Planta (2008) 229:25–36
DOI 10.1007/s00425-008-0803-4

ORIGINAL ARTICLE

An unidentified ultraviolet-B-specific photoreceptor mediates transcriptional activation of the cyclobutane pyrimidine dimer photolyase gene in plants

Motohide Ioki · Shinya Takahashi · Nobuyoshi Nakajima · Kohei Fujikura · Masanori Tamaoki · Hikaru Saji · Akihiro Kubo · Mitsuko Aono · Machi Kanna · Daisuke Ogawa · Jutarou Fukazawa · Yoshihisa Oda · Seiji Yoshida · Masakatsu Watanabe · Seiichiro Hasezawa · Noriaki Kondo

Received: 23 March 2008 / Accepted: 30 July 2008 / Published online: 30 September 2008

Abstract Cyclobutane pyrimidine dimers (CPDs) constitute a majority of DNA lesions caused by ultraviolet-B (UVB). CPD photolyase, which rapidly repairs CPDs, is essential for plant survival under sunlight containing UVB. Our earlier results that the transcription of the cucumber CPD photolyase gene (*CsPHR*) was activated by light have prompted us to propose that this light-driven transcriptional activation would allow plants to meet the need of the photolyase activity upon challenges of UVB from sunlight. However, molecular mechanisms underlying the light-dependent transcriptional activation of *CsPHR* were unknown. In order to understand spectroscopic aspects of the plant response, we investigated the wavelength-dependence (action spectra) of the light-dependent transcriptional activation of *CsPHR*. In both cucumber seedlings and transgenic *Arabidopsis* seedlings expressing reporter genes under the control of the *CsPHR* promoter, the action spectra exhibited the most predominant peak in the long-wavelength UVB waveband (around 310 nm). In addition, a 95-bp *cis*-acting region in the *CsPHR* promoter was identified to be essential for the UVB-driven transcriptional activation of *CsPHR*. Thus, we concluded that the photoperception of long-wavelength UVB by UVB photoreceptor(s) led to the induction of the *CsPHR* transcription via a conserved *cis*-acting element.

Keywords Action spectrum · *Cucumis* · Cyclobutane pyrimidine dimmer · DNA photolyase (EC 4.1.99.3) · Photoreceptor · Ultraviolet light

Abbreviations

CPD	Cyclobutane pyrimidine dimer
CsPHR	Cucumber CPD photolyase
GUS	β-Glucronidase
LUC	Luciferase
PcCHS	Parsley chalcone synthase
RT-PCR	Reverse transcription-polymerase chain reaction
UVA	Ultraviolet-A
UVB	Ultraviolet-B

Introduction

The ultraviolet waveband of the solar radiation reaching the earth's surface is subdivided into the ultraviolet-A (UVA; 315–400 nm) and ultraviolet-B (UVB; 290–315 nm) wavebands. UVB directly damages macromolecules such

M. Ioki · S. Takahashi · K. Fujikura · J. Fukazawa · S. Hasezawa · N. Kondo
Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo 113-0033, Japan

M. Ioki · N. Nakajima (✉) · M. Tamaoki · H. Saji · A. Kubo · M. Aono · M. Kanna · D. Ogawa · S. Yoshida
Environmental Biology Division, National Institute for Environmental Studies, Onogawa 16-2, Tsukuba, Ibaraki 305-8506, Japan
e-mail: naka-320@nies.go.jp

M. Ioki · Y. Oda · S. Hasezawa
Graduate School of Frontier Sciences, The University of Tokyo, Kashiwanoha 5-1-5, Kashiwa, Chiba 277-8562, Japan

M. Watanabe
National Institute for Basic Biology, Nishigonaka 38, Myodaiji, Okazaki, Aichi 444-8585, Japan

Present Address:
M. Ioki
Department of Plant Biology, University of California, One Shields Ave, Davis, CA 95616, USA

as proteins and nucleic acids. DNA damage due to UVB is thought to not only impede DNA replication and gene expression but also lead to mutations (Takayanagi et al. 1994; Taylor et al. 1996; Rousseaux et al. 1999; Ries et al. 2000). DNA lesions are largely responsible for the plant growth inhibition due to UVB (Caldwell et al. 1986; Teramura 1983; Larcher 1995; Giordano et al. 2004). Cyclobutane pyrimidine dimers (CPDs) constitute approximately 75% of UVB-induced DNA lesions (Britt 1996).

Plants have developed multiple defensive mechanisms against the challenge of direct exposure to solar UVB during their evolution. Some of the solar UVB radiation is reflected by epicuticular waxes on plant surfaces (Caldwell et al. 1983; Holmes 1997). Plants also accumulate UV-absorbing compounds like flavonoids and anthocyanins in the vacuoles of epidermal cells (Bornman et al. 1997). However, a considerable amount of solar UVB still reaches DNA in the nucleus. Enzymatic restoration of the DNA lesions, therefore, is essential for the plant survival under UVB-containing sunlight.

CPD photolyase rapidly repairs CPDs using light energy, a process known as photoreactivation. The physiological importance of CPD photolyase has been realized by many experiments in plants. For example, an *Arabidopsis* mutant lacking functional CPD photolyase, *uvr2-1*, is hypersensitive to UVB (Landry et al. 1997; Britt and Fiscus 2003). It has also been reported that differences in the CPD photolyase activity correlate with differences in the UVB sensitivity among rice cultivars (Hidema et al. 2005). Therefore, CPD photolyase builds up one of the most critical defense lines against UVB damages in plants.

CPD photolyase is intimately associated with light. CPD, the substrate of the enzyme, is generated under short-wavelength UVB (280–300 nm) and ultraviolet-C (Matsunaga et al. 1991). Moreover, the light in the UVA/blue range provide the energy for CPD photolyase to repair CPDs (Sancer 1994).

The expression of CPD photolyase can be induced by various qualities of light in plants. For example, the induction by far-red light in etiolated seedlings of *Phaseolus vulgaris* and *Sinapis alba*, by blue light in etiolated seedlings of *Arabidopsis thaliana* and *P. vulgaris*, and by UVA in etiolated *A. thaliana* seedlings has been documented (Langer and Wellmann 1990; Buchholz et al. 1995; Ahmad et al. 1997). Surprisingly, photolyase expression was not induced by UVB irradiation in etiolated *Arabidopsis* seedlings (Ahmad et al. 1997). On the other hand, light-grown *Arabidopsis* seedlings exhibit higher CPD photolyase expression levels and activities when treated with UVB (Pang and Hays 1991; Ries et al. 2000; Waterworth et al. 2002). Similarly, Giordano et al. (2003) demonstrated that *Gunnera magellanica* plants grown under the presence of UVB exhibited increased CPD photolyase activity in the leaf. Among the different qualities of light which induce CPD photolyase expression, UVB is particularly intriguing because to date UVB photoreceptor(s) which mediate the plant response to UVB radiation have not been identified.

The first true leaf of a cucumber (*Cucumis sativus* L.) plant expands rapidly and horizontally, making itself suitable for light-irradiation experiments and observation of the effects of UVB irradiation on leaf growth. Our previous report showed that CPD photolyase expression was promoted by UVA-containing white light in cucumber. Elevation of the CPD photolyase activity in the midst of the day via light-induced transcriptional activation was thought to be an adaptation strategy that plants have developed to tolerate the solar UVB (Takahashi et al. 2002). In this paper, we first demonstrated that the *CsPHR* transcription was activated by UVB in cucumber leaves. In order to examine the wavelength specificity of the photosensory mechanisms mediating this plant response to UVB, we further analyzed the fine structures of action spectra for the light-driven transcriptional activation. We also identified a promoter region that was essential for the UVB-driven transcriptional activation.

Materials and methods

Growth conditions

Prior to the polychromatic UV treatment, the seedlings of cucumber (*Cucumis sativus* L. cv. *Hokushin*; obtained from Sakata Seed Corp., Kanagawa, Japan) were grown in a mixture of vermiculite, peatmoss, perlite and fine gravel (2:2:1:1, by vol.) at 25 ± 0.5°C with a relative humidity of 70 ± 5%. The seedlings were transferred to an artificially lit growth cabinet on the eighth day after sowing. The growth cabinet was programmed for a temperature regime of 20 ± 0.5°C for 12 h under light, and 15 ± 0.5°C in dark. Metal halide lamps (BOC Lamp, Mitsubishi Electric Corp., Tokyo, Japan) were used to provide the white light with wavelengths above 340 nm. The photosynthetic photon flux density (PPFD) at the plant height was 300 μmol m^{-2} s^{-1}, as measured with a quantum meter (LI-1000, Li-Cor, Lincoln, NE, USA). The seedlings were watered daily with a 1,000-fold dilution of Hyponex (Hyponex Japan, Osaka, Japan).

Prior to the monochromatic light irradiation, seedlings grown in a naturally lit glasshouse for 10 days were transferred to the spectrograph room maintained at 20 ± 0.5°C with a relative humidity of 60 ± 5%. White light was supplied with fluorescent lamps (FLR40S W/M, Toshiba Light Technology Corp., Tokyo, Japan) during the light period on the tenth and eleventh day after sowing. The PPFD at the plant height was 200 μmol m^{-2} s^{-1}. The first true leaves

were excised and frozen in liquid nitrogen immediately after the irradiation on the 12th day, then stored at −80°C until extraction of RNA or DNA.

Seeds of the transgenic *Arabidopsis* were surface-sterilized with 70% (v/v) ethanol for 1 min and with a 5% sodium hypochlorite solution for 5 min, prior to be germinated on 0.7% agar plates containing 0.5× Murashige and Skoog medium (pH 5.8) at 25 ± 0.5°C with 14 h of light and 10 h of dark. White light was supplied with fluorescent lamps during the light period. The PPFD at the plant height was 80 μmol m^{-2} s^{-1}, as measured with a quantum meter.

Polychromatic and monochromatic UV irradiation of seedlings

The polychromatic UV (λ_{max} at 315 nm) was supplied using UV fluorescent lamps (FL20SE, Toshiba Light Technology Corp.) for 1.5–6 h starting at the beginning of the light period (6:00). Polyvinyl filters (Cutting Sheet 000C, Nakagawa Chemical, Co. Ltd., Tokyo, Japan) or quartz filters (UV-29; Hoya Co. Ltd., Tokyo, Japan) were used to remove light with wavelengths below 290 nm. The UVB irradiance measured with a UV monitor (MS210I, EKO Instruments Trading Co., Ltd., Tokyo, Japan) was 0.25 Wm^{-2} at the plant height for cucumber, and 0.15 Wm^{-2} for *Arabidopsis*. The spectral irradiance was measured using a spectroradiometer (MCPD-1000, Otsuka Electronics Co., Ltd., Osaka, Japan).

Irradiation of monochromatic light was performed using the Okazaki Large Spectrograph (Watanabe et al. 1982). The cucumber plants with horizontally expanding first true leaves, being placed in the irradiation boxes, were exposed to vertically incident monochromatic light with wavelengths of 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 400, 450, and 500 nm for 4 h, starting at the beginning of the light period (6:00). Monochromatic light of each wavelength was irradiated at four different fluence rates, measured by a photon density meter (PFDM-200LX, Rayon Industrial Co. Ltd, Tokyo, Japan). No white light was irradiated during the monochromatic irradiation. Four plants were kept in the dark as controls in each experiment. Four-day-old *Arabidopsis* seedlings were exposed to monochromatic light of 270, 280, 290, 300, 310, and 320 nm at 4 or 5 different fluence rates for 3 h. The control plants were kept in the dark.

For cucumber, the fluence rates of monochromatic light within the ultraviolet-C/short-wavelength UVB band (270–290 nm) were maintained below 0.15 μmol m^{-2} s^{-1}. For *Arabidopsis*, the fluence rates of monochromatic light within the ultraviolet-C/short-wavelength UVB band were maintained below 1 μmol m^{-2} s^{-1}.

Prior to the UV treatments of *Arabidopsis* seedlings, the lids of the Petri dishes were removed in the dark.

Quantification of *CsPHR* transcripts via quantitative RT-PCR

Total RNA was extracted from the frozen leaves using RNeasy Plant Mini Kit (Qiagen Inc., Chatsworth, CA, USA) according to the manufacturer's instructions. The amount of *CsPHR* transcripts was estimated via quantitative RT-PCR followed by Southern-blot analysis using radioactively labeled *CsPHR* probes, according to Takahashi et al. (2002).

Promoter::reporter constructs

An 8-kbp genomic DNA fragment containing *CsPHR* that we have cloned previously (Ioki et al. 2003) was used as a template for amplifying full-length *CsPHR* promoter using Ex-taq DNA polymerase (Takara Bio Inc., Shiga, Japan) with primers: 5′-CAGCTGCAGCTAATAATAAAAATATTACATCAATTA-3′, and 5′-TCTCCCGGGTCCTGTTTGAATATGAACAGGTAAGG-3′. After the PCR product was cloned into a pCR2.1 vector, the 2.5-kbp promoter fragment was excised at the *Xba*I and *Bam*HI sites located in proximity of the 5′ and 3′ ends of the fragment, which was then cloned into the binary vector pBI101 (Clontech Laboratories, Inc., Mountain View, CA, USA) containing *GUS*.

For the promoter analysis using the luciferase gene (*LUC*), the *GUS* portion of a pBI221 vector (Clontech Laboratories, Inc.) was substituted with *LUC* derived from the pGL3-basic vector (Promega Co., Madison, WI, USA). The 1,132-, 300-, and 201-bp-long fragments of the *CsPHR* promoter were amplified via PCR with specific primer pairs of 5′-CAGCTGCAGCTATCATGATGTAACTATAGGTG-3′ and 5′-TCTAGATCTCCTGTTTGAATATGAACAGGTAAGG-3′ for the 1,132-bp fragment; 5′-CAGCTGCAGTCGAGAAACGTCAATATATTAAAGTT-3′ and 5′-TCTAGATCTCCTGTTTGAATATGAACAGGTAAGG-3′ for the 300-bp fragment; and 5′-CAGCTGCAGGCTATTCAAAACCACAACTTTCAC-3′ and 5′-TCTAGATCTCCTGTTTGAATATGAACAGGTAAGG-3′ for the 201-bp fragment. The promoter segments were cloned into the pBI221 vector containing *LUC* at the *Pst*I and *Bgl*II restriction sites. The DNA fragment containing the *CsPHR* derivative, *LUC*, and *NOS* terminator was excised out and cloned into pBI101 at the *Pst*I and *Eco*RI sites.

To obtain the full-length promoter with the 95-bp (−202 to −296 bp) region deleted (Δ 95), the two promoter segments upstream and downstream of the 95-bp region were first amplified independently via PCR using the 2.5-kbp promoter fragment cloned in a pCR2.1 vector as the

template. The promoter segment upstream of the 95-bp region was amplified using the primers of 5′-GTTTTCC CAGTCACGA-3′ (M13F) and 5′-GTGGTTTTGAATAG CTCGATGTTATTTTTAAAAAATTATTATCGTTATT TGTTTTTTTAGAAACC-3′; the promoter segment downstream of the 95-bp region was amplified using the primers of 5′-CATCGAGCTATTCAAAACCACAACTTTCAC-3′ and 5′-CAGGAAACAGCTATGAC-3′ (M13R). Using the resulting PCR products as the templates, full-length promoter lacking the 95-bp region (Δ 95) was obtained via PCR with the M13F and M13R primers. The PCR product was cloned into the pGEMT-easy vector (Promega Co.). The Δ 95 promoter fragment was digested out of the pGEMT-easy vector at the *Xba*I and *Bam*HI sites and cloned into the binary vector pBI101.

Transformation of *Arabidopsis*

All the binary vectors were introduced into *Agrobacterium radiobactor* (strain C58C1) and then into *Arabidopsis thaliana* L. (Col-0; obtained from The Arabidopsis Biological Resource Center, Columbus, Ohio) using the floral dip method (Clough and Bent 1998). Transformed plants carrying the transgenes were screened by resistance to kanamycin. Approximately 10 independent lines were isolated for each promoter–reporter transgene. Detailed analyses of the transgenic plants were performed using the T2 or T3 generation of homozygotes. Since the *CsPHR* promoter is a very weak promoter, the transgenic lines tended to express the reporter gene at low levels. Therefore, we chose lines with relatively high reporter expression for detailed analysis.

Analysis of action spectra for transcriptional activation of *CsPHR*, CPD formation and induction of reporter expression

The action spectrum for the transcriptional activation of *CsPHR* was obtained by examining the *CsPHR* transcript levels in the first true leaves. The transcript levels were plotted and semilogarithmically regressed against the fluence rate. The regression coefficient for each wavelength was calculated from the fluence–response plot.

The amount of CPDs accumulated in the leaf was measured via an enzyme-linked immunosorbent assay using CPD-specific antibodies, according to Takahashi et al. (2002). In order to obtain the action spectrum for CPD accumulation in the leaf, the amount of CPDs, represented by A_{492}, was semilogarithmically regressed against the fluence rate (data not shown).

To obtain the action spectrum for the activation of the *CsPHR* promoter in the transgenic *Arabidopsis* plants, the GUS activity in the plant treated with monochromatic light was semilogarithmically regressed against the fluence rate.

For these experiments, the regression coefficient for each wavelength was calculated from the fluence–response plot and plotted against the wavelength as photon effectiveness in the action spectrum.

Measurement of GUS activity

The GUS activity was measured using 4-methylumbelliferyl β-D-glucuronide (Nakalai Tesque, Kyoto, Japan) as the substrate, according to Jefferson et al. (1987). Briefly, the transgenic plants were homogenized in GUS extraction buffer (50 mM sodium phosphate buffer, pH 7.0, 10 mM EDTA, 0.1% Triton-X100, 0.1% Sarcosyl, 10 mM β-mercaptoethanol), then centrifuged at 10,000*g* for 10 min. Seventeen microliters of the supernatant fraction were mixed with 4 µl of a 1 mM 4-methylumbelliferyl β-D-glucuronide solution. The reaction mixture was incubated at 37°C, and 10-µl aliquots were removed at 60 and 120 min. The reaction was terminated with addition of 90 µl of 0.2 M Na_2CO_3. Fluorescence was measured with a spectrofluorometer (FP-6500; JASCO Corp., Tokyo, Japan) set at 365 nm (excitation) and 455 nm (emission). The protein concentration was estimated by the Bradford method (Bio-Rad Laboratories, Hercules, CA, USA) using BSA as the standard. The specific activity of the GUS enzyme in the extract was calculated as pmol of 4-methylumbelliferone produced per min per mg of total protein.

GUS histochemical assay

For the histochemical analysis of GUS expression, seedlings were placed in the GUS staining solution (50 mM sodium phosphate buffer, pH 7.0, 20% methanol, and 1 mM 5-bromo-4-chloro-3-indolyl-β-D-glucuronide) and vacuum-infiltrated for 20 min, then incubated at 37°C for 24 h. Stained seedlings were washed in 70% ethanol to stop the reaction and to remove chlorophyll. The photographs were taken under a SZ 61 zoom stereo microscope with a DP12 digital camera (Olympus, Melville, NY, USA).

Quantification of *LUC* transcripts via quantitative RT-PCR

Total RNA was extracted from the frozen seedlings using RNeasy Plant Mini Kit (Qiagen Inc.). The amount of *LUC* transcripts was estimated via RT-PCR using Ready-To-Go™ RT-PCR beads (Amersham Pharmacia Biotech, Piscataway, NJ, USA), followed by Southern-blot analysis with radioactively labeled *LUC* probes. The total RNA (0.1 µg) was incubated at 42°C for 30 min in 20 µl of the reaction mixture containing 0.2 µg of $pd(T)_{12\text{-}18}$ (Amersham) as a primer for reverse transcription. Then, PCR was performed using specific primers for *LUC*: GL1, 5′-CG GAGTTGCAGTTGCGCCCGCGAACGAC-3′ and GL2,

5′-AGGCAGAGCGACACCTTTAGGCAGACCAG-3′. To evaluate the precision of RT-PCR and equal loading of the total RNA, control reactions were performed with specific primers for 18S ribosomal RNA: Rna1, 5′-CTATGGGTG GTGGTGCATGGC-3′ and Rna2, 5′-CGCCGCGATCC GAACACTTCACC-3′. The parameters of the PCR were 94°C for 5 min, followed by 15 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. The amplified DNA fragments in 10 µl of the reaction mixture were separated on a 1.6% agarose gel, then transferred onto a Hybond^{TM} N^+ membrane (Amersham). The membrane was probed with [α-^{32}P]dCTP-labeled 171-bp *LUC* fragment, obtained via PCR with nested primers of GL3, 5′-GGGCATTTCGCAGCCTA CCGTGGTGT-3′, and GL4, 5′-CCGGGAGGTAGATGA GATGTGACGAAC-3′, in the hybridization buffer [50% formamide, 6 × SSPE, 0.5% SDS, 5% Irish cream liquor (R & A Bailey & Co., Dublin, Ireland), and 100 µg ml^{-1} denatured salmon sperm DNA] at 42°C overnight. The membrane was washed twice with 2 × SSC containing 0.1% SDS at 42°C for 30 min, followed by twice with 0.2 × SSC containing 0.1% SDS at 42°C for 15 min. The washed membrane was exposed to a Type BAS-III imaging plate (Fuji Photo Film Co., Tokyo, Japan), and the radioactivity of the *LUC* hybridization signal was quantitated using a BAS2000 Bioimaging Analyzer (Fuji Photo Film Co.).

Results

Wavelength-dependence of the transcriptional activation of *CsPHR* in cucumber leaves

In general, light is perceived by photoreceptors that sense specific qualities of light in order to activate specific downstream signaling cascades. To deduce the photoreceptors involved in the light-induced expression of *CsPHR*, we determined the dependence of wavelength for the transcriptional activation in cucumber first true leaves. To estimate the wavelength-dependence of the light-induced expression of the *CsPHR* transcription, we first examined the transcription under the light conditions of (i) white light, and (ii) white light + UV (Fig. 1a). Because in the natural environment, plants receive sunlight containing UV above 290 nm, we first investigated the effect of supplemental irradiation of polychromatic UV of $\lambda > 290$ nm on the *CsPHR* transcription (Fig. 1a, bottom panel). The transcription was increased approximately twofold (at 9:00) by the additional UV irradiation (Fig. 1b, c). As the transcription of *CsPHR* was effectively enhanced by the low-fluence polychromatic UV irradiation, it was suggested that the UV waveband was highly effective for the transcriptional activation.

Then, irradiation of monochromatic light was performed in order to further investigate the wavelength-dependence of the transcriptional activation within the UV/blue range (270–500 nm). Photon effectiveness at each wavelength was estimated by plotting the *CsPHR* transcript level against the fluence rate and calculating the regression coefficient (or the semilogarithmic slope) of the plot (Fig. 2a). The action spectrum for the transcriptional activation of *CsPHR* was obtained by plotting the photon effectiveness against the wavelength. The action spectrum exhibited peaks at 310 nm (long-wavelength UVB waveband) and at 450 nm (blue waveband). UVA (350 nm) also seemed to slightly induce the *CsPHR* transcription (Fig. 2b). Many of the UVA/blue-light photoreceptors, such as cryptochromes and phototropins, exhibit absorption peaks at 350 and 450 nm (Lin et al. 1995; Lin 2000; Kasahara et al. 2002). Thus they might be responsible for the transcriptional activation of *CsPHR* by blue light and UVA. However, the identity of the photoreceptor(s) associated with the 310-nm peak remained elusive since no UVB photoreceptor has been identified.

To examine if the UVB-induced *CsPHR* expression was triggered by signals arising from DNA lesions, the monochromatic action spectrum for the CPD formation in the leaf was investigated under the present experimental condition. The CPD formation rate increased with shortening wavelengths within the UVB spectrum (Fig. 2c, gray line), similar to a published work using alfalfa seedlings (Quaite et al. 1992). CPD formation was negligible at 320 nm. The action spectrum for CPD formation critically differed from that for the transcriptional activation of *CsPHR* as the CPD formation was negligible when effective transcriptional activation was observed at 310 and 320 nm (Fig. 2c). Therefore, signal transduction arising from DNA lesions did not fully account for the promotion of the *CsPHR* transcription by UVB, and distinctive photoperception mechanisms were implicated for the long-wavelength UVB waveband.

Wavelength-dependence of reporter expression under control of the *CsPHR* promoter

We further investigated the transcriptional activation by long-wavelength UVB using transgenic *Arabidopsis* expressing a reporter gene under the control of the *CsPHR* promoter. First, the transgenic *Arabidopsis* plants harboring the 2.5-kb full-length promoter fused to a β-glucronidase gene (*GUS*) were subjected to three different light treatments of UV, white light, and white light + UV. In the absence of UVB (dark, white light), the GUS activity was detected only in cells around the shoot apical meristem and in cells around the distal end of the cotyledon primary vein (Fig. 3a). When UVB was applied (UV and white light + UV), induction of *GUS* expression was observed in all foliar cells (Fig. 3a). These results indicated that the

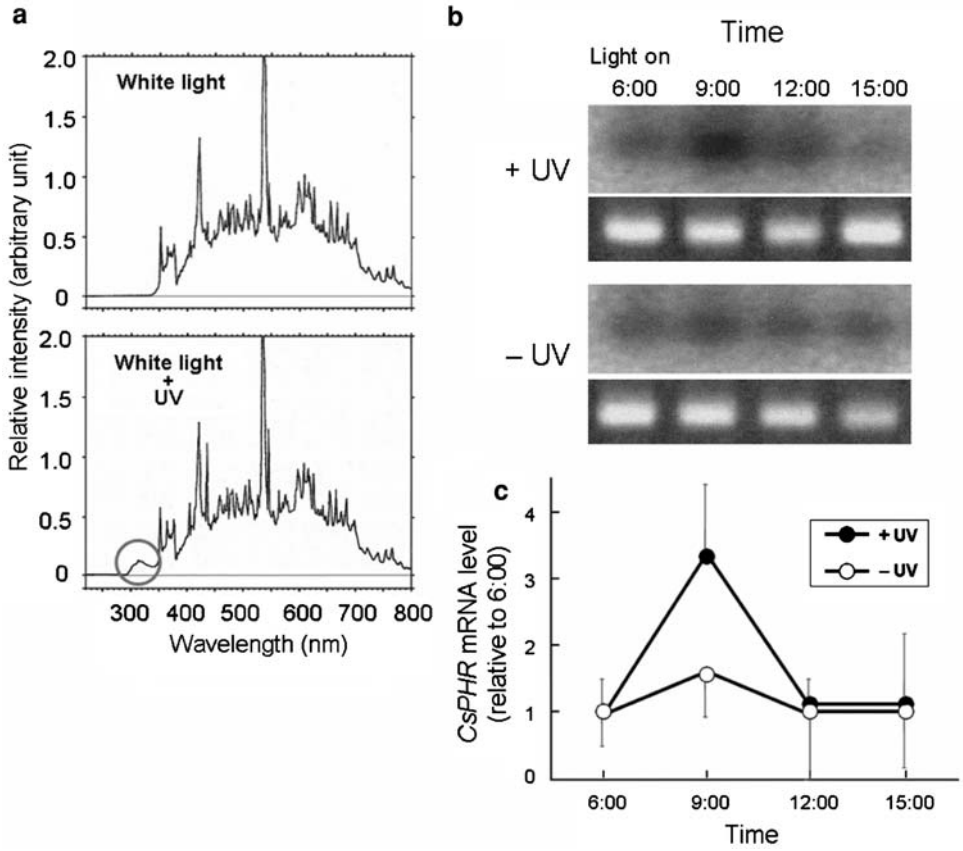

Fig. 1 Promotion of *CsPHR* transcription by supplemental UV irradiation. Cucumber seedlings were grown under white light containing no UVB for 12 days. On the 13th day after sowing, plants were treated with supplemental polychromatic UV starting at beginning of the light period (6:00). *CsPHR* transcript levels in first true leaves were determined. **a** Spectral irradiance for plant growth without (*upper panel*) and with UV treatment (*lower panel*). The PPFD and UVB irradiance were 300 μmol m^{-2} s^{-1} and 0.25 Wm^{-2} s^{-1} at the plant height, respectively. **b** Total RNA was extracted from the leaf, and *CsPHR* transcripts were detected via RT-PCR followed by Southern hybridization as described in "Materials and methods" (*top photograph*). Validity of RT-PCR was confirmed by the RT-PCR products derived from 18S rRNA (*bottom photograph*). **c** *CsPHR* transcript levels were quantified by measuring radioactivity. *Solid and open circles* represent *CsPHR* transcript levels in UV-treated and control plants, respectively. *Each bar* represents ± SD derived from at least 3 independent plants

CsPHR promoter was effectively activated by UVB in the transgenic *Arabidopsis*.

In order to test whether the UVB-dependent activation of the *CsPHR* promoter in the transgenic *Arabidopsis* exhibits the same wavelength specificity as cucumber, the action spectrum for the *GUS* expression in the transgenic *Arabidopsis* was analyzed. To determine the appropriate duration of irradiation, the time course of the induction of GUS expression by the polychromatic UV was investigated prior to the investigation of the action spectrum. The GUS activity reached the maximal level at 4.5 h after the initiation of treatment and remained at the same level thereafter (data not shown). Duration of the monochromatic irradiation, therefore, was set for 3 h. The monochromatic action spectrum exhibited the most prominent peak at 310 nm, indicating that the *CsPHR* promoter was indeed activated by long-wavelength UVB in the transgenic *Arabidopsis* (Fig. 3b, c, d). The experimental system using the transgenic *Arabidopsis* yielded action spectra more precise than those obtained using cucumber seedlings because the small size of *Arabidopsis* plants allowed large number of plants to be examined at once. The most prominent peaks of the action spectra were observed at 310 nm in the two different experimental systems (Figs. 2b, 3c). Therefore, it was concluded that similar UVB perception and signal transduction mechanisms were present in the two different plant species.

Cis elements required for *CsPHR* expression in response to UVB

The *CsPHR* promoter was further dissected for potential UVB-responsive *cis* elements via promoter–deletion experiments using transgenic *Arabidopsis*. Prior to the promoter–deletion experiments, the nucleotide sequence of the *CsPHR* promoter was scrutinized for DNA sequences that resembled light-responsive *cis* elements previously identified for light-inducible genes like those encoding enzymes associated with photosynthesis and flavonoid synthesis.

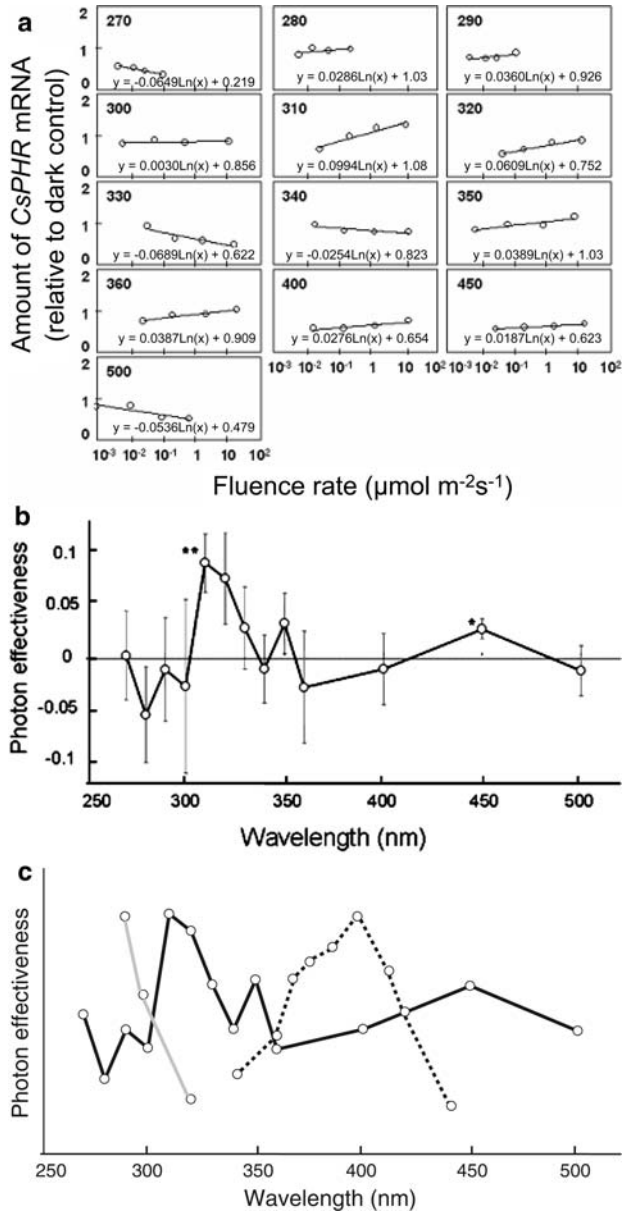

Fig. 2 Transcriptional activation of *CsPHR* by the long-wavelength UVB. **a** Representative set of fluence–response plots for accumulation of the *CsPHR* transcripts in horizontally expanding cucumber first true leaves. Wavelength of the monochromatic light is indicated at *upper left corner* of each panel. Values were normalized to the mean value for 4 dark-control plants and semilogarithmically regressed against the fluence rate. Functional equation of the regression line is presented at bottom. **b** Action spectrum for transcriptional activation of *CsPHR*. Regression coefficient for each wavelength was calculated from the fluence–response plot (**a**). Experiments were repeated 6 times and mean value for the regression coefficient was plotted against wavelength as photon effectiveness. *Each bar* indicates ± SE for 6 independent experiments. *Asterisks* indicate significant differences at $P < 0.1$ (*) and $P < 0.05$ (**) determined by one-sample *t* test against the value zero. **c** Spectroscopic interrelationship of action spectra for *CsPHR* transcriptional activation (*black solid line*), CPD formation in the leaf (*gray line*), and CsPHR activity (*broken line*; Hada et al. 2000). Light with wavelengths around 400 nm is utilized for DNA repair by CsPHR. The short-wavelength UVB (290–300 nm) causes CPDs in the foliar cells, while the long-wavelength UVB around 310 nm induces the *CsPHR* expression

Three DNA motifs with ACGT core sequences, named as the ACGT elements, were found within a 95-bp-long promoter segment at −202 to −296 bp relative to the translation start (Fig. 4a). Similar ACGT elements are involved in the light-driven transcriptional activation of the parsley chalcone synthase gene (*PcCHS*) and the tomato gene encoding the small subunit of ribulose bisphosphate carboxylase (*rbcS-3A*) (Giuliano et al. 1988; Schulze-Lefert et al. 1989; Block et al. 1990).

In order to test whether these ACGT elements were indeed required for UVB-responsive gene expression, promoter–deletion experiments were conducted using transgenic *Arabidopsis*. The truncated *CsPHR* promoters (1132, 300, and 201 bp) were fused to the luciferase reporter gene (*LUC*) and transfected into *Arabidopsis* (Fig. 4b). Expression of *LUC* under the control of the truncated promoters was examined in UV-treated and control plants kept in dark. The *LUC* expression under the control of the 1,132-bp promoter peaked at 1.5 h after the onset of the light period and decreased thereafter (data not shown). The *LUC* expression was increased significantly by UV treatment when the reporter was fused to the 1,132- or the 300-bp promoter fragment, and was not when using the 201-bp promoter fragment (Fig. 4c). Furthermore, the full-length promoter with the 95-bp region deleted (Δ95) did not promote the *GUS* expression under UV (Fig. 4b, d). These results indicated that the 95-bp-long promoter segment containing three ACGT elements was essential for the gene expression in response to UVB.

The 95-bp region was located at −201 to −296 relative to the translation start; accordingly, all promoter derivatives presented in this paper contained at least the 201-bp proximal promoter region (Fig. 4b). Our preliminary experiments have indicated the transcriptional start is approximately at −99 bp relative to the translation start (data not shown). Therefore, the 201-bp proximal region most likely contained the site for the binding of RNA synthesis machinery. Thus, the 95-bp region most likely interacted with transcription factors associated with the UVB signal.

Discussion

In this paper, we report that the expression of CPD photolyase encoded by the *PHR* gene can be transcriptionally activated by long-wavelength UVB in cucumber and *Arabidopsis*. The action spectrum for the transcriptional activation exhibited a peak at 310 nm, suggesting that the unidentified UVB photoreceptor could be involved in the signaling process leading to the activation. We also

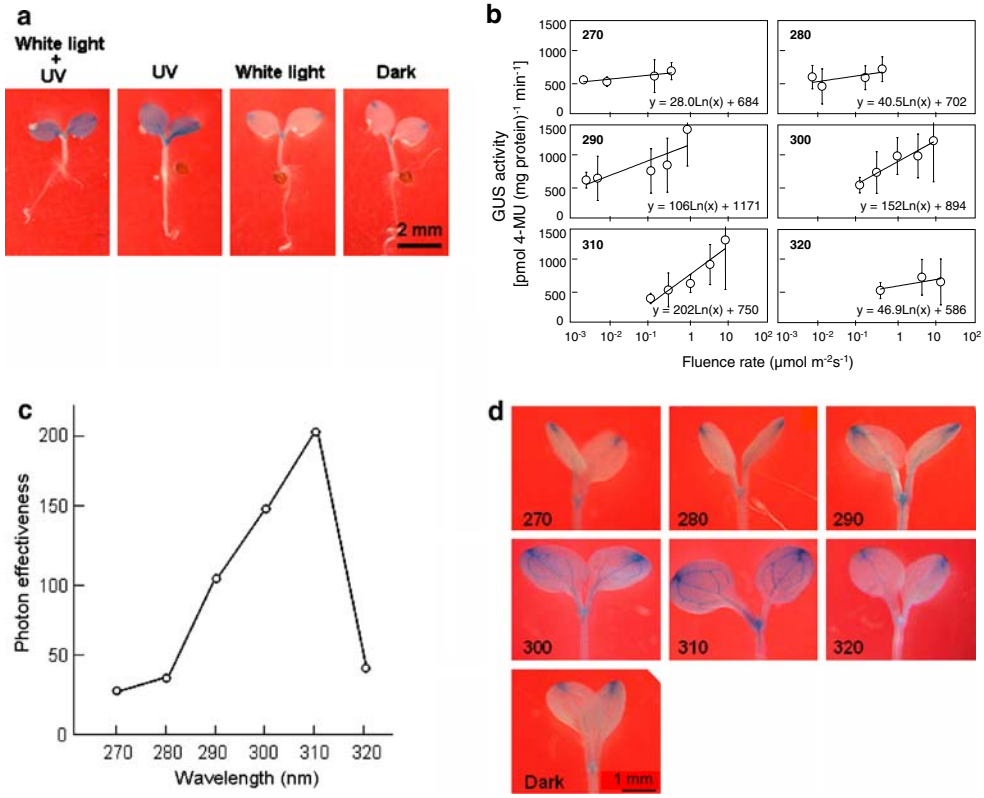

Fig. 3 Wavelength-dependence of *CsPHR* promoter activation in transgenic *Arabidopsis* plants. **a** GUS expression under control of the 2.5-kbp *CsPHR* promoter in transgenic *Arabidopsis* under four different light conditions (white light + UV, only UV, only white light, and dark). Plants were germinated and grown on agar plates. Four-day-old seedlings were subjected to the 6-h light treatments. GUS expression was induced by UV treatment (0.15 Wm^{-2}). **b** Fluence–response plots for GUS expression under the control of *CsPHR* promoter. Transgenic *Arabidopsis* was irradiated with monochromatic light for 3 h, starting at beginning of the light period. Wavelength of monochromatic light is indicated at *upper left corner*. GUS activity was semilogarithmically regressed against the fluence rate. Functional equation of the regression line is presented at bottom of each panel. *Each bar* indicates ± SD for 5 or 6 plants. **c** Action spectrum for *CsPHR* promoter activation in transgenic *Arabidopsis*. Regression coefficient for each wavelength was calculated from the fluence–response plot (**b**) and plotted against wavelength as photon effectiveness. **d** GUS-stained transgenic *Arabidopsis* seedlings irradiated with monochromatic light. Plants were irradiated with monochromatic light (270, 280, 290, 300, 310, and 320 nm) at 1.9 μmol m^{-2} s^{-1} for 3 h and GUS expression was visualized in situ by histochemical staining. The expression peaked when seedlings were treated with 310-nm light

revealed putative *cis*-acting regulatory elements associated with the activation in response to long-wavelength UVB.

Involvement of unidentified photoreceptor(s) with maximal sensitivity in the long-wavelength UVB waveband

No UVB photoreceptors have been identified to date in plants; however, they have been implicated in signaling processes associated with defense against UVB such as flavonoid accumulation and photomorphogenesis such as cotyledon opening and inhibition of hypocotyl elongation (Yatsuhashi et al. 1982; Beggs et al. 1986; Ballare et al. 1995a; Boccalandro et al. 2001). In mammalian cells, experiments using oligonucleotides mimicking DNA damage has clearly shown that signals triggered by the formation of DNA lesions lead to melanogenesis and other photoprotective responses to UVB (Eller et al. 1996; 1997). UVB-induced DNA damages have also been implicated as the trigger of UVB signals leading to some of the plant responses to UVB. For example, the action spectrum for the inhibition of root elongation in cress resembled the UV absorption spectrum of DNA (Steinmetz and Wellmann 1986). Furthermore, diminished magnitudes of the UVB effects under accelerated photoreactivation have suggested that DNA damages might be associated with the inhibition of hypocotyl elongation in cucumber (Shinkle et al. 2005). On the other hand, for the enhancement effects of UVB on phytochrome-mediated cotyledon opening, experiments using DNA-repair mutants have indicated that DNA lesions are not involved and, thus, could not constitute the primary event leading to UVB signaling (Boccalandro et al. 2001). DNA damages also seemed to be unrelated to the induction of CPD photolyase expression by long-wavelength UVB because the action spectrum for CPD photolyase expression significantly differed from that for CPD formation in cucumber leaves (Fig. 2c).

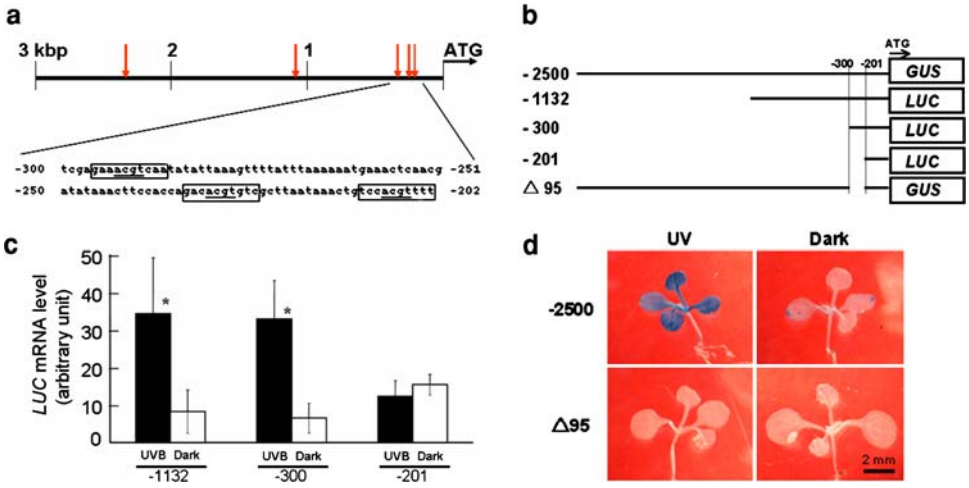

Fig. 4 Reporter assays using transgenic *Arabidopsis* harboring the *CsPHR* promoter and its derivatives. **a** DNA motifs with ACGT cores on the *CsPHR* promoter. Five DNA motifs with ACGT cores (*red arrows*) were found in the 5′-flanking region of *CsPHR*. Three of them were located in the proximity of the translation start. Nucleotide sequence of the *CsPHR* promoter at −202 to −300 bp relative to the translation start is shown. DNA motifs with ACGT cores (*underlined*) are boxed. **b** Schematic representation of different promoter deletions. Full-length promoter (2,500-bp-long), truncated promoters (−1132, −300, −201), and full-length promoter with the 95-bp region deleted (Δ 95) were placed upstream of reporter genes (*GUS* or *LUC*). **c** *LUC* expression in transgenic plants harboring the 1132-, 300-, and 201-bp promoters. Plants were irradiated with polychromatic UV (0.15 W m^{-2}) for 3 h, or placed in the dark as controls. No white light was supplied during the UV treatment. *LUC* mRNA levels are shown. *Bar* indicates ± SD for 4 plants. *Asterisks* indicate significant differences at $P < 0.1$ determined by student *t* test. **d** UVB-responsive expression of GUS under control of the −2,500 promoter and the Δ 95 promoter. GUS expression was visualized via histochemical staining after 6-h UV treatment

The 310-nm peaks of our action spectra indicated that the photolyase expression was controlled mainly by photoreceptor(s) with maximal sensitivity in the long-wavelength UVB waveband (Figs. 2b, 3c). A series of monochromatic action spectra exhibiting peaks in the UVB waveband have also strongly implied the existence of UVB photoreceptors in plants. Along with the action spectra obtained in the present research, related action spectra were listed in Table 1. All these action spectra may be indicative of a UVB-specific receptor system (Ballare et al. 1995a). While our action spectra exhibited peaks in the long-wavelength UVB waveband ($\lambda > 300$ nm), many other action spectra exhibited peaks at shorter wavelengths (Yatsuhashi et al. 1982; Steinmetz and Wellmann 1986; Baskin and Iino 1987; Takeda et al. 1997; Eisinger et al. 2000; Gerhardt et al. 2005). The differences may reflect variations in pigment content in the epidermis of the experimental organisms. Alternatively, they may reflect the involvement of different photoreceptors. In addition, one peak in an action spectrum may be a composite of two or more adjacent peaks. Recent studies have suggested that different photosensory mechanisms are involved in the plant response to short-wavelength UVB and long-wavelength UVB (Shinkle et al. 2004; Ulm et al. 2004). Revealing the molecular identities of the putative UVB photoreceptors will lead to definite answers to these speculations.

Table 1 Action spectra for plant responses to UVB

Type of response	Plant material	Peak wavelength (nm)	Reference
Inhibition of hypocotyl growth	Cress	260–280	Steinmetz and Wellmann (1986)
Phototropism	Alfalfa	280	Baskin and Iino (1987)
PAL promoter activation	Carrot (cell culture)	280	Takeda et al. (1997)
Stomatal opening	Broad bean	284	Eisinger et al. (2000)
Cotyledon curling	*Arabidopsis*	285	Gerhardt et al. (2005)
Anthocyanin formation	Broom sorghum	290	Yatsuhashi et al. (1982)
CsPHR transcription	Cucumber	310	This paper
CsPHR promoter activation	Transgenic *Arabidopsis*	310	This paper

Monochromatic action spectra previously obtained for plant responses to UVB are shown along with the action spectra obtained in this study. Here are listed studies in which action spectra were obtained via monochromatic irradiation at multiple photon–fluence rates. They are listed in the order of increasing peak wavelengths. The action spectrum for the *CsPHR* expression exhibited a peak in the longer waveband of UVB compared to the others

UVB signaling pathways controlling the *CsPHR* transcription

In the present study, the 95-bp-long promoter segment containing three ACGT element was shown to be essential for the UVB-driven transcriptional activation (Fig. 4). Four lines of evidence further support the notion that the ACGT element likely acts as the UVB-responsive *cis* element. First, the ACGT element is known to be involved in the transcriptional activation of *PcCHS*, whose expression is induced by UV (Schulze-Lefert et al. 1989; Block et al. 1990). Second, the ACGT element is conserved in *CsPHR* and *AtPHR1* (*Arabidopsis* CPD photolyase gene). Third, the ACGT element on the *AtPHR1* promoter has been shown to bind to nuclear proteins (Sakamoto et al. 1998). Fourth, all plant bZIP transcription factors reported to date are thought to gather at ACGT elements, and several plant bZIP transcription factors have been reported to be expressed in response to UVB (Feldbrugge et al. 1994, 1997; Foster et al. 1994; Jin et al. 2000; Ulm et al. 2004). Furthermore, it has been demonstrated that a bZIP transcription factor HY5 is involved in the transcriptional activation of *AtPHR1* by UVB (Brown et al. 2005).

Unfortunately, both the UVB photosensory mechanism and the UVB signaling cascades in plants are poorly understood. To date, it is not clear what signaling pathways are associated with the control of the *CsPHR* expression. It has been reported that active oxygen species, calcium ions, nitric oxide, UV-B LIGHT INSENSITIVE 3 (ULI3), UV RESISTANCE LOCUS 8 (UVR8), and certain bZIP transcription factors are involved in UVB signaling (Feldbrugge et al. 1994, 1997; Green and Fluhr 1995; Long and Jenkins 1998; Jin et al. 2000; Suesslin and Frohnmeyer 2003; Zhang et al. 2003; Ulm et al. 2004; Brown et al. 2005). Phytohormones such as ethylene may also play a role in UVB signaling (Nara and Takeuchi 2002). However, it is not clear how these signaling molecules interact with each other in a network. Further investigation of the signaling cascades controlling the *CsPHR* expression will significantly advance our understanding of the elusive UVB signaling cascades in plants. The transgenic *Arabidopsis* plants generated in the present study can be used for genetic screens to disclose the UVB signaling pathways in plants.

Physiological implications of induction of photolyase expression by long-wavelength UVB

Our experiments have indicated that, in a light/dark cycle, *CsPHR* is expressed only in the midst of the light period (Fig. 1). As emphasized in the present study, light is required to promote the expression in the morning; however, even under continuous light, the *CsPHR* expression is diminished in the afternoon (Takahashi et al. 2002). Therefore, the diurnal fluctuation of *CsPHR* expression is thought to be brought about by (i) light-dependent induction and (ii) a mechanism that suppresses the continuous expression of *CsPHR*.

Considering the natural solar radiation, our finding on the induction of CPD photolyase expression by the long-wavelength UVB is physiologically meaningful. It is well known that UVB and visible light are not always well correlated in time and space (Caldwell 1971; Caldwell et al. 1989). For example, little UVB reaches the ground in the morning or late in the afternoon when the visible light is abundant. This lack of correlation has often been mentioned as an ecological justification for the evolution of a UVB-specific receptor system (Ballare et al. 1995b). It is interesting that plants increase the CPD photolyase activity in response to long-wavelength UVB (300–315 nm) rather than the DNA-damaging short-wavelength UVB ($\lambda < 300$ nm). Long-wavelength UVB is less damaging than short-wavelength UVB and it is more abundant in the natural environment. Since long-wavelength UVB reaches the earth's surface prior to short-wavelength UVB in the diurnal cycle, the signaling cascades for long-wavelength UVB would allow plants to be prepared for the upcoming challenge of short-wavelength UVB by timely expression of CPD photolyase. Therefore, our results have suggested to us that transcriptional activation of the CPD photolyase gene by long-wavelength UVB is an important adaptation strategy for plants to grow under UV-containing sunlight.

Acknowledgments We are grateful to the members of the Large Spectrograph Laboratory at National Institute for Basic Biology (Mr. S. Higashi, Ms. C. Ichikawa and Mr. T. Nakamura), our colleagues at National Institute for Environmental Studies (Mr. and Mrs. Matsumoto, Ms. M. Nakajima, Ms. H. Watanabe, Ms. Y. Oshima, Mr. H. Takahashi, and Mr. T. Teramoto), Dr. Y. Takahashi at the University of Tokyo, and Dr. B. Liu at University of California, Davis, for their generous technical assistance and discussions.

References

Ahmad M, Jarillo JA, Klimczak LJ, Landry LG, Peng T, Last RL, Cashmore AR (1997) An enzyme similar to animal type II photolyases mediates photoreactivation in *Arabidopsis*. Plant Cell 9:199–207

Ballare CL, Barnes PW, Flint SD (1995a) Inhibition of hypocotyl elongation by ultraviolet-B radiation in de-etiolating tomato seedlings. I. The photoreceptor. Physiol Plant 93:584–592

Ballare CL, Barnes PW, Flint SD, Price S (1995b) Inhibition of hypocotyl elongation by ultraviolet-B radiation in de-etiolating tomato seedlings. II. Time-course, comparison with flavonoid responses and adaptive significance. Physiol Plant 93:593–601

Baskin TI, Iino M (1987) An action spectrum in the blue and ultraviolet for phototropism in alfalfa. Photochem Photobiol 46:127–136

Beggs CJ, Wellmann E, Grisebach H (1986) Photocontrol of flavonoid biosynthesis. In: Kendrick RE, Kronengerg GHM (eds) Photomorphogenesis in plants. Kluwer Academic Pub, Norwell, pp 478–499

Block A, Dangl JL, Hahlbrock K, Schulze-Lefert P (1990) Functional borders, genetic fine structure, and distance requirements of *cis* elements mediating light responsiveness of the parsley chalcone synthase promoter. Proc Natl Acad Sci USA 87:5387–5391

Boccalandro HE, Mazza CA, Mazzella MA, Casal JJ, Ballaré CL (2001) Ultraviolet B radiation enhances a phytochrome-B-mediated photomorphogenic response in *Arabidopsis*. Plant Physiol 126:780–788

Bornman JF, Reuber S, Cen Y-P, Weissenböck G (1997) Ultraviolet radiation as a stress factor and protective pigments. In: Lumsden P (ed) Plants and UV-B. Cambridge Univ. Press, Cambridge, pp 157–168

Britt A (1996) DNA damage and repair in plants. Annu Rev Plant Physiol Plant Mol Biol 47:75–100

Britt A, Fiscus EL (2003) Growth responses of *Arabidopsis* DNA repair mutants to solar irradiation. Physiol Plant 118:183–192

Brown BA, Cloix C, Jiang GH, Kaiserli E, Herzyk P, Kliebenstein DJ, Jenkins GI (2005) A UV-B-specific signaling component orchestrates plant UV protection. Proc Natl Acad Sci USA 102:18225–18230

Buchholz G, Ehmann B, Wellmann E (1995) Ultraviolet light inhibition of phytochrome-induced flavonoid biosynthesis and DNA photolyase formation in mustard cotyledon (*Sinapis alba* L.). Plant Physiol 108:227–234

Caldwell MM (1971) Solar UV irradiation and the growth and development of higher plants. In: Giese AC (ed) Photophysiology, vol 6. Academic Press, New York, pp 133–177

Caldwell MM, Robberecht R, Flint SD (1983) Internal filters: prospects for UV acclimation in higher plants. Physiol Plant 58:445–450

Caldwell MM, Camp LB, Warner CW, Flint SD (1986) Action spectra and their key role in assessing biological consequences of solar UV-B radiation change. In: Worrest RC, Caldwell MM (eds) Stratospheric ozone reduction, solar ultraviolet radiation and plant life. Springer-Verlag, Berlin, pp 87–111

Caldwell MM, Teramura AH, Tevini M (1989) The changing solar ultraviolet climate and the ecological consequences for higher plants. Trends Ecol Evol 4:363–367

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735–743

Eisinger W, Swartz TE, Bogomolni RA, Taiz L (2000) The ultraviolet action spectrum for stomatal opening in broad bean. Plant Physiol 122:99–106

Eller MS, Ostrom K, Gilchrest BA (1996) DNA damage enhances melanogenesis. Proc Natl Acad Sci USA 93:1087–1092

Eller MS, Maeda T, Magnoni C, Atwal D, Gilchrest BA (1997) Enhancement of DNA repair in human skin cells by thymidine dinucleotides: evidence for a p53-mediated mammalian SOS response. Proc Natl Acad Sci USA 94:12627–12632

Feldbrugge M, Sprenger M, Dinkelbach M, Yazaki K, Harter K, Weisshaar B (1994) Functional analysis of a light-responsive plant bZIP transcriptional regulator. Plant Cell 6:1607–1621

Feldbrugge M, Sprenger M, Hahlbrock K, Weisshaar B (1997) PcMYB1, a novel plant protein containing a DNA-binding domain with one MYB repeat, interacts in vivo with a light-regulatory promoter unit. Plant J 11:1079–1093

Foster R, Izawa T, Chua NH (1994) Plant bZIP proteins gather at ACGT elements. FASEB J 8:192–200

Gerhardt KE, Wilson MI, Greenberg BM (2005) Ultraviolet wavelength dependence of photomorphological and photosynthetic responses in *Brassica napus* and *Arabidopsis thaliana*. Photochem Photobiol 81:1061–1068

Giordano CV, Mori T, Sala OE, Scopel AL, Caldwell MM, Ballare CL (2003) Functional acclimation to solar UV-B in *Gunnera megellanica*, a native plant species of southernmost Patagonia. Plant Cell Environ 26:2027–2036

Giordano CV, Galatro A, Puntarulo S, Ballare CL (2004) The inhibitory effects of UV-B radiation (280–315 nm) on *Gunnera magellanica* growth correlate with increased DNA damage but not with oxidative damage to lipids. Plant Cell Environ 27:1415–1423

Giuliano G, Pichersky E, Malik VS, Timko MP, Scolnik PA, Cashmore AR (1988) An evolutionarily conserved protein binding sequence upstream of a plant light-regulated gene. Proc Natl Acad Sci USA 85:7089–7093

Green R, Fluhr R (1995) UV-B-induced PR-1 accumulation is mediated by active oxygen species. Plant Cell 7:203–212

Hada M, Iida Y, Takeuchi Y (2000) Action spectra of DNA photolyases for photorepair of cyclobutane pyrimidine dimers in sorghum and cucumber. Plant Cell Physiol 41:644–648

Hidema J, Teranishi M, Iwamatsu Y, Hirouchi T, Ueda T, Sato T, Burr B, Sutherland BM, Yamamoto K, Kumagai T (2005) Spontaneously occurring mutations in the cyclobutane pyrimidine dimer photolyase gene cause different sensitivities to ultraviolet-B in rice. Plant J 43:57–67

Holmes MG (1997) Action spectra for UV-B effects on plants: monochromatic approaches for analyzing plant responses. In: Lumsden PJ (ed) Plants and UV-B: responses to environmental change. Society for Experimental Biology, Seminar Series 64. Cambridge University Press, Cambridge

Ioki M, Nakajima N, Tamaoki M, Takahashi S, Kondo N (2003) Genomic structure of the cucumber CPD photolyase gene. OMICS 7:203–209

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 20:3901–3907

Jin H, Cominelli E, Bailey P, Parr A, Mehrtens F, Jones J, Tonelli C, Weisshaar B, Martin C (2000) Transcriptional repression by AtMYB4 controls production of UV-protecting sunscreens in *Arabidopsis*. EMBO J 19:6150–6161

Kasahara M, Swartz TE, Olney MA, Onodera A, Mochizuki N, Fukuzawa H, Asamizu E, Tabata S, Kanegae H, Takano M, Christie JM, Nagatani A, Briggs WR (2002) Photochemical properties of the flavin mononucleotide-binding domains of the phototropins from *Arabidopsis*, rice, and *Chlamydomonas reinhardtii*. Plant Physiol 129:762–773

Landry LG, Stapleton AE, Lim J, Hoffman P, Hays JB, Walbot V, Last RL (1997) An *Arabidopsis* photolyase mutant is hypersensitive to ultraviolet-B radiation. Proc Natl Acad Sci USA 94:328–332

Langer B, Wellmann E (1990) Phytochrome induction of photoreactivating enzyme in *Phaseolus vulgaris* L. seedlings. Photochem Photobiol 52:861–863

Larcher W (1995) Physiological plant ecology, 3rd edn. Springer-Verlag, Berlin, pp 32–45

Lin C (2000) Plant blue-light receptors. Trends Plant Sci 5:337–342

Lin C, Robertson DE, Ahmad M, Raibekas AA, Jorns MS, Dutton PL, Cashmore AR (1995) Association of flavin adenine dinucleotide with the *Arabidopsis* blue light receptor CRY1. Science 269:968–970

Long JC, Jenkins GI (1998) Involvement of plasma membrane redox activity and calcium homeostasis in the UV-B and UVA/blue light induction of gene expression in *Arabidopsis*. Plant Cell 10:2077–2086

Matsunaga T, Hieda K, Nikaido O (1991) Wavelength dependent formation of thymine dimers and (6–4) photoproducts in DNA by monochromatic ultraviolet light ranging from 150 to 365 nm. Photochem Photobiol 54:403–410

Nara A, Takeuchi Y (2002) Ethylene evolution from tobacco leaves irradiated with UV-B. J Plant Res 115:247–253

Pang Q, Hays JB (1991) UV-B inducible and temperature-sensitive photoreactivation of cyclobutane pyrimidine dimers in *Arabidopsis thaliana*. Plant Physiol 95:536–543

Quaite FE, Sutherland BM, Sutherland JC (1992) Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. Nature 358:576–578

Ries G, Heller W, Puchta H, Sandermann H, Seidlitz HK, Hohn B (2000) Elevated UV-B radiation reduces genome stability in plants. Nature 406:98–101

Rousseaux MC, Ballaré CL, Giordano CV, Scopel AL, Zima AM, Szwarcberg-Bracchitta M, Searles PS, Caldwell MM, Diaz SB (1999) Ozone depletion and UVB radiation: impact on plant DNA damage in southern South America. Proc Natl Acad Sci USA 96:15310–15315

Sakamoto A, Tanaka A, Watanabe H, Tano S (1998) Molecular cloning of *Arabidopsis* photolyase gene (*PHR1*) and characterization of its promoter region. DNA Seq 9:335–340

Sancer A (1994) Structure and function of DNA photolyase. Biochemistry 33:2–9

Schulze-Lefert P, Cecker-Andre M, Shulze W, Hahlbrock K, Dangl JL (1989) Functional architecture of the light-responsive chalcone synthase promoter from parsley. Plant Cell 1:707–714

Shinkle JR, Atkins AK, Humphrey EE, Rodgers CW, Wheeler SL, Barnes PW (2004) Growth and morphological responses to different UV wavebands in cucumber (*Cucumis sativum*) and other dicotyledonous seedlings. Physiol Plant 120:240–248

Shinkle JR, Derickson DL, Barnes PW (2005) Comparative photobiology of growth responses to two UV-B wavebands and UV-C in dim-red-light- and white-light-grown cucumber (*Cucumis sativus*) seedlings: physiological evidence for photoreactivation. Photochem Photobiol 81:1069–1074

Steinmetz V, Wellmann E (1986) The role of solar UV-B in growth regulation of cress (*Lepidium sativum* L.) seedlings. Photochem Photobiol 43:189–193

Suesslin C, Frohnmeyer H (2003) An *Arabidopsis* mutant defective in UV-B light-mediated responses. Plant J 33:591–601

Takahashi S, Nakajima N, Saji H, Kondo N (2002) Diurnal change of cucumber CPD photolyase gene (*CsPHR*) expression and its physiological role in growth under UV-B irradiation. Plant Cell Physiol 43:342–349

Takayanagi S, Trunk JG, Sutherland JC, Sutherland BM (1994) Alfalfa seedlings grown outdoors are more resistant to UV-induced DNA damage than plants grown in a UV-free environmental chamber. Photochem Photobiol 60:363–367

Takeda J, Ozeki Y, Yoshida K (1997) Action spectrum for induction of promoter activity of phenylalanine ammonia-lyase gene by UV in carrot suspension cells. Photochem Photobiol 66:464–470

Taylor RM, Nikaido O, Jordan BR, Rosamond J, Bray CM, Tobin AK (1996) Ultraviolet-b-induced DNA lesions and their removal in wheat (*Triticum aestvum* L.) leaves. Plant Cell Environ 19:171–181

Teramura AH (1983) Effects of ultraviolet-B radiation on growth and yield of crop plants. Physiol Plant 58:415–427

Ulm R, Baumann A, Oravecz A, Mate Z, Adam E, Oakeley EJ, Schafer E, Nagy F (2004) Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of *Arabidopsis*. Proc Natl Acad Sci USA 101:1397–1402

Watanabe M, Furuya M, Miyoshi Y, Inoue Y, Iwahashi I, Matsumoto K (1982) Design and performance of the Okazaki Large Spectrograph for photobiological research. Photochem Photobiol 36:491–498

Waterworth WM, Jiang Q, West CE, Nikaido M, Bray CM (2002) Characterization of *Arabidopsis* photolyase enzymes and analysis of their role in protection from ultraviolet-B radiation. J Exp Bot 53:1005–1015

Yatsuhashi H, Hashimoto T, Shimizu S (1982) Ultraviolet action spectrum for anthocyanin formation in broom sorghum first internodes. Plant Physiol 70:735–741

Zhang M, An L, Feng H, Chen T, Chen K, Liu Y, Tang H, Chang J, Wang X (2003) The cascade mechanisms of nitric oxide as a second messenger of ultraviolet B in inhibiting mesocotyl elongations. Photochem Photobiol 77:219–225