYgo*) of *Mougeotia scalaris* inferred by computer analysis with FOLD and MFOLD from software package HUSAR (EMBL, Heidelberg). Thermodynamic stability ($\Delta G°$) is −27.7.

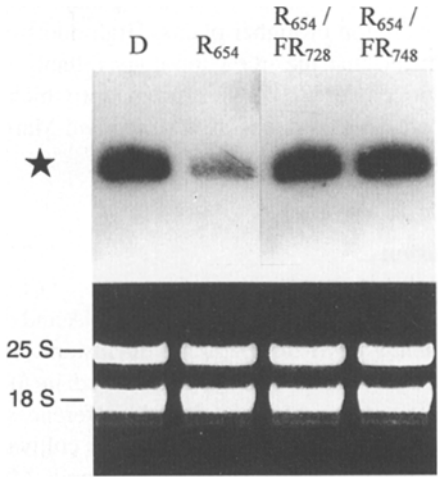

Figure 4. Upper part: northern blot analysis of transcriptional levels of *PHY* mRNA in *Mougeotia scalaris* (*, kept either for 24 h in darkness (D), or in the dark followed by a 5 min pulse of red light ($R_{654\ nm}$) or the red light pulse followed by an immediate 5 min pulse of far-red light (FR_{728nm} or $FR_{748\ nm}$). Irradiances were 10 W/m^2 throughout. The probe used was the PCR cDNA product obtained with the primer combination S1300/A1733. Each lane contained 10 μg of total RNA. Lower part: loading control with stained 18S- and 25S-rRNA, respectively.

therefore subjected dark-pre-treated *Mougeotia* trichome cultures to different red/far-red light treatments. When identical amounts of total RNA from these cultures were probed with the [^{32}P]-labelled *Mougeotia PHY* PCR product S1266/A1669 in northern blot analysis, the control of dark adapted cultures showed the high level of *PHY* mRNA as expected (Fig. 4), whereas the *PHY* mRNA level declined to less than 10% of its initial value after a 5 min-pulse of red light. A 5-min far-red pulse, immediately upon the red light pulse, greatly reduced the signal triggered by red light and kept the *PHY* mRNA level in *Mougeotia* trichomes at 70–90% of its dark level (Fig. 4). No photosynthetic light treatment was required, as reported by Morand and co-workers [19] for *M. caldariorum*. Thus, among lower-plant *PHY*s, *Mougeotia PHY* appears the first species reported here that is autoregulated in its gene expression.

Primordial type of Mougeotia PHY

A phylogenetic tree (Fig. 5), using the PHY amino acid sequence of *Mougeotia* as an outgroup, positions this sequence between the sequences of the fern *Adiantum* and the fork separating the *Physcomitrella* (Bryopsida) sequence from the *Selaginella* (Lycopodiopsida) sequence. There is no means to attribute to the *Mougeotia* sequence A- or B-type PHY characteristics; A- and B-type phytochromes evolved in the higher-plant lineage only after the fern *Adiantum* has been split off (see also [15]). The branching-off of E-

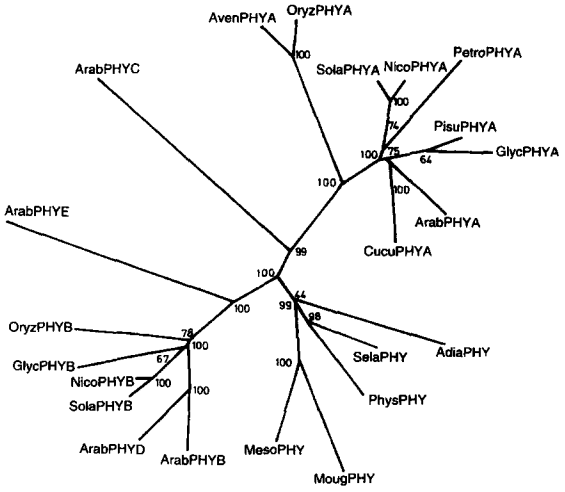

Figure 5. Unrooted phylogenetic tree based on complete PHY amino acid sequences. The sequence of the zygnematophycean green alga *Mougeotia scalaris* (Fig. 1; MougPHYgo) was used as an outgroup. Alignment and the distance scores were generated by the PHYLIP program (see Materials and methods). Bootstrap values, indicating the number of times a node was supported in 100 replicas, are given at the corresponding branching nodes. In order to emphasize the rather similar phylogenetic distances from lower plant phytochrome to that of higher-plant species and in order to prevent premature speculations on ancestors, the tree was redrawn without a root. For accession numbers of the sequences used, see Table 1 and [15, 28].

or C-type PHYs was an event taking place even later in the evolution of higher plants. High bootstrap values indicate that the tree's topology reliably reflects the phylogeny of *PHY* genes, no matter which of the construction method was used (Fitch and Margoliash or Neighbour joining).

Conclusion

Sequencing of the *Mougeotia PHY* cDNA and derived aa sequence has lead to three conclusions. First, a single phytochrome gene is transcribed in *M. scalaris*, with strain-specific nucleotide differences based on the origin from separate habitats or cultivars; the *PHY* expression is autoregulated. Second, *Mougeotia* PHY shows no hydrophobic domains characteristic of a transmembrane protein; in consequence, phytochrome in *Mougeotia* is not expected to act as a light-regulated Ca^{2+} channel as supposed earlier in the literature. Third, comparative sequence alignments conclusively show this zygnematophycean phytochrome to be close to the roots of functional phytochrome in plants.

Two properties of *Mougeotia* phytochrome, reported here, deserve further analysis: first, the mechanism of autoregulated *Mougeotia PHY* expression and possible function of the stem-loop structure in the 5′ noncoding portion of the mRNA, and second, a repetitive interconnected 16-mer amino acid motif in the COOH-terminal part of the *Mougeotia* phytochrome molecule, reminiscent of a microtubule-binding domain.

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