Constitutively active UVR8 photoreceptor variant in *Arabidopsis*

Marc Heijde[a,1], Melanie Binkert[a], Ruohe Yin[a], Florence Ares-Orpel[a], Luca Rizzini[b,2], Eveline Van De Slijke[c,d], Geert Persiau[c,d], Jonah Nolf[c,d], Kris Gevaert[e,f], Geert De Jaeger[c,d], and Roman Ulm[a,3]

[a]Department of Botany and Plant Biology, Sciences III, University of Geneva, CH-1211 Geneva 4, Switzerland; [b]Faculty of Biology, Institute of Biology II, University of Freiburg, D-79104 Freiburg, Germany; Departments of [c]Plant Systems Biology and [e]Medical Protein Research, VIB, B-9052 Ghent, Belgium; and Departments of [d]Plant Biotechnology and Bioinformatics and [f]Biochemistry, Ghent University, B-9052 Ghent, Belgium

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Arabidopsis thaliana **UV RESISTANCE LOCUS 8 (UVR8) is a UV-B photoreceptor that initiates photomorphogenic responses underlying acclimation and UV-B tolerance in plants. UVR8 is a homodimer in its ground state, and UV-B exposure results in its instantaneous monomerization followed by interaction with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1), a major factor in UV-B signaling. UV-B photoreception by UVR8 is based on intrinsic tryptophan aromatic amino acid residues, with tryptophan-285 as the main chromophore. We generated transgenic plants expressing UVR8 with a single amino acid change of tryptophan-285 to alanine. UVR8^{W285A} appears monomeric and shows UV-B–independent interaction with COP1. Phenotypically, the plants expressing UVR8^{W285A} exhibit constitutive photomorphogenesis associated with constitutive activation of target genes, elevated levels of anthocyanins, and enhanced, acclimation-independent UV-B tolerance. Moreover, we have identified COP1, REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 and 2 (RUP1 and RUP2), and the SUPPRESSOR OF PHYA-105 (SPA) family as proteins copurifying with UVR8^{W285A}. Whereas COP1, RUP1, and RUP2 are known to directly interact with UVR8, we show that SPA1 interacts with UVR8 indirectly through COP1. We conclude that UVR8^{W285A} is a constitutively active UVR8 photoreceptor variant in *Arabidopsis*, as is consistent with the crucial importance of monomer formation and COP1 binding for UVR8 activity.**

signal transduction | abiotic stress | ultraviolet-B

Plants react to UV-B radiation (UV-B; 280–315 nm) with a photomorphogenic response that generates acclimation to this environmental stress factor (1–3). The associated specific signaling pathway is characterized molecularly by the involvement of the UV RESISTANCE LOCUS 8 (UVR8) photoreceptor (4, 5). Loss of UVR8 in *Arabidopsis* results in the loss of a broad range of molecular and physiological UV-B responses, including reduced UV-B acclimation and tolerance (6–11). Perception of UV-B photons by UVR8 homodimers results in UVR8 monomerization (4). The crystal structure of UVR8 shows that the homodimer is maintained by salt-bridge interactions between charged amino acids at the dimeric interaction surface (12, 13). Destabilization of the salt bridges upon absorption of UV-B photons by tryptophan-285, and to a lesser extent tryptophan-233, underlies UVR8 monomerization and signal initiation (12, 13). The UVR8 photoreceptor can revert to the ground state in vivo by redimerization (14, 15). This process is facilitated by REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 and 2 (RUP1 and RUP2), consequently disrupting the key interaction of UVR8 with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) (14, 16). The reversibility of UVR8 between inactive homodimer and active monomer conformations results in continuous sensitivity to the ambient UV-B environment (14, 15). It can be assumed that UVR8 cycles between the dimeric and monomeric forms in vivo, and thus the resulting UVR8 dimer/monomer photoequilibrium is a measure of the ambient UV-B levels experienced by the plant.

Activated UVR8 interacts with COP1 (8), which is an E3 ubiquitin ligase with important activity as a repressor of photomorphogenesis in the dark (17) and a key role in promoting UV-B signaling (18). Mutations in COP1 or UVR8 affect the interaction and impair UV-B signaling (8, 19). The COP1 interaction domain recently was found to be a region of 27 amino acids in the C terminus of UVR8 (19). UVR8–COP1 interaction is associated with stabilization of the bZIP transcription factor ELONGATED HYPOCOTYL 5 (HY5), which also plays an important role in UV-B signaling (7, 18, 20, 21). Together with FHY3, HY5 also positively regulates *COP1* expression in response to UV-B (22), but its transcriptional activity is feedback-inhibited by the B-box protein BBX24 (23).

Mutation of the tryptophan-285 chromophore to phenyalanine renders UVR8^{W285F} constitutively homodimeric and inactive (4, 12, 13, 24). In marked contrast, the mutation of tryptophan-285 to alanine (UVR8^{W285A}) was found to be monomeric in vivo and to interact constitutively with COP1 in yeast (4), in mammalian cells (25), and in plants (24). A recent report has addressed the physiological effect of expressing GFP-UVR8^{W285A} in transgenic plants (*uvr8-1*/*Pro$_{35S}$::GFP-UVR8^{W285A}*) (24). Despite the apparent monomeric form of GFP-UVR8^{W285A} and associated constitutive interaction with COP1 *in planta*, lines expressing GFP-UVR8^{W285A} were not altered in growth phenotype and lack only UV-B responsiveness (24). Therefore, it was concluded that monomer formation and COP1 binding are not sufficient for UVR8 function (24).

Significance

Sunlight is an essential environmental factor for photosynthetic plants and ultimately for life on Earth, which is sustained through plants as fundamental source of food. However, plants have a love/hate relationship with sunlight and must be protected from potentially harmful UV-B radiation. The UV-B photoreceptor UVR8 is of great importance in mounting UV-protective responses and thus for survival in sunlight. Based on our understanding of UVR8 signaling, we have engineered a UVR8 variant that is constitutively active in transgenic plants. The generation of a constitutively active photoreceptor variant is an important step in understanding the molecular signaling mechanism and may hold opportunities for crop improvement.

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[1]Present address: Department of Plant Systems Biology, VIB, B-9052 Ghent, Belgium.

[2]Present address: Department of Pathology, Cancer Institute, New York University School of Medicine, New York, NY 10016.

[3]To whom correspondence should be addressed. E-mail: roman.ulm@unige.ch.

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In a search for a constitutively active UVR8 variant, we generated *uvr8-7*/*Pro*$_{35S}$*::UVR8*^{W285A} transgenic lines. We show here that expression of UVR8^{W285A} results in a constitutive photomorphogenic phenotype, including hypocotyl growth inhibition, target gene expression, and elevated levels of anthocyanins. As a consequence, lines overexpressing UVR8^{W285A} are constitutively acclimated and thus display an enhanced basal UV-B tolerance.

Results

UVR8^{W285} Appears Partially Monomeric and Interacts Constitutively with COP1 in Vivo. To analyze the in vivo function of UVR8^{W285A}, we transformed the *uvr8-7* null mutant with the *UVR8*^{W285A} coding sequence driven by the constitutive *CaMV 35S*-promoter (*uvr8-7*/*Pro*$_{35S}$*::UVR8*^{W285A}). Among the transgenic lines, we carefully selected lines having either low levels of UVR8^{W285A} overexpression relative to endogenous UVR8 expression in wild-type plants (e.g., lines #4 and #24) or high levels of UVR8^{W285A} overexpression similar to those in a control line overexpressing UVR8 (UVR8-Ox; *uvr8-7*/*Pro*$_{35S}$*::UVR8*) (e.g., lines #3, #6, and #12) (Figs. 1 and 2*A*).

UVR8^{W285A} in the transgenic lines appears as a constitutive monomer after SDS-PAGE without sample boiling (Fig. 2*A*), as previously described for UVR8^{W285A} expressed in yeast (4) and GFP-UVR8^{W285A} in transgenic plants (24). This configuration is in marked contrast to that of UVR8^{W285F}, which appears as a constitutive homodimer in plant transgenic lines (Fig. S1 *A* and *B*) (24), as it does in yeast (4). Notably, although SDS-PAGE is a very convenient assay for cell extracts, there is some discrepancy when the results are compared with size-exclusion chromatography of purified UVR8^{W285A} in vitro: SDS-PAGE identified UVR8^{W285A} as monomeric, whereas size-exclusion chromatography identified it as homodimeric (12, 13). This discrepancy indicates that UVR8^{W285A} may not be monomeric per se but that the dimer is strongly destabilized. However, UVR8 dimerization assays in an yeast two-hybrid system indicated that UVR8^{W285A} does not homodimerize detectably in vivo, in contrast to UVR8 and UVR8^{W285F} (Fig. 2*B*). Although we cannot exclude the possibility that UVR8^{W285A} forms weak dimers that are undetectable in yeast two-hybrid assays, our data suggest that UVR8^{W285A} is, at least in part, a functional monomer in the cellular context in vivo. *In planta*, UVR8 and UVR8^{W285A} are likely to be associated with additional proteins. Indeed, size-exclusion chromatography of protein extracts from transgenic seedlings indicated that UVR8 and UVR8^{W285F} were present in native complexes with an apparent molecular mass <158 kDa, whereas fractions containing UVR8^{W285A} were shifted to a lower molecular mass, and UVR8^{W285A} was detected in fractions that correspond to monomeric size (Fig. S1*C*). Similarly, in 2D Blue Native/SDS-PAGE, both UVR8 and UVR8^{W285A} were detected in native complexes of about 146 kDa, in which UVR8 appears as dimer and UVR8^{W285A} as monomer (second-dimension SDS-PAGE, nonboiled) (Fig. S1*D*). We conclude that both UVR8 and UVR8^{W285A} are associated with interacting proteins in vivo rather than being present as isolated dimers or monomers, respectively.

UVR8^{W285A} interacts constitutively with COP1 in yeast, whereas the UVR8^{W285F} mutation prevents UV-B–dependent interaction with COP1 (Fig. 2*C*) (4). UVR8^{W285A} was found interact constitutively with the C-terminal WD40-repeat domain of COP1 (C-terminal 340 amino acids; COP1^{C340}) but not with the N-terminal RING/coiled-coil domains (COP1^{N335}) (Fig. 2*D*). In further support of a UVR8–COP1^{C340} interaction (4, 8), a single amino acid mutation corresponding to the *cop1-19* allele (COP1^{G608R}) (8) abolished interaction with UVR8^{W285A} in yeast (Fig. 2*D*). In agreement with the yeast data, COP1 coimmunoprecipitates with GFP- and TAP-tagged UVR8^{W285A} independently of UV-B in plant cells (ref. 24, and see below), in contrast to wild-type UVR8, which coimmunoprecipitates with COP1 only under UV-B (4, 8, 14, 19, 24). Taken together, these results indicate that at least a fraction of UVR8^{W285A} is constitutively monomeric *in planta* and interacts with the WD40-repeat domain of COP1.

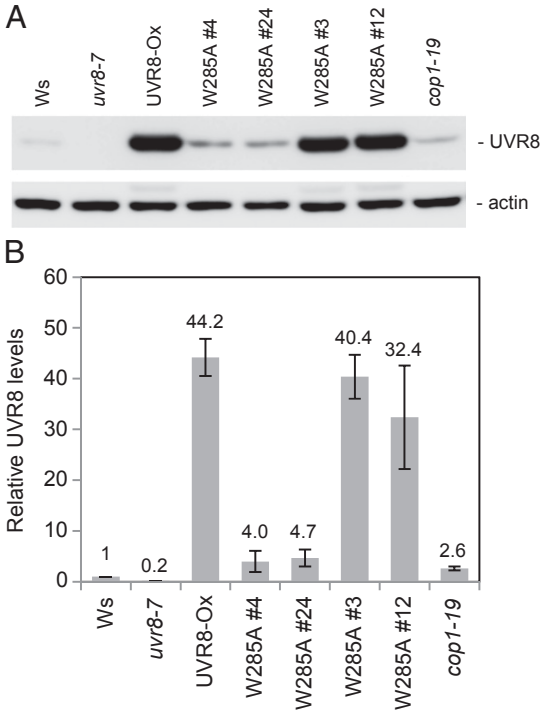

Fig. 1. Expression of UVR8^{W285A} in transgenic plants. (*A*) UVR8 and UVR8^{W285A} protein levels in two transgenic UVR8^{W285A} lines with low levels of overexpression (W285A #4 and #24), in two transgenic UVR8^{W285A} lines with high levels of overexpression (W285A #3 and #12), in the wild-type line (Ws), in a control line overexpressing UVR8 (UVR8-Ox), and in the *cop1-19* mutant line. (*B*) Quantification of UVR8 and UVR8^{W285A} protein levels in biological triplicates, relative to the wild type (Ws) UVR8 level; error bars represent SD.

Expression of UVR8^{W285A} Results in Constitutive UV-B Responses in Seedlings in both Light and Dark Conditions. The UVR8-COP1 interaction is an early and crucial step in the UV-B photoreceptor signaling pathway (8). Therefore, we tested whether UVR8^{W285A} is constitutively active *in planta*. UV-B induces gene-expression changes and a series of photomorphogenic responses, including hypocotyl growth inhibition and the accumulation of anthocyanins (2). Seedlings with low overexpression of UVR8^{W285A} (lines #4 and #24) exhibited significantly shorter hypocotyls than wild-type seedlings in weak white light, but no difference was seen in dark-grown seedlings (Fig. 3 *A* and *B*). However, higher overexpression of UVR8^{W285A} (lines #6 and #12) led to a constitutive photomorphogenic (*cop*) phenotype even in darkness, as displayed by a short hypocotyl and open and expanded cotyledons (Fig. 3*A*). This phenotype is reminiscent of the *cop1*-mutant phenotype (26), although *COP1* mRNA and COP1 protein levels were not reduced in the line overexpressing UVR8^{W285A} (Fig. 3 *C* and *D*). In this line, the *cop1*-like phenotype is not caused by low levels of COP1. Rather, COP1 was higher in lines overexpressing UVR8^{W285A} grown in white light (Fig. 3*D*), as is in agreement with the posttranslational COP1 stabilization seen in UV-B–irradiated wild-type seedlings (8). Importantly, overexpression of UVR8 or UVR8^{W285F} did not result in a *cop1*-like phenotype (Fig. 3 *A* and *B*).

The UV-B–dependent interaction of UVR8 with COP1 is followed by a transcriptional response (2). Therefore we analyzed the expression of UVR8-dependent, UV-B–responsive marker genes in the lines expressing UVR8^{W285A}. The *RUP2* and *EARLY LIGHT-INDUCIBLE PROTEIN 2* (*ELIP2*) marker genes tested were both constitutively expressed at elevated levels in lines overexpressing UVR8^{W285A} (Fig. 3 *E* and *F* and Fig. S2).

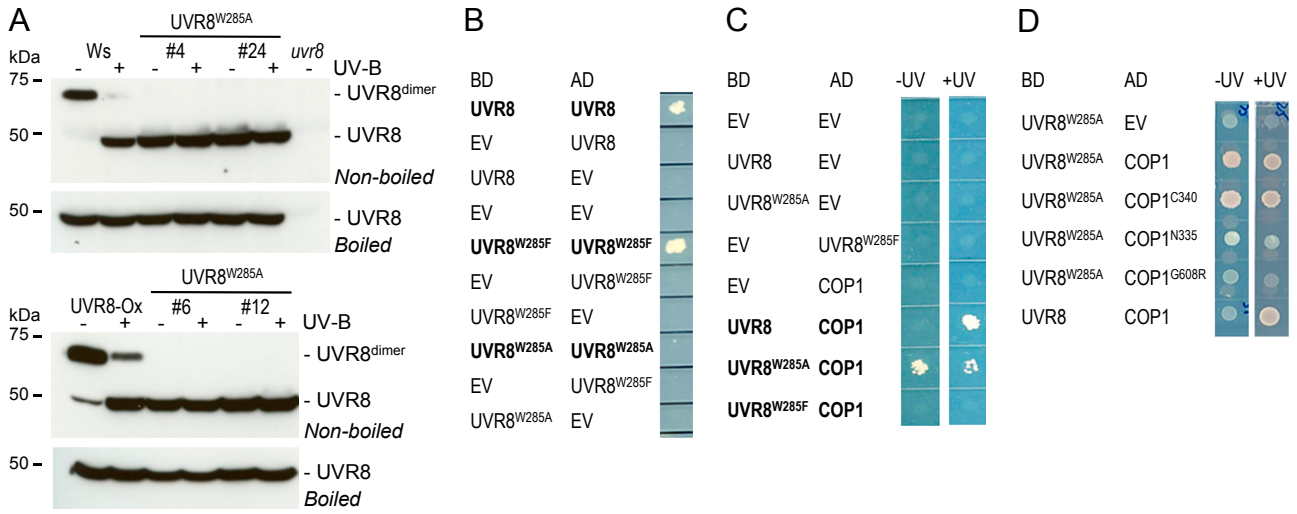

Fig. 2. UVR8^{W285A} appears as a constitutive monomer and constitutively interacts with COP1 in transgenic plants. (*A*) Four-day-old seedlings were irradiated for 15 min with (+) or without (–) supplementary broadband UV-B. The dimer was observed on Western blots from SDS-PAGE without sample boiling (*Upper*), and the total amount of UVR8 protein was detected in samples denatured by boiling (*Lower*). UVR8 protein levels of wild-type (Ws) plants are compared with those in *Pro$_{35S}$::UVR8^{W285A}* lines #4 and #24 (W285A #4 and #24) (*Upper*), and UVR8 protein levels of a line overexpressing *Pro$_{35S}$::UVR8* (UVR8-Ox) are compared with levels in lines overexpressing *Pro$_{35S}$::UVR8^{W285A}* (#6 and #12, *Lower*). *uvr8-7* is shown as negative control demonstrating the specificity of the detected bands. (*B*) UVR8^{W285A} does not form homodimers in vivo in yeast two-hybrid growth assays, in contrast to UVR8 and UVR8^{W285F}. (*C*) UVR8^{W285A} interacts with COP1 in a UV-B–independent manner, in contrast to wild-type UVR8, whereas UVR8^{W285F} shows no interaction with COP1. (*D*) UVR8^{W285A} interacts with the 340 C-terminal amino acids of COP1 in a UV-B–independent manner in a yeast two-hybrid growth assay. EV, empty vector control.

HY5 is a crucial transcriptional regulator in the UV-B signaling pathway (20). Transcriptionally, the *HY5* gene is induced early, and the HY5 protein is posttranslationally stabilized upon UV-B exposure, despite the parallel nuclear accumulation of COP1 (8, 18, 20). Indeed, HY5 accumulated substantially in lines overexpressing UVR8^{W285A}, despite wild-type levels of *HY5* mRNA (Fig. 3 *G*–*J*) and elevated levels of COP1 (Fig. 3*D*). The wild-type level of *HY5* mRNA is consistent with the rapid and transient kinetics of *HY5* gene induction by UV-B, corresponding to the basal level after 4 d exposure (8, 16, 18).

Thus, we conclude that UVR8^{W285A} is a constitutively active UV-B photoreceptor *in planta*, which leads to UV-B–associated phenotypic (hypocotyl growth inhibition) and molecular (COP1 and HY5 stabilization and target gene expression) responses in the absence of UV-B.

UVR8^{W285A} Expression Results in Elevated Constitutive Levels of Chalcone Synthase and Anthocyanins. UV-B also is known to induce a series of changes in metabolite levels, including the accumulation of UV-protective pigments such as anthocyanins (2). CHALCONE SYNTHASE (CHS) is a key enzyme in the phenylpropanoid biosynthesis pathway leading to anthocyanins that is regulated largely at the transcriptional level in response to UV-B (27, 28). In agreement with their apparent constitutive UV-B photomorphogenic responses, plants overexpressing UVR8^{W285A} showed higher *CHS* mRNA levels than the wild-type plants, *uvr8-7* mutants, or plants overexpressing UVR8 (Fig. 4*A*). Elevated levels of CHS in seedlings expressing UVR8^{W285A} also were apparent at the protein level (Fig. 4*B*). In agreement with the changes in CHS, the overexpression of UVR8^{W285A} resulted in elevated levels of anthocyanins in the absence of UV-B, representing a constitutive UV-B response (Fig. 4*C*). In contrast, neither UVR8 nor UVR8^{W285F} overexpression resulted in constitutively elevated levels of anthocyanins (Fig. 4*C*).

Constitutive Acclimation Results in Enhanced UV-B Tolerance in Lines Overexpressing UVR8^{W285A}. The UVR8-dependent UV-B signaling pathway acclimates plants to UV-B and, as a consequence, enhances UV-B stress tolerance (8, 16, 29). Because the phenotype of lines overexpressing UVR8^{W285A} suggested constitutive acclimation to UV-B, we subjected 7-d-old seedlings to UV-B stress without prior acclimation. Indeed, the overexpression of UVR8^{W285A} resulted in constitutively elevated basal UV-B tolerance, in contrast to wild-type seedlings, the *uvr8-7* null mutant, and lines overexpressing UVR8 (Fig. 4*D*). Thus, we conclude that overexpression of UVR8^{W285A} (but not of UVR8) is sufficient to trigger a combination of responses that usually are associated with UV-B–induced photomorphogenesis and acclimation and that render plants UV-B tolerant.

Lines Overexpressing UVR8^{W285A} Show a Dwarfed Growth Phenotype. UVR8^{W285A}-overexpression phenotypes were not restricted to the seedling stage. When grown on soil for 5 wk, lines overexpressing UVR8^{W285A} had a dwarfed growth phenotype reminiscent of *cop1*-mutant plants (Fig. 5). The lines expressing low levels of UVR8^{W285A} also were stunted, including short petioles, but to a much lower extent (Fig. 5). We conclude that expression of UVR8^{W285A} results in a dwarf growth phenotype at the adult stage, likely associated with its constitutive interaction with COP1.

Tandem Affinity Purification-Tagged UVR8^{W285A} Copurifies with the COP1–SUPPRESSOR OF PHYA-105 Complex. Our physiological and molecular analyses of lines expressing UVR8^{W285A} demonstrate that UVR8^{W285A} mimics active UVR8. Therefore, we used a tandem affinity purification (TAP)-based screening approach to isolate proteins interacting with UVR8^{W285A} in *Arabidopsis* cells. We transformed *Arabidopsis* suspension-cultured cells with GS-TAP–tagged UVR8^{W285A} and identified interacting proteins by copurification and MS (30). As expected, COP1, RUP1, and RUP2 were present in complex(es) with UVR8^{W285A} (Fig. 6*A*). The only further copurifying proteins were the WD40-repeat protein SPA1 (SUPPRESSOR OF PHYA-105 1) and the related SPA2, SPA3, and SPA4 (Fig. 6*A*). The SPA1–SPA4 quartet is known to interact with COP1 and to be required for its activities (21, 31, 32). The copurification of SPA1 with UVR8^{W285A} from suspension cultures was supported further by the coimmunoprecipitation of SPA1 with endogenous UVR8 in wild-type seedlings. Coimmunoprecipitation of SPA1 with UVR8 occurred specifically in response to UV-B, similar to coimmunoprecipitation of COP1 with UVR8 (Fig. 6*B*). Importantly, we did not detect coimmunoprecipitation of SPA1 with UVR8 in a *cop1*-mutant background (Fig. 6*B*). We conclude that, although UVR8 interacts

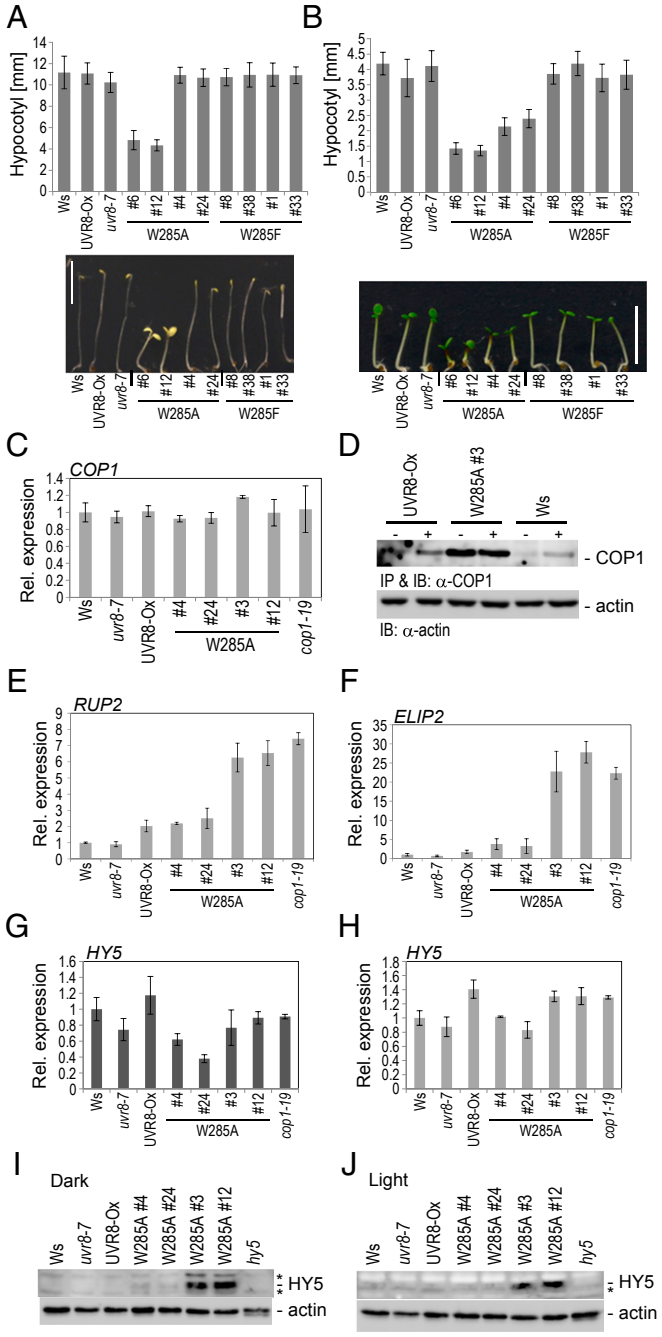

Fig. 3. UVR8^{W285A} expression results in a constitutive photomorphogenic response. (*A* and *B*) Analysis of hypocotyl length and quantification of 4-d-old seedlings grown in the dark (*A*) or in continuous white light (3.6 μmol·m^{-2}·s^{-1}) (*B*). Histograms show average hypocotyl length; error bars represent SD ($n > 30$). (Scale bar, 5 mm.) (*C*) Quantitative RT-PCR analysis of *COP1* mRNA levels in 4-d-old UVR8^{W285A} transgenic seedlings under white light compared with wild-type seedlings (Ws). (*D*) Immunoprecipitation and Western blot analysis of COP1 protein levels in 7-d-old seedlings grown in white light (−UV-B) or supplemented for 6 h with narrowband UV-B before harvesting (+UV-B). (*E* and *F*) Quantitative RT-PCR comparison of *RUP2* and *ELIP2* mRNA levels in 4-d-old wild-type (Ws) and UVR8^{W285A} transgenic plants grown in white light. (*G* and *H*) Quantitative RT-PCR comparison of *HY5* mRNA levels in 4-d-old wild-type (Ws) and UVR8^{W285A} transgenic plants grown in the dark (*G*) or in white light (*H*). (*I* and *J*) Immunoblot analysis of HY5 protein levels in 7-d-old seedlings grown in the dark (*I*) or in white light (*J*). Asterisks indicate unspecific bands.

directly with COP1, it interacts with SPA1 indirectly through COP1. Moreover, the COP1–SPA complex remains intact upon interaction with UVR8^{W285A} as well as upon interaction with UVR8 after activation by UV-B.

Discussion

The generation of constitutively active photoreceptor variants has provided useful tools and important information about the signaling and responses of diverse photoreceptors, including plant cryptochrome blue-light receptors (33, 34), plant phytochrome red-/far-red-light receptors (35–37), and mammalian rhodopsin (38). The observation that plants expressing UVR8^{W285A} exhibit constitutive photomorphogenetic development in the dark and exaggerated photomorphogenesis in light devoid of UV-B demonstrates that the functional consequence of this chromophore mutation is the UV-B–independent activation of the UVR8 photoreceptor pathway.

The crystal structures of the core of UVR8, as well as UVR8^{W285A} and UVR8^{W285F}, have been determined recently (12, 13). Interestingly, the overall structures of the UVR8 variants UVR8^{W285F} and UVR8^{W285A} are almost identical to that of the UVR8 core domain (13). Analysis of local structural features surrounding the chromophore residue tryptophan-285 revealed no significant conformational differences between UVR8 and UVR8^{W285F}, but major changes in critical residues have occurred in the variant UVR8^{W285A} (13). Because the solved UVR8 core structure does not include the C-terminal 40 amino acids, it presently is not known how these changes in UVR8^{W285A} have affected the C-terminal C27 region, which is crucial for UVR8–COP1 interaction as well as for UVR8 activity in vivo (19). It has been suggested that the C27 region is hidden from COP1 in homodimeric UVR8 and that UV-B–induced monomerization and associated conformational changes expose the C27 region to interaction with COP1 (19). We thus hypothesize that the C27 region is exposed in UVR8^{W285A} independently of UV-B, resulting in UV-B–independent signaling involving constitutive interaction with COP1. In contrast, UVR8^{W285F} does not monomerize or expose the C27 region to interaction with COP1 in response to UV-B (phenylalanine does not absorb in the UV-B range). In agreement with this notion, UVR8^{W285F} transgenic lines remain unresponsive to UV-B (ref. 24 and this study).

It was shown previously that COP1 forms protein complexes with the SPA quartet (SPA1–SPA4) and that the SPA proteins are required for COP1 activity (31). Whether the SPA proteins also are essential for COP1 activity in the response to UV-B is controversial at present (18, 21). Independent of this question, although cryptochrome photoreceptors impinge on COP1 activity by light-dependent interaction via SPA proteins (39), UVR8 presently is the only photoreceptor showing light-dependent interaction directly with COP1 (2, 4). Moreover, we show here that UVR8 interacts with the COP1–SPA complex and not with COP1 in isolation. This result further indicates that UVR8 must affect COP1 activity in response to UV-B in the presence of interacting SPA proteins. Recent findings suggest that light-dependent reorganization of protein complexes containing the COP1–SPA core underlie the switch to UV-B–specific COP1 function (21). However, the exact molecular mechanism by which the UVR8–COP1 interaction transduces the UV-B signal remains to be determined.

It is noteworthy that a constitutive photomorphogenic phenotype was not found in *uvr8-1*/*Pro$_{35S}$::GFP-UVR8^{W285A}* transgenic lines in an independent study (24), in marked contrast to the clear phenotype of the *uvr8-7*/*Pro$_{35S}$::UVR8^{W285A}* lines reported here. This difference is particularly surprising because GFP-UVR8^{W285A} was reported to appear as a constitutive monomer in the transgenic plants and to interact strongly with COP1 in the absence of UV-B (24). Obvious differences between the two reports are the background accessions [*uvr8-7* in Wassilewskija and *uvr8-1* in Landsberg *erecta* (L*er*)] and the presence of an N-terminal 27-kDa GFP tag. Given that L*er* responds to UV-B in a UVR8-dependent manner and that GFP-UVR8^{W285A}

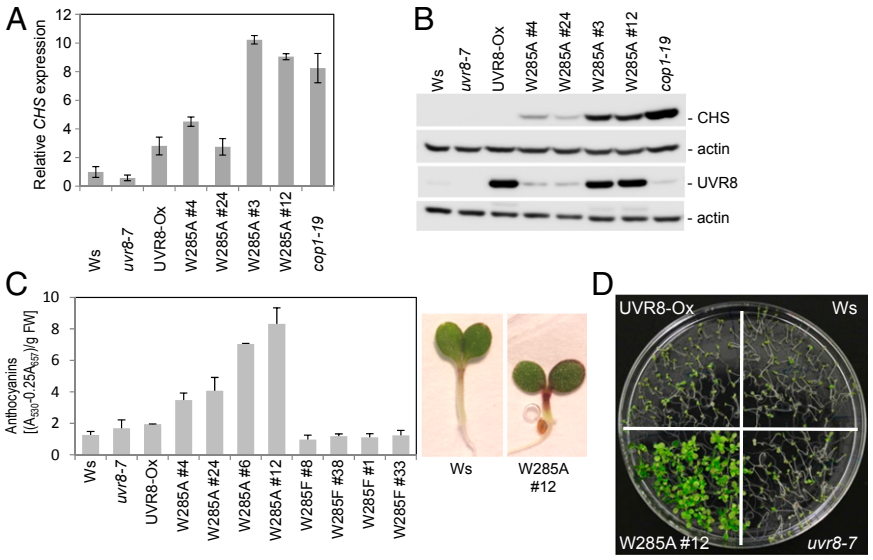

Fig. 4. UVR8^{W285A} expression results in elevated constitutive levels of chalcone synthase and anthocyanins as well as elevated UV-B tolerance. (*A* and *B*) Analysis of *CHS* mRNA levels (*A*) and CHS protein levels (*B*) in 4-d-old seedlings grown in white light. (*C*) Photometric determination of the anthocyanin content of 4-d-old *Arabidopsis* seedlings. Data shown are the mean values of three independent biological replicates; error bars indicate SD. Representative images showing the elevated anthocyanin content (purple pigmentation) in a 4-d-old transgenic plant overexpressing UVR8^{W285A} (*Right*). (*D*) UV-B tolerance of non–UV-B–acclimated 7-d-old seedlings treated for 3 h with broadband UV-B. After UV-B stress treatment, the seedlings were allowed to recover for 1 wk without UV-B before the image was captured.

was found to interact with COP1 in the absence of UV-B apparently as strongly as GFP-UVR8 interacts in the presence of UV-B (24), the background accession is the less likely reason for the observed differences. We hypothesize instead that the N terminus of UVR8 contributes to UVR8 activity and that this activity is partially impaired in N-terminal fusions of GFP. The fact that GFP-UVR8 can complement *uvr8* mutants (7) suggests that it is still at least partially active and/or that UV-B activation of GFP-UVR8 is stronger than the synthetic activation mimicry in GFP-UVR8^{W285A}, and thus higher levels of GFP-UVR8^{W285A} overexpression may be needed to detect a constitutive photomorphogenic response. Independent of this suggestion, we also conclude that the constitutively photomorphogenic phenotype of the active UVR8^{W285A} allele is not caused simply by COP1 interaction per se, because this interaction also is clearly apparent in GFP-UVR8^{W285A} lines (24). Thus, UVR8^{W285A} must affect COP1 more specifically, perhaps involving the N terminus of the protein (potentially partly impaired in N-terminal GFP fusions) and not only the C27 region. The UVR8 structure indeed suggests that the enigmatic N- and C-terminal regions are in close proximity (12, 13), but any potential interplay remains to be determined.

Interestingly, we initially observed a substantial phenotypic difference between wild-type plants, lines overexpressing UVR8, and *uvr8-7* mutants under standard growth conditions using fluorescent lamps (Fig. S3*A*). We tested whether this difference was associated with the very low levels of UV-B issued by such lamps (Fig. S3*B*) and found, in fact, that the difference in growth was reduced by inserting a UV-B filter (Fig. S3 *A* and *C*). This response demonstrates the high sensitivity of the plant UVR8 photoreceptor system and provides a cautionary note regarding analysis of lines overexpressing UVR8 under "standard conditions." As expected of a chromophore mutation, a UV-B filter made no difference to transgenic lines expressing UVR8^{W285A}, which were equally dwarfed in both conditions (Fig. S3*A*).

We conclude that UVR8^{W285A} undergoes spontaneous changes in molecular conformation, mainly from homodimer to monomer, that activate the signaling cascade involving interaction with COP1. This study provides a UVR8 mutant form that can stimulate the UV-B signaling pathway spontaneously at a level high enough to produce a constitutive photomorphogenic phenotype. This result not only underlines the great importance of monomer formation and COP1 binding for UVR8 activity in plants but also provides a promising tool to elucidate further the molecular mechanism of UVR8 signaling. Moreover, the elevated levels of anthocyanins recorded, as well as the enhanced UV-B tolerance, indicate that UVR8^{W285A} may offer considerable potential for crop improvement.

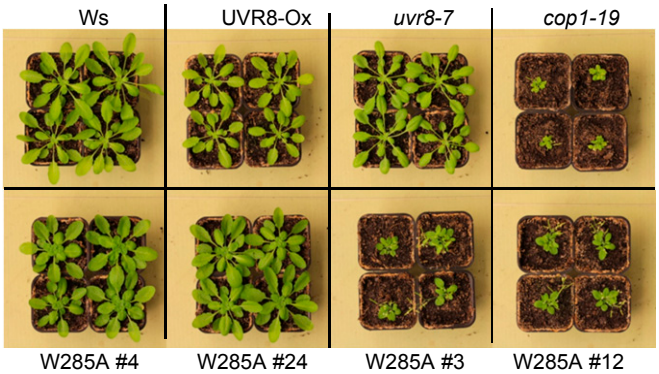

Fig. 5. UVR8^{W285A} overexpression results in dwarf growth of adult plants. Representative 5-wk-old plants grown in standard growth conditions (with a UV-B filter) in a phytochamber under short days (8 h/16 h light/dark).

Materials and Methods

Protein immunoprecipitation, protein gel blot analysis, size-exclusion chromatography, Blue Native/SDS-PAGE, yeast two-hybrid assays, real-time PCR, anthocyanin analysis, TAP, and LC-MS/MS analysis are described in *SI Materials and Methods*.

Plant Material. The *uvr8-7*, *cop1-19*, *cop1-4*, and *spa1-3* mutants were described previously (8, 26, 40). The *UVR8^{W285A}* and *UVR8^{W285F}* versions were generated by site-directed mutagenesis (4) and were cloned into Gateway-compatible pB2GW7 (41). The mutated constructs were verified by sequencing and then were introduced into the *uvr8-7* mutant to generate *uvr8-7/Pro$_{35S}$::UVR8^{W285A}* and *uvr8-7/Pro$_{35S}$::UVR8^{W285F}*. The *uvr8-7/Pro$_{35S}$::UVR8* control overexpression line (UVR8-Ox) was described previously (8). The transgenic lines described in this work were determined genetically to have the transgenes integrated at a single locus.

Growth Conditions and UV-B Irradiation. *Arabidopsis* seeds were surface-sterilized and sown on half-strength Murashige and Skoog basal salt medium (MS; Duchefa) containing 1% (wt/vol) sucrose and 1% (wt/vol) phytagel (Sigma). Seeds were stratified for 2 d at 4 °C and were germinated at 22 °C in a standard growth chamber under constant white light.

UV-B stress-tolerance experiments were performed essentially as described previously (29): 7-d-old seedlings were irradiated for 3 h using broadband UV-B lamps (Philips TL40W/12RS; 2 mW·cm^{-2}, measured with a VLX-3W UV light meter equipped with a CX-312 sensor; Vilber Lourmat) and were transferred to standard white light for 7-d recovery before images were captured.

A

AGI	Name	Found/2 exps	Protein coverage %
AT5G63860	UVR8	2/2	71.1
AT2G32950	COP1	2/2	41.3
AT5G52250	RUP1	2/2	7.0
AT5G23730	RUP2	2/2	36.1
AT2G46340	SPA1	2/2	8.6
AT4G11110	SPA2	2/2	9.2
AT3G15354	SPA3	2/2	5.1
AT1G53090	SPA4	2/2	35.0

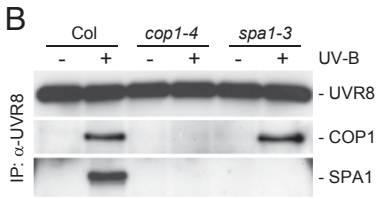

Fig. 6. UVR8/UVR8^{W285A} "interactors" include SPA family proteins, but interaction with SPA1 is indirect via COP1. (*A*) Proteins identified after TAP purification with GS-TAP-UVR8^{W285A} expressed in *Arabidopsis* cell-suspension cultures [PSB-L/PKSA::GS(rhino)-UVR8^{W285A}]. Known TAP background proteins were filtered from the list. AGI, *Arabidopsis* Genome Initiative identifier. See Table S1 for details of protein identification. (*B*) Coimmunoprecipitation of COP1 and SPA1 using UVR8 antibodies in extracts from 7-d-old wild-type (Col), *cop1-4,* and *spa1-3* seedlings. Seedlings were irradiated for 6 h with supplemental narrowband UV-B and for 15 min with (+) or without (–) broadband UV-B.

Hypocotyl lengths were measured on 4-d-old seedlings ($n > 30$) using ImageJ software (http://rsbweb.nih.gov/ij/), as described previously (8, 18).

For the adult phenotype, *Arabidopsis* plants were cultured in a growth chamber at 22 °C with 75% humidity in short-day conditions (8 h/16 h light/dark) with a 1:1 ratio of Osram L 58W/840 cool and Osram L 58W/830 warm white light lamps (see Fig. S3*B* for the spectrum as measured with an Ocean Optics QE65000 spectrometer). To filter out residual UV-B, the fluorescent lamps were covered when indicated with UV-B filter foil no. 226 (Lee Filters).

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