Volume 334, number 1, 95 100 FEBS 13217 November 1993
© 1993 Federation of European Biochemical Societies 00145793/93/$6.00

Mosses do express conventional, distantly B-type-related phytochromes

Phytochrome of *Physcomitrella patens* (Hedw.)

H. Üner Kolukisaoglu[a], Birgit Braun[a], William F. Martin[b], Hansjörg A.W. Schneider-Poetsch[a,*]

[a]*Botanisches Institut, Universität zu Köln, 50923 Köln, Germany*
[b]*Institut für Genetik, Technische Universität, 38023 Braunschweig, Germany*

Received 1 September 1993; revised version received 14 September 1993

We have screened a cDNA library of the moss *Physcomitrella patens* (Hedw.) for phytochrome sequences. The isolated sequences turned out to encode a phytochrome dissimilar to the phytochrome type postulated for the moss *Ceratodon* [(1992) Plant Mol. Biol. 20, 1003–1017] *Physcomitrella* phytochrome was completely alignable to fern phytochrome (*Selaginella*) and phytochromes of higher plants. The frequency of clones encoding this phytochrome indicated that a *Ceratodon*-like type should only be expressed, if at all, with lower frequencies than the sequenced phytochrome cDNA. Sequence differences between lower plant phytochromes are small as compared to phytochrome types of higher plants.

Moss photoreceptor; *Physcomitrella* phytochrome; Phytochrome phylogeny; Phytochrome sequence; *Physcomitrella patens* (Hedw.)

1. INTRODUCTION

Plants have evolved a rather sophisticated light sensory system which allows them to detect and respond to intensity, wavelength, distribution and periodicity of ambient light. This system enables them to take optimal advantage of photosynthesis and photosynthate. One of the most important photoreceptors in this system is phytochrome. It is responsible for many light-regulated phenomena ranging from chloroplast movement to control of morphogenesis (for recent approaches and reviews see [1–3]).

Hoping to find clues to the hitherto unknown mode of action of phytochrome, a series of phytochrome genes of higher and lower plants has been isolated (for literature see [1–5]). Phytochrome genes of lower plants have gained additional attention because lower plants may have preserved traits of the phylogenetic past. Ancestral phytochrome functions can perhaps be studied in these organisms more readily than in systems of higher complexity.

Screening a genomic library of the moss *Ceratodon* with a PCR-generated probe for phytochrome, Thümmler et al. [5] isolated a clone encoding a phytochrome different from all known phytochromes by virtue of a totally aberrant C-terminal third that resembled a kinase. Only the first and the ensuing half of the second exon showed homologies with known phytochrome primary structures.

This was an exciting and challenging finding; exciting because it supported the notion that phytochromes were light-regulated kinases (see [6]) and challenging because the finding was contradictory to immunological data which indicated conventional C-terminal structures in *Bryophyta* phytochromes. SDS-PAGE-separated and immuno-blotted proteins of *Marchantia, Funaria* and *Sphagnum* had shown distinct bands in the proper 120 to 125 kDa region when probed with Z-3B1 [7], a monoclonal antibody that recognizes a C-terminal epitope [8,9] not present in the nucleotide-deduced amino acid sequence of *Ceratodon* (G-845 to A-850 of *Selaginella* phytochrome)

In order to scrutinize these two contradictory lines of evidence, we screened a cDNA library of the moss *Physcomitrella* for phytochrome genes. Our results give clear evidence that conventional-type phytochromes are expressed in mosses.

2. MATERIALS AND METHODS

2.1. *cDNA cloning*

The preparation of a cDNA library from *Physcomitrella patens* (Hedw.) in lambda NM1149 was described by Martin et al. [10]. The library contained about 300,000 independent recombinants.

2.2. *Probing the library by PCR and degenerate primers*

The library was first probed by PCR (Perkin-Elmer Cetus, Düsseldorf, Germany)) with Taq polymerase (Promega, Heidelberg, Germany, no. 254S) and degenerate primers (see [11]) designed to bind to two conserved regions flanking the chromophore domain of phytochromes and separated by about 600 nucleotides. Denaturing was at 94°C (1 min), annealing at 45°C (1.5 min) and extension at 72°C (1.5 min) with the quickest ramping possible. After 40 cycles, a faint band of the expected size was isolated by agarose gel (1%) electrophoresis and subjected to another round of 40 cycles of PCR using Vent polym-

*Corresponding author. Fax: (49) (221) 470 5181.

erase (New England Biolabs, Schwalbach, Germany, no. M186A) and an anncaling temperature of 60°C. The resulting product was cloned into the *Sma*I site of the pUC18 polylinker.

2.3. *Screening of the library*

After amplification in *E. coli* DH5α and reconfirmation of phytochrome sequences in the recombinant plasmid by sequencing, the insert of pUC18 was cut out, labelled by the DIG system of Boehringer, (Mannheim, Germany) and used to screen the library. From half of the library, which was plated on *E. coli* Y1090, five positive clones were isolated. After amplification, cloning in pUC18 and sequencing of their ends, three of them proved to contain overlapping parts of phytochrome genes.

None of the clones, however, contained the utmost C-terminal sequences. Therefore, the library was screened a second time by a labeled 3'-end fragment of the clone stretching furthest into this region. Five different clones containing the missing sequences were isolated. Within the parts that overlapped with the isolates of the first round, the sequences of the second round proved to be identical with these.

2.4. *Subcloning and Sequencing*

The inserts of phytochrome-positive lambda clones were subcloned in pUC18 and sequenced on a Pharmacia system using universal primers, reagents and protocols of the manufacturer (Pharmacia, Freiburg, Germany).

3. RESULTS AND DISCUSSION

Although the *Physcomitrella* cDNA library was screened by sequences which are contained in conventional phytochromes as well as in the aberrant *Ceratodon* phytochrome genomic clone (region encoding the chromophore binding domain), the results clearly demonstrate that the clones isolated and hitherto sequenced conform to phytochromes with conventional C-termini and not at all to the computer-translated *Ceratodon* 3' sequences (Fig. 1).

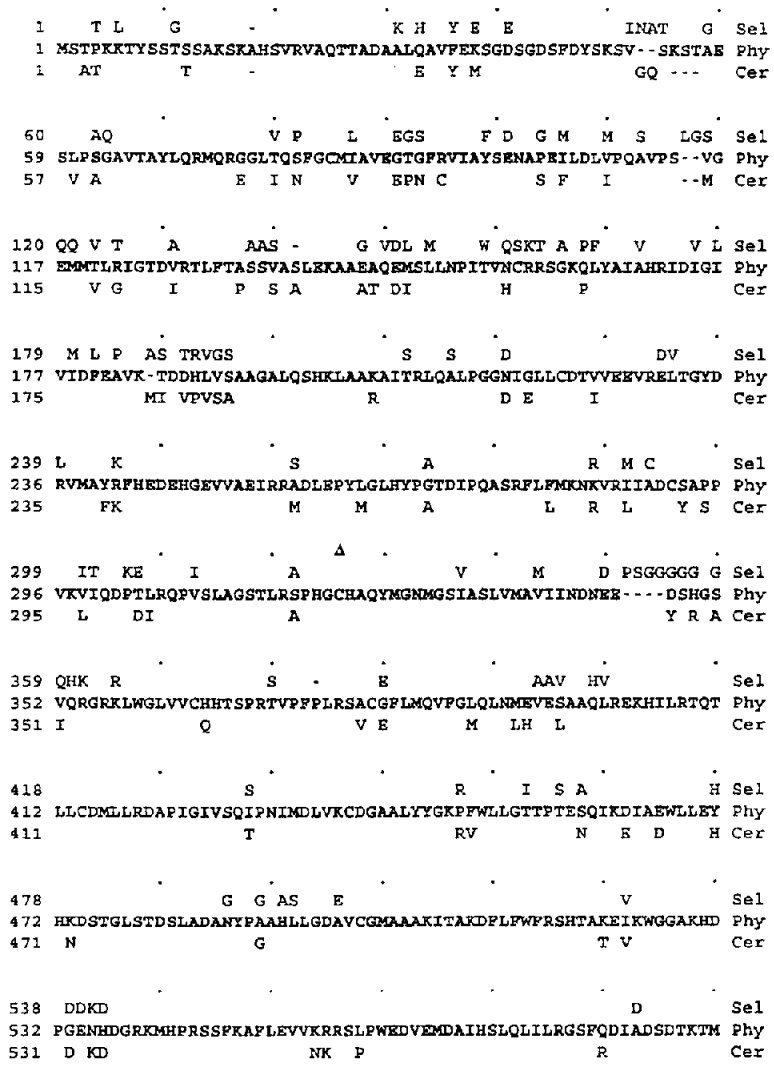

Fig. 1. cDNA-derived amino acid sequence of *Physcomitrella* phytochrome. Amino acids substituted in *Selaginella* (EMBL X61458) and *Ceratodon* (EMBL S51224) are marked by their one letter amino acid symbol. The epitopes for pea-25 and Z-3B1 are underlined. Crosses (#) indicate the borders of *Selaginella* exons. The second exon of *Ceratodon* ends at position 779 (D, vertical bar). The chromophore attachment site is marked by a triangle. Within the region alignable to *Ceradodon* phytochrome, *Physcomitrella* and *Ceratodon* share 82.9 percent identical amino acids and *Physcomitrella* and *Selaginella* 80.3%. (The cDNA sequence of *Physcomitrella* phytochrome has been submitted to EMBL.)

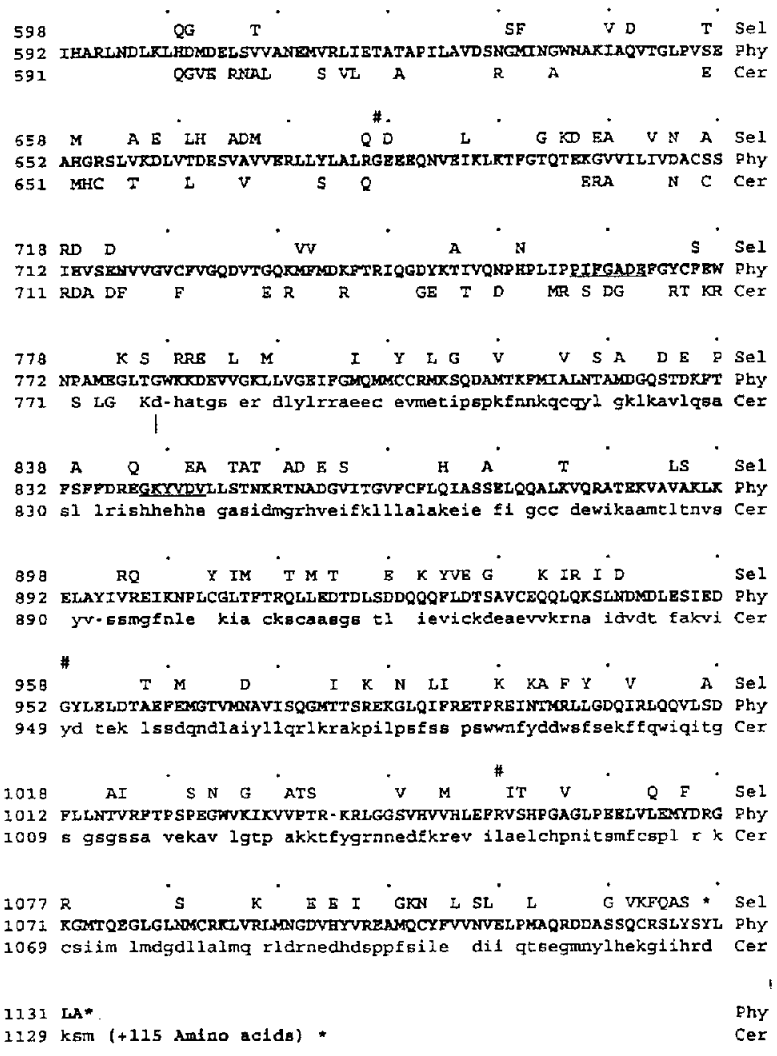

Fig. 1. (continued)

The results unambiguously demonstrate that a conventional type phytochrome is expressed in *Physcomitrella*, thus confirming preliminary immunological evidence with other *Bryophyta* and the antibody Z-3B1 [7].

The epitope of *Physcomitrella* conforming with Z-3B1 epitopes [$_n$-G-K(M)-Y-V(I)-E(Q)-A(C)-$_c$] is $_n$-G-K-Y-V-D-V-$_c$ (see Fig. 1). Epitope studies (unpublished) show that C-terminal from the sequence $_n$-G-K-Y-V-$_c$, a variety of alterations are tolerated; even the sequence downstream of Y may be replaced by $_n$-H-A-S-L-$_c$.

Physcomitrella phytochrome protein was also detected by the antibody pea-25 [12,13] binding to an epitope (P-765 to G-771 of pea phytochrome, $_n$-P-I-F-G-A-D-E-$_c$ in *Physcomitrella* phytochrome) which is highly aberrant in the nucleotide sequence-derived amino acid sequence of *Ceratodon* and very close to the portion not alignable to conventional phytochromes.

Where the amino acid sequences of *Physcomitrella* phytochrome can be aligned to *Ceratodon* sequences (first exon and half of the second, see Fig. 1), the identity differences on the amino acid level amount to 17.1%, thus showing that the two species are closely related. Within the same region, the pair *Ceratodon*/*Selaginella* shows 22.9% differences and the pair *Physcomitrella*/*Selaginella* 19.7%.

The identity differences between the total *Physcomitrella* and *Selaginella* sequences amount to 22.4 percent (Table I). This figure is striking regarding the more than twofold higher differences found between the phytochromes a, b and c of *Arabidopsis* and the 450 million years which have passed since ferns and mosses were separated. The data infere that the evolution of phytochrome proteins in lower and higher plants proceeds with different velocities.

An evolutionary tree (Fig. 2) establishes *Physcomitrella* phytochrome as a distantly b-type-related phytochrome or more precisely as a member of the lower plant phytochrome family. Numerous members of this family are known through chromophore-spanning PCR fragments which allow their ordering [4]. However, a-

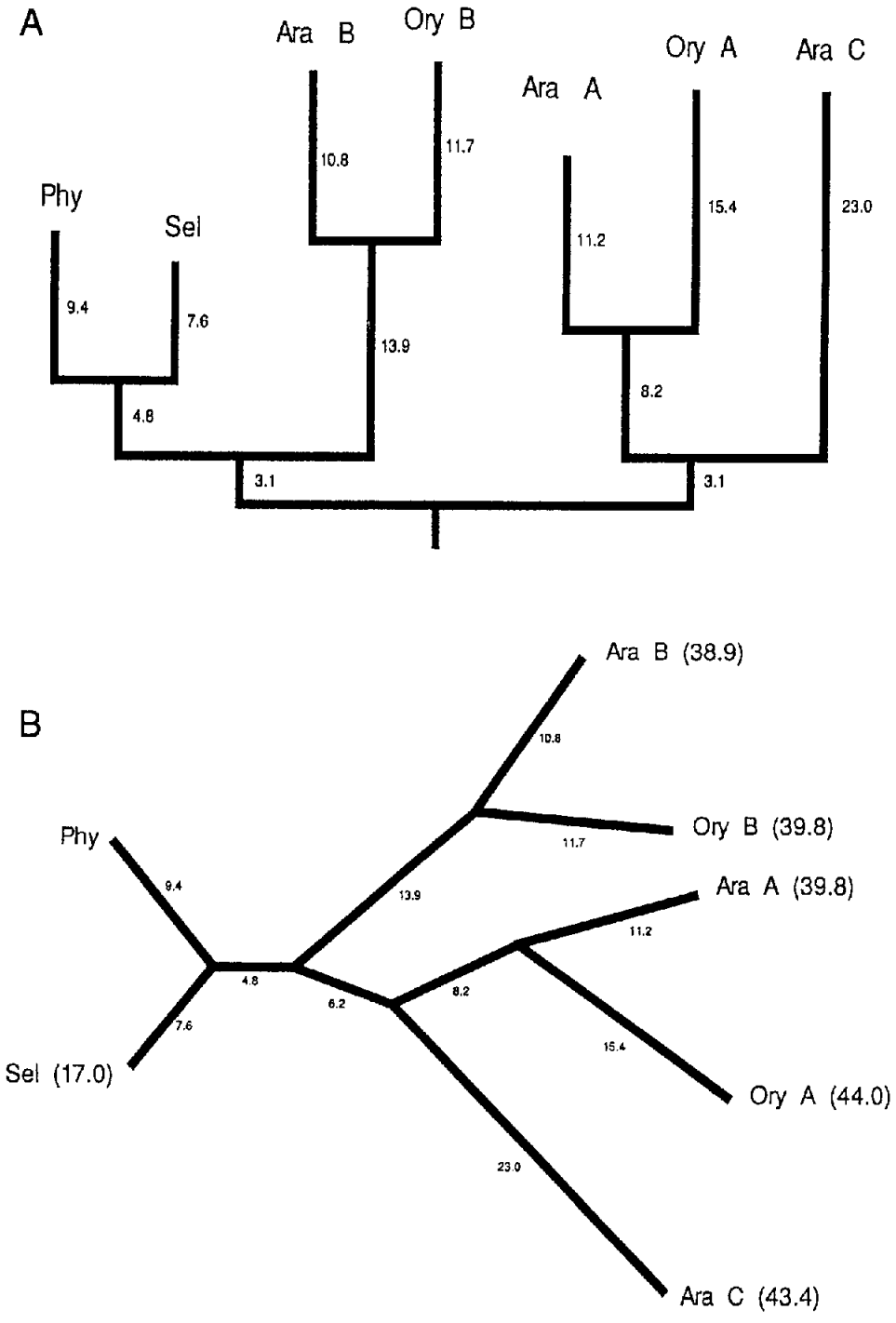

Fig. 2. Phylogenetic tree according to the alignment and the distance scores of the TREE program (see [14]) of the GCG-package provided by the DKFZ database, Heidelberg (A). In order to dodge commitments about the position of the phytochrome ancestor, the tree was redrawn and the root omitted. It becomes clear that the distances from lower plant phytochromes to higher plant types a and b are very similar. The total distances from *Physcomitrella* to other phytochromes are indicated in brackets (B). For references see legend of Table I.

type phytochromes and full length lower plant phytochromes share homologies only a few percent below the percentages which determine lower plant phytochromes' b-type nature. *Physcomitrella* phytochrome, e.g. is only marginally more similar (0.38%) to b-type than to a-type *Arabidopsis* phytochrome (see Table I).

Because all lower plant phytochrome sequences (from cDNA as well as from genomic DNA) were isolated without any bias towards a certain type, it appears up to now that lower plants only harbour a sole type of phytochrome in contrast to higher plants where the evolutionary clock for phytochrome (a-, b-, and c-type

Table I

Per cent amino acid identity of pairs of complete phytochrome sequences (A) and distance scores (B) as computed and used by the TREE program (see [14]) of the GCG-package provided by the DKFZ (Deutsches Krebsforschungszentrum) database, Heidelberg

	Percent identity (based on aligned regions)						
	Phy	Sel	Ara B	Ory B	Ara A	Ory A	Ara C
Phy	0.00	77.58	58.58	61.18	58.25	54.73	53.18
Sel		0.00	61.89	62.46	59.35	56.37	54.73
Ara B			0.00	72.84	52.61	49.73	51.68
Ory B				0.00	51.71	48.16	50.09
Ara A					0.00	63.93	52.08
Ory A						0.00	49.46
Ara C							0.00
	Distance scores for phylogenetic tree						
	Phy	Sel	Ara B	Ory B	Ara A	Ory A	Ara C
Phy	0.00	16.99	39.68	39.68	39.30	43.24	44.21
Sel		0.00	36.65	37.85	37.97	41.25	43.93
Ara B			0.00	22.60	50.47	54.89	53.13
Ory B				0.00	51.02	57.07	54.07
Ara A					0.00	26.61	43.16
Ory A						0.00	46.82
Ara C							0.00

The sequences used were *Oryza* a [15] and b [16], *Arabidopsis* a, b and c [17] and *Selaginella* [4]. (The respective EMBL accession numbers are X14172, X57563, X17341, X17342, X17343 and X61458.)

phytochromes) is apparently accelerated. The *Ceratodon* type phytochrom is an exception in that only the N-terminal two thirds are typical lower plant phytochrome. Apart from this fact, the relative small diversity within lower plant phytochromes suggests that phytochrome types of the higher plants may possibly originate from a phytochrome closely related to lower plant phytochromes. The diverse functions of more 'advanced' phytochromes may already have derived from this ancestral phytochrome type.

Although there is no doubt that a conventional type phytochrome is expressed in a species closely related to *Ceratodon*, we cannot exclude that, in addition, *Physcomitrella* contains a *Ceratodon*-like phytochrome. It might be expressed at much lower levels than the phytochrome found. We are in search of the other type. Difficulties imposed by low levels of expression and by screening with sequences common to both phytochrome types might be circumvented by probing cDNA libraries with PCR-primers derived from C-terminal sequences of the *Ceratodon* genomic clone. It would be curious if two closely related species (closely related according to amino acid similarities of phytochrome) did not express the same type(s) of phytochrome.

4. CONCLUSION

As indicated by immunological data, cDNA-derived amino acid sequences of the moss *Physcomitrella* demonstrate that conventional type phytochromes are expressed in *Bryophyta*. The transcript of a *Ceratodon*-like phytochrome gene still awaits detection.

Acknowledgements: We are indebted to the Volkswagen-Stiftung which generously supported our investigations. Dr. Stefan Marx (see [11]) provided the PCR primers.

REFERENCES

[1] Rüdiger, W. and Thümmler, F. (1991) Angew. Chem. Int. Ed. Engl. 30, 1216–1228.
[2] VanDerWoude, W.J, Britz, S.J., Deitzer, G.F. and Watson, J.C. (eds.) (1992) Photomorphogenesis in Plants, Photochem. Photobiol. 56 (5), 571–856, Pergamon Press, New York.
[3] Furuya, M. (1993) Annu. Rev. Plant Physiol. Plant Mol. Biol. 44, 617–645.
[4] Hanelt, S., Braun, B., Marx, S. and Schneider-Poetsch, H.A.W. (1992) Photochem. Photobiol. 56, 751–758.
[5] Thümmler, F., Dufner, M., Kreisl, P. and Dittrich, P. (1992) Plant Mol. Biol. 20, 1003–1017.
[6] Schneider-Poetsch, H.A.W. (1992) Photochem. Photobiol. 56, 839–846.
[7] Schneider-Poetsch, H.A.W., Schwarz, H., Grimm, R. and Rüdiger, W. (1988) Planta 173, 61–72.
[8] Schneider-Poetsch, H.A.W. and Braun, B. (1991) J. Plant Physiol. 137, 576–580.
[9] Bonenberger, J., Schendel, R., Schneider-Poetsch, H.A.W. and Rüdiger, W. (1992) Photochem. Photobiol. 56, 717–723.
[10] Martin, W., Lydiate, D., Brinkmann, H., Forkmann, G., Saedler, H. and Cerff, R. (1993) Mol. Biol. Evol. 10, 140–162.
[11] Winands, A., Wagner, G., Marx, S. and Schneider-Poetsch, H.A.W. (1992) Photochem. Photobiol. 56, 765–770.
[12] Thompson, L.K., Pratt, L.H., Cordonnier, M.-M., Kadwell, S., Darlix, J.-L. and Crossland, L. (1989) J. Biol. Chem. 264, 12426–12431.

[13] Cordonnier, M.-M., Greppin, A. and Pratt, L.H. (1986) Plant Physiol. 80, 982–987.
[14] Feng, D.F. and Doolittle, R.F. (1990) in: Methods in Enzymology, Molecular Evolution (Doolittle, R.F., Ed.) Vol. 183, pp. 375–387, Academic Press, San Diego, CA.
[15] Kay, S.A., Keith, B., Shinozaki, K. and Chua, N.-H. (1989) Nucleic Acids Res. 17, 2865 2866.
[16] Dehesh, K., Tepperman, J., Christensen, A.H. and Quail, P.H. (1991) Mol. Gen. Genet. 225, 305 313.
[17] Sharrock, R.A. and Quail, H.P. (1989) Genes Dev. 3, 1745 1757.