Volume 281, number 1,2, 245–249 FEBS 09595 April 1991

ADONIS 001457939100115N

Phytochromes and bacterial sensor proteins are related by structural and functional homologies

Hypothesis on phytochrome-mediated signal-transduction

Hansjörg A.W. Schneider-Poetsch, Birgit Braun, Stefan Marx and Anke Schaumburg

Botanisches Institut II der Universität zu Köln, 5000 Köln 41, Germany

Received 14 January 1991; revised version received 11 February 1991

Phytochrome and bacterial sensor proteins are related by functional and structural homologies. They are both sensors of environmental stimuli and share structural homologies which comprise a domain of about 250 amino acids (about 28 kg·mol^{-1}). This domain is C-terminal in phytochromes and in several bacterial sensor proteins. In both groups of sensors this domain undergoes conformational changes which are caused by the N-terminal part sensing the stimulus. In the case of bacterial sensors, the conformational alteration is regulated by additional proteins, conferred to a corresponding regulator protein which then acts on transcription. The coincidences between the two groups of sensors are striking enough to assume phytochrome to transduce signals in a way comparable to the bacterial two-component systems.

Phytochrome sensor protein; Bacterial sensor protein; Phytochrome, mode of action; Phytochrome phylogeny

1. INTRODUCTION

Phytochrome is one of the most fascinating proteins in plants. A great many striking photomorphogenetic processes are mediated by it (see [1]). Phytochrome is well characterized by biochemical and immunological techniques and by methods of molecular biology. Despite this fact, the mechanism of action of phytochrome, the molecular links between its conformational alterations and the ensuing biochemical and morphogenetic phenomena are still unknown. Many sophisticated experiments have not been able to unveil the secret of light-induced signal-transduction by phytochrome. Here we report on findings which we assume could be evidence of the mode of action and the phylogeny of this light-sensing protein.

2. MATERIALS AND METHODS

2.1. *Preparation of clones and sequencing*

Libraries of cDNA were constructed in the λgt11 expression vector. Phages giving a positive response with a monoclonal antibody (Z-3B1 [2]) highly specific for a wide range of different phytochromes were isolated. Their inserts were transferred to pUC 18. In a first attempt, two inserts (S1 ffrom a *Zea mays* L., var. Badischer Landmais, S2 from a library of *Selaginella martensii* Spring) were subjected to double strand sequencing, using forward and backward primers and fragments obtained by nested deletion (within the section of interest, S1 is 93% similar to a published genomic *Zea* phytochrome sequence [3] and S2 is with 57.6% most related to phytochrome B from *Arabidopsis* [4].

Correspondence address: Hj. Schneider-Poetsch, Botanisches Institut der Universität zu Köln, 5000 Köln 41, Germany. Fax: (49) (221) 470 5181

Abbreviations: At, *Agrobacterium tumefaciens*; Br, *Bradyrhizobium spec.*; Bs, *Bacillus subtilis*; Ec, *Escherichia coli*; Kp, *Klebsiella pneumoniae*; CpxA, bacterial protein sensing membrane composition; EnvZ, bacterial protein sensing membrane composition; NtrB, bacterial protein sensing nitrogen limitation; PhoR, bacterial protein sensing phosphate limitation; RcsC, bacterial protein sensing capsule composition; VirA, bacterial protein sensing plant exudate; LHR, low host range; WHR, wide host range

2.2. *Demonstrating structural homologies*

Homology studies were performed by FASTA (program of W.R. Pearson [5] provided by EMBL, Heidelberg). Apart from phytochromes, the best score for S1 was RcsC of *Escherichia coli* RcsCEc itself proved to share homologies with proteins like NtrB of *Klebsiella pneumoniae* [6] which had in part already been aligned by Nixon et al. [7]. Using the alignment of Sharrock and Quail [4] of the phytochromes A, B and C of *Arabidopsis thaliana*, S2 and other phytochrome sequences [8–11] were subsequently aligned to S1. This procedure, highlighted regions strongly conserved between phytochromes and bacterial proteins. However, manual rearrangements were suggested in some cases, especially in regions where the computer alignment did not account for extended gaps or extensions. The alignment significance for any given pair of sequences and the significance of the final arrangement could not always be reconciled. The alignment of regions of weak coincidence is necessarily infected by ambiguity.

Hydropathy profiles were established according to Kyte and Doolittle [12]. We are indebted to J. Sprengel (Genetics Department of the Universität zu Köln) who introduced us to the program.

3. RESULTS

Screening protein sequence libraries for homologies with new sequences of phytochromes, we came across striking relationships between phytochromes and bacterial sensor proteins which belong to two-

component systems composed of a sensor and a regulator protein. The sensor protein senses environmental stimuli and confers the stimulus by conformational alteration to the regulator protein which then acts on transcription.

Sequence 1 (S1, from a *Zea* cDNA library) and the bacterial protein RcsCEc (SwissProt RCSC$ECOLI), which shares homologies with many bacterial sensor proteins (like NtrBKp [6], NtrBBr [7], EnvZEc [13], CpxAEc [14], PhoREc [15], PhoRBs [16], and different VirAAt proteins [17] overlap in a region of about 260 amino acids (centre of Fig. 1). The positions of 25.5% of the amino acids are identical. Similar results are obtained when other phytochromes are compared to bacterial sensor proteins: sequence 2 (S2, found in a *Selaginella* cDNA library) e.g. shares homologies of 23.2% with PhoRBs, 24.3% with NtrBBr and 26.3% with RcsCEc. Fig. 1 aligns 7 more phytochrome sequences [4,8–11] with 9 of the bacterial proteins (vide supra).

The sequences of phytochromes which overlap with bacterial sensor proteins are C-terminal. It is also the C-terminal regions of bacterial proteins like NtrBBr, NtrBKp, EnvZEc, CpxAEc, PhoREc and PhoRBs which are involved in the overlap. In contrast, the proteins VirAAt and RcsCEc overlap with a region follow-

Fig. 1. Alignment of several phytochromes and bacterial proteins sharing homologies. The coincident amino acid positions between sequence 1 (*Zea*) and RcsC of *E. coli* (SwissProt RCSC$ECOLI) are shown in the centre. Amino acids which are found on corresponding positions in phytochromes and bacterial sensor proteins are drawn in bold letters. (Phytochromes are characterized by the species containing them. Code and function of the bacterial sensor proteins are given in Abbreviations.)

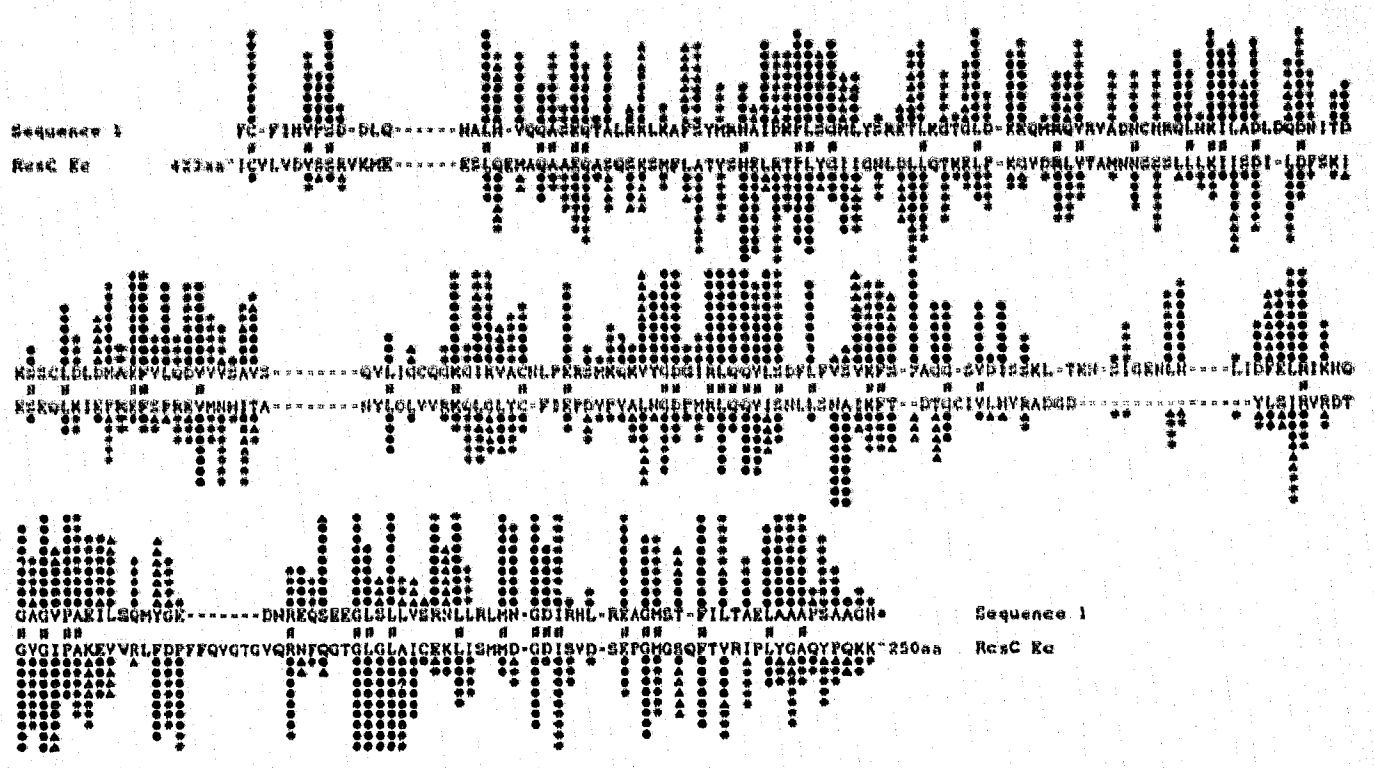

Fig. 2. Frequency of coincident amino acids in phytochromes and bacterial sensors. (The 9 phytochromes and 9 bacterial sensors shown in Fig. 1 are compared.) At each position where amino acids coincide in phytochromes and bacterial sensors, the number of coincident amino acids is represented by the total number of symbols (●, ▲, ★), or taken in at a glance by the height of the columns made up by the symbols. Symbols above sequence 1 refer to phytochromes, symbols below the RcsC sequence to bacterial sensors. (●) Counts the frequency of the amino acid labelled with this symbol; (▲) counts the frequency of the amino acid labelled by ● in the opposite group of proteins; (★) counts the frequency of coinciding amino acids which are not contained in the two sequences shown.

ed C-terminally by about 120 and 250 amino acids. The algined bacterial proteins are shorter than the phytochromes. In contrast to the about 870 amino acids of phytochromes which precede the overlapping region N-terminally, bacterial sensors end about 100–450 amino acids upstream from the homologous domain. The shorter ones span approximately the three final exons of phytochrome which begin around amino acid 690.

The relationships between phytochrome C-terminal sequences and bacterial sensor proteins can be demonstrated even more strikingly with a pairwise comparison of the kind given in the centre of Fig. 1. When the coincidences in amino acid positions of any given pair of a phytochrome and a bacterial protein are summarized, about 45% of the amino acids of the phytochrome find their counterpart on at least one of the bacterial sensor proteins (bold letters on Fig. 1).

It is clear that single coincidences may be random, especially if they are far from the clusters of coincidences and lie in regions where the alignment is not unequivocal. The clusters of coincidences between phytochromes and bacterial sensor proteins, however, show unambiguously that there are astounding relationships between these two groups of proteins.

How often an amino acid has coincident positions in phytochromes and bacterial sensors, is highlighted in Fig. 2. There are only a few positions where amino acids found in one of the two groups of proteins have no counterpart in the other group.

We find that the positions of glycine and leucine (plus valine and isoleucine) are most often conserved in all or almost all members of both protein groups. In two places the frequency of phenylalanine is high. The same is true for proline and at least in one place each for glutamine, aspartic acid and asparagine. Frequently there are conservative exchanges of amino acids. The conservation of pairs like PL, GL, GP, GI(V) or QV(I) is striking. The greater heterogeneity of the group of bacterial sensor proteins as compared to the group of phytochromes may explain why in some places the frequency of coincident amino acids appears unbalanced between the two groups of proteins. Functional different bacterial sensors are hardly more related than bacterial sensors and phytochromes.

The coincidences in conserved amino acid positions and conservative exchanges are even reflected in hydropathy profiles. As an example, S1, S2 and RcsCEc are compared (Fig. 3). Apart from a middle section (⊗) spanning approximately the amino acids 90–160 in Fig. 1, the profile of the bacterial protein is very similar to the profiles of phytochromes. Note that

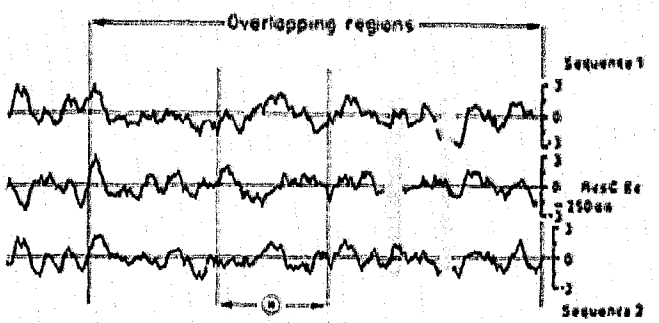

Fig. 3. Hydropathy profiles (according to Kyte and Doolittle, 1982) of sequences 1 and 2 and RcsC of *E. coli* within their overlapping regions showing the structural similarity of phytochromes and bacterial sensor proteins on this level. Gaps demanded by the alignment of Fig. 1 were eliminated except those demanded by the alignment of the 3 proteins shown. <0: hydrophilic; >0: hydrophobic; (⊗): sections of minor coincidences; dark stippling: gaps or extensions within a sequence.

in this middle section even the two phytochrome sequences appear less similar than in the other parts. However, even there, hydrophobic cluster analysis (HCA) according to Gaboriaud et al. [18], that allows predictions on the overall folding of proteins by hand, demonstrates considerable conformity of bacterial sensor proteins and phytochromes. (Results will be presented as soon as a computer output of the bulky graphics is possible.)

4. DISCUSSION

The present findings on structural homologies between phytochromes and bacterial sensor proteins gain significance by the fact that the two groups of proteins are also related by functional homologies and that there are some insights into the mode of action of the bacterial proteins.

The bacterial proteins respond to environmental stimuli like nitrogen limitation (NtrBKp [6], NtrBBr [7]), phosphate limitation (PhoREc [15], PhoRBs [16]), plant exudate (VirAAt [17]), and membrane composition (CpxAEc [14], EnvZEc [13]), and phytochromes respond to light stimuli. Structural and functional coincidences corroborate the view that phytochromes and bacterial sensor proteins may have evolved from a common ancestral system. Even reports on membrane-associated phytochromes (see [1]) are not adverse. NtrBs are the only sensor proteins like the isolated phytochromes which lack hydrophobic transmembrane regions in the N-terminal sequences (see [19]). There are also reports on cross-talks between sensors of the one system and the regulators of another system (see [19]). We remember that oat phytochrome e.g. can be expressed in tomato [20] though oat and tomato phytochrome are certainly as different as oat and any other dicotyledonous phytochrome.

Experimental evidence infers that the bacterial proteins perceive the stimulus by their N-terminal part and transmit the information to their conserved C-terminal part by conformational alteration. Thereupon, the C-terminal part interacts with the conserved N-terminal region of its corresponding regulator protein which finally affects transcription. We also know that phytochrome responds to light with conformational changes which also comprise its C-terminal parts [21]. However, we do not know how the information stored by the conformation is transferred.

By reason of experimental data on NtrB [22] a far-reaching model for the interaction of sensor and regulator proteins has been constructed by Ronson et al. [19]. It proposes that NtrB modifies its regulator protein (NtrC) by phosphorylation (activation) and dephosphorylation (inactivation). However, phosphorylation or dephosphorylation is determined by two additional gene products. One of these products, GlnB, is required in addition to NtrB to dephosphorylate NtrC-phosphate. But dephosphorylation will only be achieved if GlnB is de-uridylylated by GlnD.

In summary, NtrB is assumed to be a kinase/phosphatase that is integrated in and additionally regulated by a system of other regulative proteins. Kinase function of phytochrome was discussed [23] but not found [24]. By analogy, the failure may well be caused by the absence of additional factors and the right target, a regulator protein or a protein functionally related to it like a transacting factor. We may also assume that not phytochrome itself but one of the additional factors is the kinase. A kinase was always copurified with phytochrome [24]. However, we should also consider possibilities of signal transfer other than phosphorylation and dephosphorylation. Nonetheless, in the frame of the model, it does not seem improbable that also experiments like those incubating nuclei with isolated phytochrome [25,26] suffer from incomplete systems.

It is tempting to introduce the model worked out with NtrB as a working hypothesis for the mode of action of phytochrome, though it does not account for the greater complexity of higher systems. At present an alternative model explaining experimental data more consistently than the present one is not available. It again directs attention to the proteins interacting with phytochrome. The knowledge that bacterial regulator proteins are conserved in their N-terminal parts can be, but need not necessarily be, a help in identifying them.

Apart from functional studies, studies in the field of phytochrome phylogeny may also be promoted by the new findings. Although relationships with bacterial sensors cannot be overlooked, we do not know how and at which stage the light-sensing head had been acquired.

Sure, our deductions are more speculative the further they move away from the actual experimental data, but they may contain explanations for many a phenomenon. Assuming e.g. that plastids regulate

nucleic transcription by a regulator protein or a factor functionally related to such a protein, we may come to an explanation of the finding that light-regulated proteins located in the plastids but encoded by the nucleus are not transcribed when the plastid is destroyed [27,28].

We think the present hypotheses on phytochrome-mediated signal-transduction are to be accepted by way of trial. They have to be measured against forthcoming data. But even if these data should not be in favour of them, the question remains: why have structures been conserved over such an evolutionary distance if they lost or changed their function fundamentally? It is about 28 kDa that share homologies.

Acknowledgement: The present investigations were made possible by a generous grant of the Volkswagen-Stiftung to H.J.S.-P.

REFERENCES

[1] Mohr, H. (1972) Lectures on Photomorphogenesis, pp. 24 ff, Springer, New York.
[2] Schneider-Poetsch, H.A.W., Schwarz, H., Grimm, R. and Rüdiger, W. (1989) Planta 173, 61–72.
[3] Christensen, A.H. and Quail, P.H. (1989) Gene 85, 381–390.
[4] Sharrock, R.A. and Quail, P.H. (1989) Genes Dev. 3, 1745–1757.
[5] Pearson, W.R. and Lipman, D.J. (1988) Proc. Natl. Acad. Sci. USA 85, 2444–2448.
[6] Buikema, W.J., Szeto, W.W., Lemley, P.V., Orme-Johnson, W.H. and Ausubel, F.M. (1985) Nucleic Acids Res. 13, 4539–4555.
[7] Nixon, T., Ronson, C.W. and Ausubel, F.M. (1986) Proc. Natl. Acad. Sci. USA 83, 7850–7854.
[8] Hershey, H.P., Baker, R.F., Idler, K.B., Lissemore, D.L. and Quail, P.H. (1985) Nucleic Acids Res. 13, 8543–8559.
[9] Sharrock, R.A., Lissemore, D.L. and Quail, P.H. (1986) Gene 47, 287–295.
[10] Sato, M. (1988) Plant Mol. Biol. 11, 697–710.
[11] Kay, S.A., Keith, B., Shinozaki, K. and Chua, N.H. (1989) Nucleic Acids Res. 17, 2865–2866.
[12] Kyte, J. and Doolittle, R.F. (1982) J. Mol. Biol. 157, 105–132.
[13] Comeau, D.E., Ikenaka, K., Tsung, K. and Inouye, M. (1985) J. Bacteriol. 164, 578–584.
[14] Albin, R., Weber, R. and Silverman, P.M. (1986) J. Biol. Chem. 261, 4698–4705.
[15] Wanner, B.L. and Chang, B.-D. (1987) J. Bacteriol. 169, 5569–5574.
[16] Seki, T., Yoshikawa, H., Takahashi, H. and Saito, H. (1988) J. Bacteriol. 170, 5935–5938.
[17] Leroux, B., Yanofsky, M.F., Winans, S.C., Ward, J.E., Ziegler, S.F. and Nester, E.W. (1987) EMBO J. 6, 849–856.
[18] Gaboriaud, C., Bissery, V., Benchetrit, T. and Mornon, J.P. (1987) FEBS Lett. 224, 149–155.
[19] Ronson, C.W., Nixon, B.T. and Ausubel, F.M. (1987) Cell 49, 579–581.
[20] Boylan, M.T. and Quail, P.H. (1989) Plant Cell. 1, 765–773.
[21] Schneider-Poetsch, H.A.W., Braun, B. and Rüdiger, W. (1989) Planta 177, 511–514.
[22] Ninfa, A.J. and Magasanik, B. (1986) Proc. Natl. Acad. Sci. USA 83, 5909–5913.
[23] Lagarias, J.C., Wong, Y.-S., Berkelman, T.R., Kidd, D.G. and McMichael, R.W. (1987) in: Phytochrome and Photoregulation in Plants, pp. 51–61, Academic Press, New York.
[24] Grimm, R., Gast, D. and Rüdiger, W. (1989) Planta 178, 199–206.
[25] Ernst, D. and Oesterhelt, D. (1984) EMBO J. 3, 3075.
[26] Mösinger, E., Bartschauer, A., Vierstra, R., Apel, K. and Schäfer, E. (1987) Planta 170, 505–514.
[27] Oelmüller, R. and Mohr, H. (1986) Planta 167, 106–113.
[28] Burgess, D.G. and Taylor, W.C. (1987) Planta 170, 520–527.