A UV-B-specific signaling component orchestrates plant UV protection

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Edited by Alexander N. Glazer, University of California System, Oakland, CA, and approved October 20, 2005 (received for review August 22, 2005)

UV-B radiation in sunlight has diverse effects on humans, animals, plants, and microorganisms. UV-B can cause damage to molecules and cells, and consequently organisms need to protect against and repair UV damage to survive in sunlight. In plants, low nondamaging levels of UV-B stimulate transcription of genes involved in UV-protective responses. However, remarkably little is known about the underlying mechanisms of UV-B perception and signal transduction. Here we report that *Arabidopsis* UV RESISTANCE LOCUS 8 (UVR8) is a UV-B-specific signaling component that orchestrates expression of a range of genes with vital UV-protective functions. Moreover, we show that UVR8 regulates expression of the transcription factor HY5 specifically when the plant is exposed to UV-B. We demonstrate that HY5 is a key effector of the UVR8 pathway, and that it is required for survival under UV-B radiation. UVR8 has sequence similarity to the eukaryotic guanine nucleotide exchange factor RCC1, but we found that it has little exchange activity. However, UVR8, like RCC1, is located principally in the nucleus and associates with chromatin via histones. Chromatin immunoprecipitation showed that UVR8 associates with chromatin in the *HY5* promoter region, providing a mechanistic basis for its involvement in regulating transcription. We conclude that UVR8 defines a UV-B-specific signaling pathway in plants that orchestrates the protective gene expression responses to UV-B required for plant survival in sunlight.

photomorphogenesis | ultraviolet-B radiation | UV RESISTANCE LOCUS 8

UV-B radiation (280–320 nm) is an integral component of sunlight. UV-B can cause damage to macromolecules, including DNA, and generate reactive oxygen species. Because UV-B affects the growth, development, reproduction, and survival of many organisms, there is concern that any further increases in ambient levels of UV-B, resulting from stratospheric ozone depletion may have a significant impact on natural and agricultural ecosystems (1–4). Hence, it is important to understand how plants and other organisms protect themselves against the potentially damaging effects of UV-B.

Exposure to UV-B is obligatory for higher plants because of the need to maximize light capture for photosynthesis. The effects of UV-B on diverse species of plants have been reported in the literature, and it is evident that different responses are observed at different UV-B fluence rates (5, 6). Exposure to high amounts of UV-B causes tissue necrosis and induces the expression of stress-associated genes in part through activation of pathogen-defense and wound-signaling pathways (5–7). At ambient UV-B levels, crosstalk between wound and UV-B signaling pathways modifies plant–insect interactions (8). Importantly, exposure to low nondamaging levels of UV-B has numerous regulatory effects on plant morphology, development, physiology, and biochemical composition (1, 5, 6, 9). Low fluence rates of UV-B promote the expression of a range of genes involved in UV-B protection (5, 6, 10, 11). These include genes concerned with the production of flavonoids and other phenolic compounds that accumulate in the epidermal layers and provide a UV-absorbing sun screen (12, 13). Other UV-B-induced genes are involved, for instance in ameliorating oxidative stress and repairing UV damage. Mutants lacking UV-protective components, such as the flavonoids and sinapic acid esters, are highly sensitive to ambient levels of UV-B (13, 14).

Although plant responses to low ambient fluence rates of UV-B are key to survival, the underlying mechanisms of UV-B perception and signal transduction are very poorly understood, despite decades of research. These responses are not mediated by the known plant photoreceptors and do not involve DNA damage signaling pathways (5, 6). It has often been speculated that UV-B may be perceived by a novel class of photoreceptor, but no such molecule has ever been identified. Moreover, although pharmacological, cell physiological, and genetic approaches have provided some insights into UV-B signal transduction processes (15–17), no UV-B-specific signaling pathway has been defined. Thus, understanding of the mechanisms of plant UV-B responses lags well behind knowledge of light responses mediated by the phytochrome, cryptochrome, and phototropin photoreceptors.

Here we report that the *Arabidopsis* protein UV RESISTANCE LOCUS 8 (UVR8) is a UV-B-specific signaling component that regulates expression of a range of genes essential for UV-B protection. In addition, we show that UVR8 controls expression of the transcription factor HY5, and that HY5 is a key effector of the UVR8 signaling pathway. Further, we show that the association of UVR8 with chromatin provides a basis for its action in regulating transcription.

Materials and Methods

Plant Material and Treatments. For transcript measurements and transcriptome analyses, wild-type *Arabidopsis* L *er*, *uvr8-1* (18), *uvr8-2* (this study; backcrossed twice to wild type), and *hy5-1* (19) plants were grown in compost for 3 weeks in 25 μmol $m^{-2}{\cdot}s^{-1}$ continuous white light (warm white fluorescent tubes) at 21°C and exposed to 3 μmol $m^{-2}{\cdot}s^{-1}$ UV-B for 4 h or 100 μmol $m^{-2}{\cdot}s^{-1}$ UV-A for 6 h using light sources described previously (20). For low-temperature treatment, plants were transferred to 7°C for 24 h (20). Seedlings were grown in darkness for 4 days on 0.8% agar plates containing 1× Murashige and Skoog salts, MS vitamins, 2% sucrose, and 0.05% Mes (pH 5.7) and illuminated with 70 μmol $m^{-2}{\cdot}s^{-1}$ far-red light (20) for 6 h. The effect of sucrose was examined in seedlings grown in darkness with or without 2%

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Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: FDR, false discovery rate; CHS, chalcone synthase; GEF, guanine nucleotide exchange factor.

Data deposition: The Affymetrix microarray expression data reported in this paper have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus (GEO) database (accession no. GSE3533).

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sucrose, as described (20). For GFP-UVR8 localization and chromatin association experiments, plants were grown in 25 μmol $m^{-2}{\cdot}s^{-1}$ or 100 μmol $m^{-2}{\cdot}s^{-1}$ continuous white light before UV-B illumination. For UV-B sensitivity assays, plants were grown in 120 μmol $m^{-2}{\cdot}s^{-1}$ white light for 12 days, then exposed to 5 μmol $m^{-2}{\cdot}s^{-1}$ UV-B with supplementary 40 μmol $m^{-2}{\cdot}s^{-1}$ white light for 24 h. Plants were returned to 120 μmol $m^{-2}{\cdot}s^{-1}$ white light for 5 days to determine survival.

Transcript Measurements. RNA was isolated from leaf tissue by using the Purescript kit (Flowgen, Nottingham, U.K.). Chalcone synthase (*CHS*), *HY5*, and *ACTIN2* transcripts were assayed by RT-PCR with gene-specific primers by using cycle numbers within the linear range of amplification (CHS-L 5′-ATCTTTGAGATG-GTGTCTGC-3′, CHS-R 5′-CGTCTAGTATGAAGAGAACG-3′; HY5-L 5′-GCTGCAAGCTCTTTACCATC-3′, HY5-R 5′-AGCATCTGGTTCTCGTTCTG-3′; *ACTIN2* primers as in ref. 21).

Transcriptome Analysis. Three independent RNA samples were analyzed for each treatment to facilitate statistical analysis (22). RNA quality was checked by using an Agilent (Austin, TX) RNA BioAnalyzer 2100. RNA was reverse-transcribed, double-stranded cDNA *in vitro* transcribed, and biotinylated cRNA hybridized to Affymetrix *Arabidopsis* ATH1 GeneChips as recommended by Affymetrix. Chips were washed and stained by using Affymetrix protocols on the Fluidics Station 400 and scanned on the Gene Array Scanner 2500. Data were analyzed by using FUNALYSE, Version 2.0, an automated pipeline in Sir Henry Wellcome Functional Genomics Facility, University of Glasgow. This analysis consisted of the Robust Multichip Average (23) normalization followed by identification of differentially expressed genes by using the Rank Products method (22).

Genes regulated by UVR8 were identified by the following procedure. First, the comparison of transcripts in wild type exposed to UV-B versus *uvr8-1* exposed to UV-B, cut at 5% false discovery rate (FDR), identified genes with reduced expression in the mutant. Second, each of the genes listed was verified as UV-B induced in wild type by comparison of wild type exposed to UV-B versus wild type in low fluence rate white light, cut at 5% FDR, but at the same time not identified as elevated in expression in *uvr8-1* exposed to UV-B versus *uvr8-1* in low fluence rate white light at 5% FDR. Genes regulated by HY5 were identified through comparison of wild type exposed to UV-B and *hy5* exposed to UV-B, with FDR $<5\%$.

Guanine Nucleotide Exchange Factor (GEF) Activity Assays. RCC1, UVR8, and human Ran were expressed in *Escherichia coli* as fusions with GST. The Ran clone was provided by Murray Stewart (Medical Research Council Laboratory for Molecular Biology, Cambridge, U.K.). Assays of guanine nucleotide exchange activity were performed essentially as described (24) by using [^{3}H]GDP to load 30 pmol GST-Ran and subsequent incubation with 0.5 nM recombinant RCC1 or UVR8 for 3 min. The exchange activity was calculated as $\ln(C_t/C_0)$, where C_0 and C_t are radioactive counts at the start and end of the reaction, respectively (24). Assays were repeated four times.

Histone Interaction. Purified GST-UVR8 and GST (5 μg) were applied to 0.3 ml of calf thymus histone-agarose columns (Sigma) equilibrated with buffer (20 mM Tris·HCl, pH 7.5/2 mM EDTA/0.1% Nonidet P-40/10% glycerol/0.05 M NaCl), and material that flowed through was recovered. After 5-min incubation, the column was washed with five column volumes of equilibration buffer. Elutions were then carried out with equilibration buffer containing NaCl at final concentrations of 0.1, 0.3, or 1.0 M. Proteins were fractionated by SDS/PAGE and a Western blot challenged with anti-GST antibody (Novagen). After the secondary antibody (anti-mouse IgG conjugated to horseradish peroxidase, Promega), bands were visualized by using ECL+ reagent (Amersham Pharmacia).

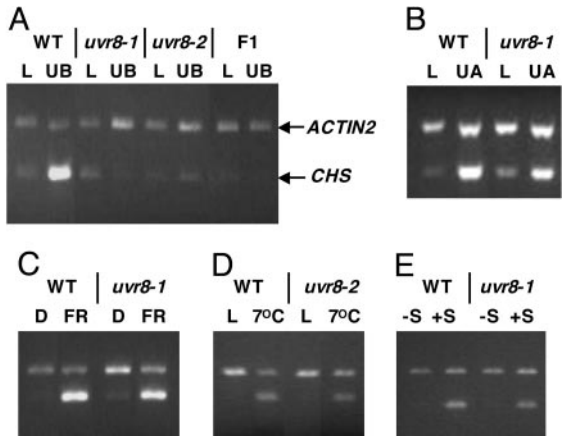

Fig. 1. UVR8 acts specifically in the UV-B regulation of *CHS* expression. Shown are RT-PCR measurements of *CHS* and control *ACTIN2* transcripts (lower and upper bands, respectively, *A–E*). (*A*) Wild-type, *uvr8-1*, *uvr8-2*, and one of the F_1 progeny of *uvr8-1* and *uvr8-2* grown for 3 weeks in low fluence rate (25 μmol $m^{-2}{\cdot}s^{-1}$) white light (L) and illuminated with ambient (3 μmol $m^{-2}{\cdot}s^{-1}$) UV-B for 4 h (UB). (*B*) Plants grown as in *A* illuminated with UV-A (UA) for 6 h. (*C*) Four-day-old dark-grown (D) seedlings illuminated with far-red (FR) light for 6 h. (*D*) Plants grown as in *A* transferred to 7°C for 24 h. (*E*) Seedlings grown in darkness for 4 days with (+S) or without (−S) 2% sucrose.

GFP-UVR8 Localization and Chromatin Association. UVR8 cDNA was inserted into the binary vector pEZR(K)-LC, a derivative of pEGAD (25) (provided by Gert-Jan de Boer, University of Amsterdam, Amsterdam), to yield the cauliflower mosaic virus *35S* promoter::GFP-UVR8 fusion. The construct was stably transformed into mutant *uvr8-2* plants, and homozygous lines were selected on agar plates (0.8% agar/0.5× MS salts/50 μg·ml^{-1} kanamycin). The *35S* promoter::GFP line (in L *er*) was provided by Robert Sablowski (John Innes Centre, Norwich, U.K.). Confocal laser-scanning microscopy (Zeiss LSM510) was used to visualize GFP by using an excitation of 488 nm (Argon) and an emission range of 505–530 nm. Chromatin was isolated and the chromatin immunoprecipitation assay carried out as described (26) by using an anti-GFP antibody (A11122, Molecular Probes). Before antibody treatment, the samples were precleared with proteinA Dynabeads (Dynal Biotech, Great Neck, NY, 100.02). The immunoprecipitated DNA was used in PCR reactions of 40 cycles to amplify fragments from the *ACTIN2* gene (primers as above) and the *HY5* gene (sequences from −331 to +23, according to the TAIR annotation; forward primer 5′-TTGGTTTATGGCGGCTATAAA-3′; reverse primer 5′-TGGCTACCGCCGTCAGAT-3′).

Results and Discussion

Isolation of UV-B Response Mutants. We opted to use a genetic approach in *Arabidopsis* to identify components involved specifically in UV-B perception or signaling. We decided to screen for mutants defective in expression of the gene encoding CHS, the first enzyme in the flavonoid biosynthesis pathway. *CHS* transcription is induced by low doses of UV-B via a signaling pathway distinct from the high-dose stress-response pathways (15, 16). We produced a transgenic line expressing luciferase driven by the *Arabidopsis CHS* gene promoter. Plants derived from mutagenized seed were grown in a low fluence rate of white light lacking UV-B that does not promote *CHS* transcription (27) and were then given a UV-B treatment to induce *CHS*::*LUC* expression. Putative mutants that failed to induce luciferase were isolated by using a photon-counting camera. To confirm the phenotype, *CHS* transcripts were assayed in progeny of selected mutants exposed to UV-B (Fig. 1*A*). In

Table 1. UVR8 and HY5 regulate a range of genes concerned with UV protection

Probe-set ID	Gene	Name	wt UV-B versus *uvr8* UV-B			wt UV-B versus *hy5* UV-B		
			RPscore	FDR	FCrma	RPscore	FDR	FCrma
258321_at	At3g22840	Early light-induced protein (ELIP1)	1.7	0	−156.71	40.27	0	−5
245306_at	At4g14690	Early light-induced protein (ELIP2)	2.36	0	−92.41	279.78	1.77	−2.59
252123_at	At3g51240	Flavanone 3-hydroxylase (F3H)	3.26	0	−63.22	46.69	0	−4.71
253496_at	At4g31870	Glutathione peroxidase, putative	4.71	0	−43.67			
250207_at	At5g13930	Chalcone synthase (CHS)	5.5	0	−34.76	5.08	0	−9.96
249063_at	At5g44110	ABC transporter family protein	6.42	0	−28.16	230.02	1.22	−2.86
250533_at	At5g08640	Flavonol synthase 1 (FLS1)	6.85	0	−26.52	2.35	0	−13.32
249769_at	At5g24120	RNA polymerase subunit SigE (sigE)	9.6	0	−20.23			
251020_at	At5g02270	ABC transporter family protein	9.96	0	−20.08	401.58	3.14	−2.45
247463_at	At5g62210	Embryo-specific protein-related	11.13	0	−19.05	1.66	0	−15.7
246966_at	At5g24850	Cryptochrome dash (CRYD)	13.66	0	−15.39	5.23	0	−9.7
245560_at	At4g15480	UDP-glucoronosyl/UDP-glucosyl transferase family protein	15.21	0	−14.56	18.61	0	−6.33
251658_at	At3g57020	Strictosidine synthase family protein	16.73	0.07	−13.45			
252010_at	At3g52740	Expressed protein	21.41	0.07	−11.51	323.82	2.37	−2.66
251827_at	At3g55120	Chalcone isomerase (CHI)	23.11	0.06	−10.84	20.88	0	−6.08
249191_at	At5g42760	*O*-methyltransferase N terminus domain-containing protein	23.81	0.06	−11.05			
251727_at	At3g56290	Expressed protein	24.83	0.06	−10.23			
253039_at	At4g37760	Squalene monooxygenase, putative	25.89	0.05	−10.12			
246468_at	At5g17050	Flavonoid 3-*O*-glucosyl transferase	26.33	0.05	−9.76			
250420_at	At5g11260	bZIP protein HY5 (HY5)	27.24	0.05	−9.93	58.93	0.03	−4.56
248049_at	At5g56090	Cytochrome oxidase assembly family protein	30.44	0.05	−9.05	83.53	0.12	−3.67
250083_at	At5g17220	Glutathione *S*-transferase, AtGST12/TT19	31.93	0.04	−8.64	16.03	0	−6.77
249215_at	At5g42800	Dihydroflavonol 4-reductase (DFR)	32.04	0.04	−8.7	56.27	0.04	−4.52
263122_at	At1g78510	Solanesyl diphosphate synthase (SPS)	33.57	0.04	−8.5			
259537_at	At1g12370	Type II CPD photolyase PHR1 (PHR1)	35.67	0.04	−8.03	184.55	0.75	−3.03
253943_at	At4g27030	Expressed protein	36.84	0.04	−8.09			
260955_at	At1g06000	UDP-glucoronosyl/UDP-glucosyl transferase family protein	37.27	0.03	−8.12	98.76	0.17	−3.98
250794_at	At5g05270	Chalcone-flavanone isomerase family protein	38.01	0.03	−8.25	15.62	0	−6.55
249798_at	At5g23730	Transducin family protein/WD-40 repeat family protein	38.51	0.03	−7.73	332.09	2.46	−2.5
262626_at	At1g06430	FtsH protease, FtsH8	41.46	0.03	−7.46			
252661_at	At3g44450	Expressed protein	42.44	0.03	−7.55			
262705_at	At1g16260	Protein kinase family protein	47.38	0.06	−6.86			
253879_s_at	At4g27570	Glycosyltransferase family protein	54.76	0.05	−6.35	42.2	0	−4.96
248347_at	At5g52250	Transducin family protein/WD-40 repeat family protein	56	0.05	−6.31			
255594_at	At4g01660	ABC1 family protein	56.86	0.05	−6.24			
258349_at	At3g17610	bZIP transcription factor HY5-like protein (HYH)	58.07	0.04	−6.01			
265634_at	At2g25530	AFG1-like ATPase family protein	61.67	0.09	−5.95	185.64	0.74	−2.96
260072_at	At1g73650	Expressed protein	66.05	0.1	−5.56			
264752_at	At1g23010	Multi-copper oxidase type I family protein	69.4	0.1	−5.7	104.77	0.2	−3.65

The genes listed are induced by UV-B in wild type but show much reduced UV-B induction in *uvr8-1* (left columns) and, in many cases, *hy5-1* (right columns). The data-set comparisons used for gene identification are explained in *Materials and Methods*, and the complete data for 72 genes are presented in Table 2, which is published as supporting information on the PNAS web site. The microarray data were analyzed by the Rank Products method (22); RPscore is a measure of differential expression, FDR indicates the expected percentage of false positives, and FCrma is a measure of the fold change in expression. The Probe-set ID is the Affymetrix probe-set identifier.

addition, plants were illuminated with UV-A light, which induces *CHS* transcription via the cryptochrome 1 photoreceptor (27), to identify mutants defective only in their response to UV-B. After screening >50,000 mutagenized plants, four mutants lacking specifically the UV-B induction of *CHS* expression were obtained. Remarkably, genetic analysis revealed that all four mutants were allelic with the *uvr8-1* mutant, isolated previously in a screen for plants showing hypersensitivity to UV-B (18). One such allele, *uvr8-2*, which has a premature stop codon at amino acid 400 in the *UVR8* coding sequence, is shown in Fig. 1*A*. The results of our screen demonstrate that UVR8 is a key component mediating the UV-B induction of *CHS* expression. Furthermore, the lack of other classes of mutants isolated in the screen suggests that the UV-B specific pathway may have relatively few components.

UVR8 Acts in a UV-B-Specific Pathway. Kliebenstein *et al.* (18) reported that *uvr8-1* was defective in the UV-B induction of one gene, *CHS* but did not investigate whether the impairment was specific to UV-B. *CHS* expression is controlled by a network of light signaling pathways involving several photoreceptors in mature leaves and seedlings (27, 28). It was therefore essential to determine whether the loss of *CHS* expression in *uvr8* was caused by a lesion specifically in the UV-B response pathway. We found that *uvr8* lacks only the response to UV-B, because it retains *CHS* induction by both cryptochrome 1 and phytochrome A photoreceptors (Fig. 1 *B* and *C*). Furthermore, the mutant is unaltered in the stimulation of *CHS* expression by several nonlight stimuli, including low temperature and sucrose (Fig. 1 *D* and *E*). These observations indicate that UVR8 acts in a UV-B-specific pathway.

The UVR8 Pathway Orchestrates UV Protection. Although the *uvr8-1* mutant was reported to be UV-sensitive (18), the basis of the phenotype and the role of UVR8 were not defined. To establish the significance of the UVR8 pathway in UV-B responses, we undertook a transcriptome analysis of *uvr8* in comparison to wild type. Plants grown in a low fluence rate of white light lacking UV-B were given a UV-B treatment and expression profiles examined by using whole-genome microarrays. This treatment did not induce the stress-responsive genes expressed at high doses of UV-B. We used the Rank Products method (22) to statistically analyze the data. This method ranks genes according to their expression changes, gives a measure of fold change, and provides statistical confidence levels. The statistical analysis identified 72 UV-B-induced genes regulated by UVR8 when a 5% FDR (a measure of the frequency of false positives) was selected as the cutoff in each gene list used to produce the data (see *Materials and Methods*). Some of the genes regulated by UVR8 are shown in Table 1 and the complete data are presented in Table 2, which is published as supporting information on the PNAS web site. The list represents the minimum number of UVR8-regulated genes. For instance, the number increases to 113 if 10% FDR is used. Moreover, RT-PCR experiments have identified several genes regulated by UVR8 that do not appear in Table 2, probably because of their low level of expression (data not shown).

The genes shown in Tables 1 and 2 include most of the flavonoid biosynthesis genes (12) and several concerned with other secondary metabolic pathways, such as alkaloid biosynthesis, that have been implicated in UV protection (9, 29). Furthermore, UVR8 regulates expression of the type II photolyase PHR1 required for photoreactivating DNA repair in *Arabidopsis*; the *uvr2* mutant lacking this enzyme is highly sensitive to UV-B (30, 31). In addition, UVR8 regulates genes concerned with protection against oxidative stress (e.g., glutathione peroxidases; ref. 32) and photooxidative damage (ELIP proteins; ref. 33) and a number of genes encoding signaling components, transcription factors, transporters, proteases, and several proteins with unknown functions. Thus, UVR8 regulates expression of a range of components with vital functions in protecting plants against UV-B. Significantly, several of the genes regulated by UVR8 encode chloroplast proteins (e.g., ELIP proteins, FtsH proteases, and RNA polymerase SigE subunit). It is well known that some photosynthetic components are particularly susceptible to damage by UV-B (9), and our findings indicate that UVR8 has an important function in maintaining photosynthetic activity. Further observations indicate that the UVR8 signaling pathway is likely to be important for plant survival in the natural environment; wild-type and *uvr8-1* seedlings grew normally in sunlight under Mylar filter that absorbs UV-B, but the mutant plants died when the filter was removed (D.J.K., unpublished results).

UVR8 Regulates HY5 Expression. Among the transcription factor genes regulated by UVR8 is *HY5* (*ELONGATED HYPOCOTYL5*). HY5 is a bZIP transcription factor that plays a key role in the regulation of seedling photomorphogenesis, mediating the action of different photoreceptors (34). In addition, Northern hybridizations have shown that HY5 regulates several UV-B-induced genes (10), although a role for HY5 in UV protection has not been demonstrated. It is well established that HY5 is regulated by targeted proteolysis (34), but regulation via transcript accumulation has also been demonstrated (10, 35). Using RT-PCR, we confirmed that *HY5* transcripts are induced by UV-B in wild-type plants but not in the *uvr8* mutant (Fig. 2*A*). Nevertheless, *uvr8* retains the induction of *HY5* transcripts by cryptochrome and phytochrome photoreceptors (Fig. 2 *A* and *B*). Therefore, UVR8 controls *HY5* transcript accumulation specifically when the plant is exposed to UV-B.

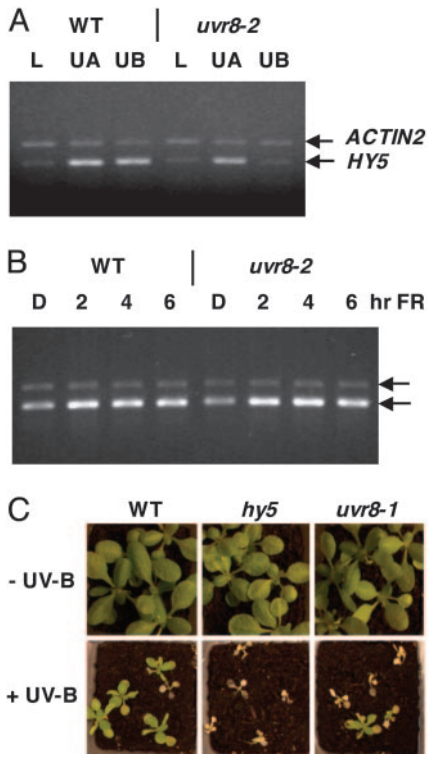

Fig. 2. *HY5* is regulated by UVR8 and required for UV-B protection. (*A*) *HY5* and control *ACTIN2* transcripts measured by RT-PCR in wild-type and *uvr8-2* plants grown for 3 weeks in 25 μmol $m^{-2}{\cdot}s^{-1}$ white light (L) and illuminated with 3 μmol $m^{-2}{\cdot}s^{-1}$ UV-B (UB) for 4 h or UV-A (UA) for 6 h. (*B*) *HY5* and *ACTIN2* transcripts (lower and upper bands, respectively) in 4-day-old dark-grown (D) seedlings illuminated with far-red (FR) light for the times indicated. (*C*) UV-B sensitivity assay. Wild-type, *hy5-1*, and *uvr8-1* plants grown in 120 μmol $m^{-2}{\cdot}s^{-1}$ white light for 12 days and then exposed to above-ambient (5 μmol $m^{-2}{\cdot}s^{-1}$) UV-B with supplementary 40 μmol $m^{-2}{\cdot}s^{-1}$ white light for 24 h. Plants were photographed after return to white light for 5 days.

HY5 Is a Key Effector of the UVR8 Pathway. We used transcriptome analysis to identify genes reduced in expression in response to UV-B in the *hy5* mutant and then compared those genes with those reduced in expression in *uvr8*. The results show that ≈50% of the genes regulated by UVR8 are also regulated by HY5 (Tables 1 and 2). Therefore, HY5 is a key effector of the UVR8 pathway. Consistent with this, we found that the *hy5* mutant is highly sensitive to UV-B, similar to *uvr8* (Fig. 2*C*). Thus HY5 is required for survival under UV-B radiation. Whereas previous studies have highlighted the importance of HY5 in the development of photosynthetically competent seedlings (34), our findings demonstrate that HY5 plays an additional vital role in established plants in protecting against UV-B damage and maintaining photosynthetic function.

UVR8 Differs in Activity and Function from RCC1. UVR8 has sequence similarity and predicted structural similarity to human RCC1 (REGULATOR OF CHROMATIN CONDENSATION 1; ref. 18). RCC1 and its homologues in other eukaryotes are GEFs for the small GTP-binding protein Ran (36, 37). RCC1 is constitutively localized in the nucleus, binds to chromatin, and generates a Ran-GTP/Ran-GDP gradient across the nuclear envelope that is required both to drive nucleo-cytoplasmic transport and to regulate processes associated with progression of the cell cycle and mitosis. There is no evidence that RCC1 is involved in UV-B responses or transcriptional regulation. It is unlikely that UVR8 is involved in nucleo-cytoplasmic transport and mitotic regulation, because the *uvr8* mutant grows normally under standard (non-UV-B) condi-

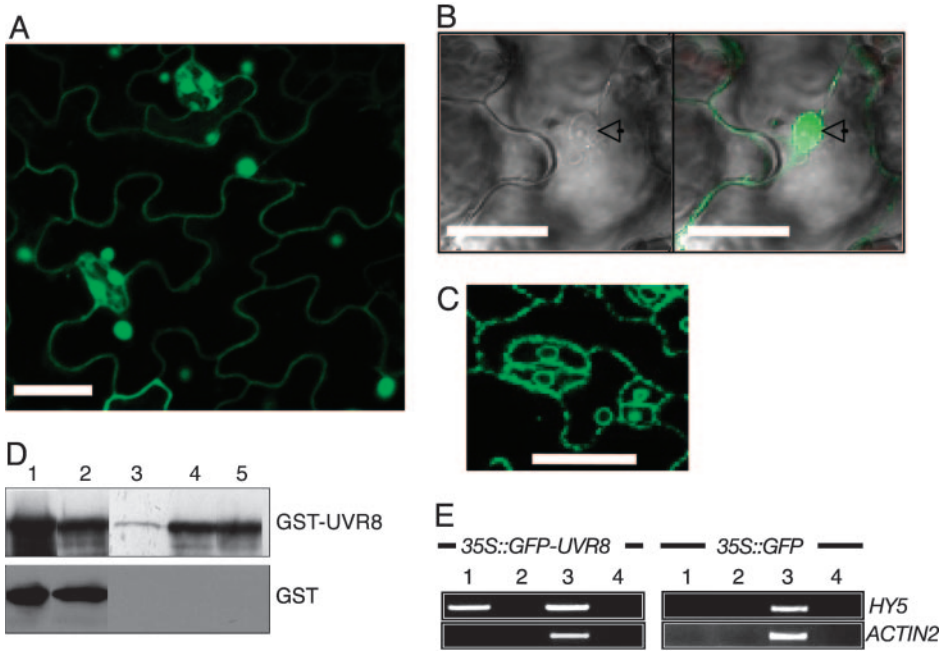

Fig. 3. GFP-UVR8 is present in the nucleus and associates with chromatin. (*A*) *uvr8-2* plants expressing a *35S* promoter::GFP-UVR8 fusion grown in low fluence rate white light and illuminated with 3 μmol $m^{-2}\cdot s^{-1}$ UV-B for 2 h. Confocal image shows GFP fluorescence of epidermal cells. (Scale bar, 20 μm.) (*B*) Single cell showing the nucleus (arrowed); left, bright-field image and right, with GFP fluorescence superimposed. (Scale bars, 20 μm.) (*C*) Localization of fluorescence in wild-type plants expressing a control *35S* promoter::GFP fusion. (Scale bar, 20 μm.) (*D*) Binding of *E. coli*-expressed GST-UVR8 to a calf thymus histone-agarose column. Western blots with anti-GST antibody showing GST-UVR8 (73 kDa) or control GST (26 kDa). Lane 1, protein applied to the columns; lane 2, unbound material that flowed through; lanes 3, 4, and 5, protein bound after a 5-min incubation, eluted with 0.1, 0.3, or 1.0 M NaCl, respectively. (*E*) Chromatin immunoprecipitation assay of DNA associated with GFP-UVR8. PCR of *HY5* promoter (−331 to +23) and *ACTIN2* DNA from *35S::GFP-UVR8* (*Left*) and *35S::GFP* (*Right*) transgenic plants grown in 100 μmol $m^{-2}\cdot s^{-1}$ white light and illuminated with 3 μmol $m^{-2}\cdot s^{-1}$ UV-B for 4 h: lane 1, DNA immunoprecipitated by using anti-GFP antibody; lane 2, no antibody control; lane 3, input DNA before immunoprecipitation; lane 4, PCR without added DNA.

tions (Fig. 2*C*), whereas *rcc1* mutants of human cells and yeast fail to grow. Because UVR8 is a single copy gene in *Arabidopsis*, it is likely that RCC1-related functions are carried out by another gene product(s) with sequence similarity to RCC1. It is clear that no protein acts redundantly with UVR8 in mediating UV-B protection; otherwise, the mutant phenotype would not be observed. Thus, RCC1 and UVR8 appear to have distinct functions.

We examined whether UVR8 has the characteristic properties of RCC1-family proteins: Ran GEF activity, nuclear localization, and chromatin association. UVR8 lacks several amino acids known to be required for maximal GEF activity of RCC1 (38) and for interaction with Ran (36). Using proteins expressed in *E. coli*, we found that UVR8 had ≈7% of the GEF activity of RCC1 with human Ran as substrate (mean $\ln(C_t/C_0)$ of −0.015 for UVR8 versus −0.222 for RCC1). This low activity is unlikely to be explained by Ran sequence divergence, because the human and *Arabidopsis* Ran proteins are highly conserved (39). Furthermore, we found that UVR8 did not interact with *Arabidopsis* Ran1 and Ran2 in yeast two-hybrid assays and did not complement the yeast *prp20* mutant lacking yeast RCC1 (data not shown). Together, these observations indicate that UVR8 is unlikely to be a functional homologue of RCC1, and Ran GEF activity is unlikely to be the basis of UVR8 activity.

UVR8 Is Localized Principally in the Nucleus. To determine the localization of UVR8, we made translational fusions with GFP and expressed them in *uvr8* mutant plants by using the cauliflower mosaic virus *35S* promoter. The N-terminal GFP-UVR8 fusion was functional, because it complemented the UV-sensitivity phenotype of *uvr8*. Examination of several independent transgenic lines by using confocal microscopy revealed that GFP-UVR8 fluorescence was present in the nucleus but also detectable in the cytosol (Fig. 3 *A* and *B*). A control line expressing GFP alone from the *35S* promoter showed different localization to GFP-UVR8 (Fig. 3*C*). The presence of GFP-UVR8 in the cytosol was not caused by aberrant overexpression from the *35S* promoter, because it was also observed in lines with very low expression. The finding that UVR8 is present in both the cytosol and nucleus is in contrast to other RCC1-family proteins, whose localization is exclusively nuclear.

UVR8 Interacts with Chromatin. One feature that UVR8 shares with RCC1 is that it interacts with chromatin and specifically with histones. UVR8 expressed in *E. coli* bound strongly to a histone-agarose column (Fig. 3*D*). To explore the significance of this finding, we examined whether UVR8 associates with chromatin *in vivo* in the region of a target gene. Using chromatin immunoprecipitation with an anti-GFP antibody, we found that GFP-UVR8 associated with a chromatin fragment containing the *HY5* promoter (sequences −331 to +23) but not with control *ACTIN2* gene DNA (Fig. 3*E*). No such association was found with chromatin from control *35S::GFP* plants. This finding indicates that UVR8 is involved directly in the regulation of *HY5* transcription. Moreover, it is likely that the association of UVR8 with chromatin provides a general mechanistic basis for its involvement in the transcriptional regulation of target genes.

Conclusion

The data presented here show that UVR8 is a UV-B-specific signal transduction component that plays a vital role in mediating plant responses to UV-B. In particular, UVR8 orchestrates the protective gene expression responses that enable plants to survive in sunlight. Thus, UVR8 defines a key light signaling pathway in plants. Further research is required to identify other components of the UVR8 pathway, but our data show that the HY5 transcription factor is an

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important downstream effector, regulating a substantial number of genes concerned with UV protection. We show that UVR8 regulates transcript levels of HY5 specifically in response to UV-B. Furthermore, the UV sensitivity of the *hy5* mutant provides direct evidence that HY5 is required for survival under UV-B radiation. The research therefore demonstrates that HY5 plays a dual role in photomorphogenesis and UV-protection.

UVR8 is a novel member of the RCC1 family of proteins. Unlike RCC1, UVR8 has very little Ran GEF activity and functions to regulate gene expression rather than nucleo-cytoplasmic transport and processes associated with the cell cycle and mitosis. However, UVR8, like RCC1, strongly associates with chromatin via histones. Our working hypothesis is that the association of UVR8 with chromatin, probably through interaction with other proteins, facilitates recruitment of transcription factors that regulate target genes such as *HY5*. Evidence for this model is the demonstration that UVR8 associates with chromatin in the region of the *HY5* promoter. Further research is required to test and refine this model. It is essential to determine how UV-B regulates UVR8, in particular, to examine the effects of UV-B on the nuclear localization and chromatin association of UVR8. In addition, it is important to identify the genes regulated directly by UVR8 and to establish the mechanism of action of UVR8 in transcriptional regulation.

We are grateful to Matt Shenton and George Littlejohn (G.I.J. Laboratory) for producing and characterizing the *CHS* promoter::Luc line; Giorgia Riboldi-Tunnicliffe, Irene Johnstone, and staff at the Sir Henry Wellcome Functional Genomics Facility for assistance with the microarray work; Ufo Sutter, Matt Tyrell, and Mike Blatt for help with the confocal microscopy; Robert Sablowski and Clare Lister (John Innes Centre, Norwich, U.K.) for seeds; Murray Stewart (MRC Laboratory for Molecular Biology, Cambridge, U.K.) for the human Ran clone; John Christie and others in the Plant Science Group for valuable discussions; the Biotechnology and Biological Sciences Research Council for grant support; and the University of Glasgow for a Staff Research Scholarship (to B.A.B.) and a Ph.D. Scholarship (to E.K.). We are indebted to the Bower Fire Insurance Fund and everyone who helped after the Bower Building fire for enabling us to continue our research.

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