A novel blue light- and abscisic acid-inducible gene of Arabidopsis thaliana encoding an intrinsic membrane protein

Ralf Kaldenhoff, Andreas Kölling and Gerhard Richter*
Institut für Botanik, Universität Hannover, D-30419 Hannover, Germany (*author for correspondence)

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Abstract

Continuous irradiation with blue light (400–500 nm) induces flower formation in plantlets of Arabidopsis thaliana (C24) while red light (600–700 nm) is ineffective. This observation started a search for genes that are activated by blue light and initiate the morphogenic programme leading to flower formation. Several genes were identified via their cDNAs. From these clone AthH2, with an open reading frame for a hydrophobic 30.5 kDa polypeptide, was selected for further characterization of the corresponding gene. From a genomic library a DNA fragment of about 6.4 kb was isolated, comprising the coding region as well as 5’-upstream and 3’-downstream flanking segments. The coding region is composed of four exons, which specify a polypeptide of 286 amino acids. Several potential regulatory elements were found between position –670 and –1140 including GA and ABA sequence motifs. The latter could account for the observed induction of the AthH2 gene by ABA. Southern blot analysis of Arabidopsis genomic DNA suggests that the AthH2 gene is encoded by a single-copy gene. Hydropathy plots and secondary structure analysis of the putative polypeptide predict six membrane-spanning domains implicating a function as transmembrane channel protein. It displays significant homology with the proteins TR7a of pea (82%) and RD 28 of A. thaliana (68%).

Introduction

Light, as an environmental factor, plays a crucial role in the development of plants. Accordingly, they have evolved photoreceptor systems which respond to different qualities and quantities of light and thus control developmental processes. While the red/far-red sensing phytochrome is well characterized, the nature of the blue and near-UV light mediating receptor (cryptochrome) is still unknown. Recent studies indicate that both are part of a multiple photosensory system which exerts control over a number of physiological processes by mediating different light qualities [12]. However, it is not known which of the various responses is controlled, by which photoreceptor

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Database under the accession numbers Z17399 (genomic) and Z17424 (cDNA).
or to what extent the different receptor molecules have overlapping functions. To resolve these uncertainties a system is required where light responses can be dissected by setting up conditions in which either blue light- or red light-dependent processes take place. Arabidopsis thaliana was found to be a plant particularly well-suited for these investigations. Mutation analysis of hypocotyl elongation inhibition observed in blue light-grown seedlings revealed that certain genes involved in signal transduction are defective [27]. They are distinct from those found in the hy mutants of Arabidopsis thaliana [25], in which the phytochrome response is altered.

Floral induction as a typical light response of Arabidopsis plantlets could be accomplished by continuous exposure to blue light (485 nm) while red light (660 nm) administered under the same experimental conditions was completely ineffective [3]. The obvious blue light dependence of morphogenesis under these conditions offered a good opportunity to search for genes which are likewise preferentially activated by blue light because their products serve as structural, housekeeping or regulatory proteins in the process of flowering.

In a recent investigation, we found evidence for the existence of such genes in A. thaliana. Several of these are rapidly expressed upon blue light irradiation of young plants. The features of one gene, which encodes an intrinsic membrane protein, suggest a transporter function. Remarkably, for the same gene, induction by the plant hormone abscisic acid was observed. In this paper we describe its genomic structure and the mode of expression as well as features of the encoded polypeptide.

Materials and methods

Plant material and growth conditions

Dry seeds of Arabidopsis thaliana L. (strain C24) were surface-sterilized by immersion in ethanol + 0.02% Triton X-100 for 5 min followed by incubation with 5% hypochlorite + 0.02% Triton X-100 for 15 min. Germination and growth were carried out at 24 °C in darkness on a medium described by Estelle and Sommerville [7] supplemented with 2% sucrose and solidified with 0.8% agar. Seedlings after 2 days and plantlets after 14 days of culturing were exposed to either blue, red or white light. The blue light source consisted of Philips fluorescent tubes TL 36/W 18 providing 4–6 W/m² within the spectral range 400–550 nm. Red light of equal energy fluence rate (spectral range 600–700 nm) was produced by Philips fluorescent tubes TL 36/W 15. Osram incandescent tubes supplied white light of equal energy fluence rate. The set-up for irradiation with far-red light was as reported [26].

For hormone treatment plantlets were grown for 2 h on the standard agar medium containing either 1 mM abscisic acid (ABA-cis, trans, Sigma) or gibberellic acid (GA₃, Sigma) in various amounts: 10 μM, 100 μM or 1 mM. For testing the effect of calcium ions, the concentration of CaCl₂ in the agar medium was raised to 10 mM.

Preparation and analysis of RNA

Plantlets (2 g fresh weight) were quickly chilled in liquid nitrogen and ground in a mortar. The resulting powder was suspended in 15 ml extraction buffer (0.6 M NaCl, 10 mM EDTA, 100 mM Tris-HCl pH 8.0, 4% SDS) plus 15 ml of a 1:1 mixture of phenol (80%) and chloroform. After shaking for 10 min and centrifugation at 20000 × g for 10 min the aqueous phase was recovered. 0.75 vol LiCl solution (8 M) was added and the mixture incubated for 1 h on ice, then centrifuged at 20000 × g and 4 °C for 10 min. The pellet was resuspended in 5 ml water treated previously with diethyl pyrocarbonate (DEPC). 1/10 vol sodium acetate (3 M) and 2.5 vol ethanol were added and left for 2 h at −20 °C. The precipitated RNA was collected by centrifugation, resuspended in a small volume of DEPC-treated water, and size-fractionated on 1% denaturing agarose-formaldehyde gel [5]. Total RNA from different preparations was standardized spectrophotometrically, by using the ethidium