Ultraviolet-B Radiation-Mediated Responses in Plants. Balancing Damage and Protection[1]

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Seven percent of the electromagnetic radiation emitted from the sun is in the UV range (200–400 nm). As it passes through the atmosphere, the total flux transmitted is greatly reduced, and the composition of the UV radiation is modified. Shortwave UV-C radiation (200–280 nm) is completely absorbed by atmospheric gases. UV-B radiation (280–320 nm) is additionally absorbed by stratospheric ozone and thus only a very small proportion is transmitted to the Earth's surface, whereas UV-A radiation (320–400 nm) is hardly absorbed by ozone (Fig. 1). In the past 50 years, the concentration of ozone has decreased by about 5%, mainly due to the release of anthropogenic pollutants such as chlorofluorocarbons (Pyle, 1996). Consequently, a larger proportion of the UV-B spectrum reaches the Earth's surface with serious implications for all living organisms (Xiong and Day, 2001; Caldwell et al., 2003).

Elevated UV-B radiation (UV-B) has pleiotropic effects on plant development, morphology, and physiology, summarized in Table I. The morphological consequences of UV-B-supplemented white-light treatment include reduced growth, thickening of leaves and of cuticular wax layers. In addition, a lower photosynthetic capacity due to degradation of the D1 protein of photosystem II and reduced pollen fertility have been described for various plant species (Jansen et al., 1998; Caldwell et al., 2003).

Their sessile life style forces plants to adapt to changing environmental conditions. In general, plants respond differently to irradiation with low or high doses of UV-B, either by stimulating protection mechanisms or by activating repair mechanisms to cope with the different types of stress. The most common protective mechanism against potentially damaging irradiation is the biosynthesis of UV-absorbing compounds (Hahlbrock and Scheel, 1989). These secondary metabolites, mainly phenolic compounds, flavonoids, and hydroxycinnamate esters, accumulate in the vacuoles of epidermal cells in response to UV-B irradiation and attenuate the penetration of the UV-B range of the solar spectrum into deeper cell layers with little effect on the visible region. Therefore, humans using sunscreen with UV-absorbing agents mimic ancient plant protection responses.

It is well documented that the responses to low UV-B fluence rates are in part due to transcriptome changes. The molecular underpinnings of UV-B perception and the proposed signaling events set in motion by the proposed UV-B photoreceptor(s) have been reviewed in detail (Jordan, 1996; Jansen et al., 1998; Mackerness, 2000; Brosché and Strid, 2003). In this *Update*, we summarize recent progress on dose-dependent gene expression and on the characterization on putative signaling elements linked to gene expression. In addition, recent genetic approaches have shed some light on novel components that might be involved in the perception of UV-B and in the transduction of signals generated by UV-B.

DNA DAMAGE AND REPAIR EVOKED BY HIGH FLUENCE UV-B

DNA is particularly sensitive to UV-B radiation because absorption of UV-B causes phototransformations, resulting in the production of cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidinone dimers (6-4 PPs). Because DNA and RNA polymerases are not able to read through these photoproducts, their elimination is essential for DNA replication and transcription and thus for survival (Britt and May, 2003).

To avoid the cytotoxic effects of UV-induced DNA damage, most organisms have developed a complex set of repair mechanisms including photoreactivation, excision, and recombination repair. Photoreactivation is a light-dependent enzymatic process using UV-A and blue light to monomerize pyrimidine dimers: Photolyase binds to the photoproducts and then uses light energy to initiate electron transfer to break the chemical bonds of the cyclobutane ring and restore integrity of the bases. Arabidopsis contains photolyases with substrate specificity for either CPDs or 6-4 PPs, respectively (Hoffman et al., 1996; Ahmad et al., 1997). Whereas 6-4 PP photolyase protein is constitutively expressed, CPD photolyase is induced by UV-B (Waterworth et al., 2002).

In cucumber (*Cucumis sativus*), CPD photolyase shows diurnal changes: Transcript levels and enzy-

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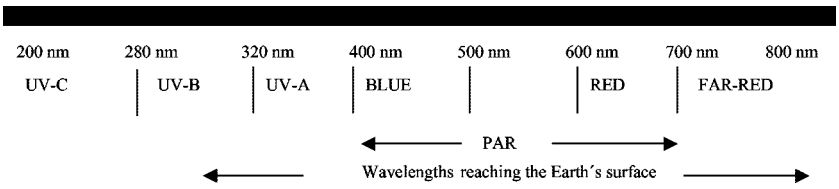

Figure 1. The solar spectrum perceived by higher plants. PAR, Photosynthetically active radiation.

matic activity peak 3 to 6 h into the light period, respectively, and thus are inversely correlated with the growth inhibition elicited by supplementary UV-B irradiation (Takahashi et al., 2002). It has therefore been suggested that fluctuations in CPD repair activity may contribute to alleviating the UV-B induced detrimental effects on leaf growth. Oscillations with a reduced amplitude were also observed when plants were kept in darkness during the day (Takahashi et al., 2002). However, the lack of extended time courses under constant conditions precludes conclusions on whether CPD photolyase oscillations are under endogenous control or not.

Table I. *UV-B-induced alterations in plants*

Data from Jansen et al. (1998) and refs. therein.

	Molecular, Biochemical, and Physiological Effects
DNA	Formation of CPDs and (6–4) PPs
	Induction of repair mechanisms
	Stimulation of homologous recombination
Photosynthesis	Degradation of photosystem II D1 and D2 proteins
	Reduction of activity and amount of Rubisco
	Damage of thylacoid membrane
	Destruction of chlorophyll and carotinoides
Phytohormones	Photooxidation of indolacetamide
Membranes	Peroxidation of lipids
Secondary metabolism	Activation of phenylpropanoid biosynthetic pathway
	Accumulation of UV-protective pigments
Stress responses	Formation of ROS
	Induction of superoxide dismutase, ascorbate peroxidase, and glutathione reductase accumulation of PR-1
Photomorphogenesis	Inhibition of hypocotyl elongation
	Cotyledon opening
	Morphology and anatomy
	Alteration in the composition of epicuticular waxes
	Reduction of leaf surface area
	Increased thickness of leaf
	Shortened internodes
	Branching
	Influence of the whole plant, plant communities, and oecosystems
	Reduction of biomass
	Reduction of crop yield
	Altered competitive balance
	Altered flowering
	Reduced fertility

CPDs and 6-4 PPs can also be removed in the dark through nucleotide excision repair through endonucleolytic cleavage, release of the damaged nucleotides, and strand resynthesis (Liu et al., 2000). This multistep process involving multiple enzymes has been found to operate with only a low capacity in plants (Gallego et al., 2000).

In addition, plants respond to DNA-damaging treatments such as high doses of UV with repair by homologous recombination (Ries et al., 2000a). During UV-B irradiation, the increased homologous recombination frequency correlates with the amount of CPDs formed, and this frequency is significantly enhanced in the photolyase-deficient *uvr2-1* mutant devoid of CPD-mediated photoreactivation (Ries et al., 2000b). These findings implicate homologous recombination in the removal of CPDs. Although homologous recombination in plants is generally classified as a dark repair process, it is stimulated by red but not by far-red exposure after UV-B treatment. These observations indicate that photosynthetic activity or other as yet undefined processes dependent on photosynthetic active radiation (400–700 nm; compare with Fig. 1) may promote UV-B induced homologous recombination in plants (Ries et al., 2000b).

PROTECTIVE RESPONSES ARE EVOKED BY LOW DOSES OF UV-B

Low UV-B fluence rates (<1 μmol m^{-2} s^{-1}) cause no or very low amounts of CPDs that are below the limit of detection but stimulate protective and photomorphogenetic responses (Batschauer et al., 1996; Kim et al., 1998; Frohnmeyer et al., 1999) that affect the plant's resistance to UV-B stress and to other biotic stress types (Kim et al., 1998; Ballaré, 2003).

The most effective protection mechanism stimulated under such a light regime is the biosynthesis of flavonoids and other UV-B-absorbing phenolic components. Their physiological relevance as UV-B sunscreens was confirmed by the UV-B hypersensitive phenotype of mutants devoid of these compounds on the one hand and the increased resistance to UV radiation of mutants with enhanced flavonoid and sinapate levels on the other hand (Li et al., 1993; Landry et al., 1995; Bieza and Lois, 2001).

A similar strategy is employed by cyanobacteria to withstand deleterious UV-B radiation impinging on them. They are thought to use a special class of

compounds with absorption maxima between 310 and 360 nm as UV protectants. In the filamentous and heterocystous N_2-fixing *Anabaene* sp., *Nostoc commune*, and *Scytonema* sp. shinorine, a representative of these mycosporin-like amino acids that are defined by the presence of a cyclohexenone or cyclohexenimine chromophore conjugated with an amino acid or its imino alcohol accumulates in response to solar UV-B radiation, mostly during the daily light period (Sinha et al., 2001).

UV-B-INDUCED PHOTOMORPHOGENESIS

General Responses

Plants grown in UV-exposed locations, i.e. at higher altitudes or geographical latitudes, are commonly more UV-B tolerant than plants grown at places with low UV-B exposure (Jordan, 1996). Such a variety of UV-B tolerance has been even shown between different Arabidopsis ecotypes (Torabinejad and Caldwell, 2000). Many morphological and anatomical changes have been reported from plants grown under long-term UV-B regimes of which the best characterized are summarized in Table I.

Photomorphogenesis in seedlings is largely controlled by red/far-red-absorbing phytochromes (phyA–E) and by blue/UV-A-absorbing cryptochromes (Batschauer, 1999; Quail, 2002). Interestingly, low doses of UV-B also stimulate photomorphogenesis in etiolated seedlings, because the inhibition of hypocotyl elongation and opening of the apical hook are mediated independently of phytochromes and cryptochromes and exhibit a UV-B fluence response relationship (Ballaré et al., 1991, 1995; Kim et al., 1998; Suesslin and Frohnmeyer, 2003).

In parsley (*Petroselinum crispum*) plants as well as in isogenic cell cultures, another UV-B-mediated response—the biosynthesis of flavonoids—has been elaborated in detail. In this case, phytochromes and cryptochromes are modulating the UV-B response but are not sufficient to stimulate increased flavonoid levels without UV-B (Beggs et al., 1986). This response pattern is not confined to parsley but was also described for defined developmental stages of other plant species (Batschauer et al., 1996; Wade et al., 2001) as well as in cell cultures (Beggs et al., 1986).

With respect to circadian rhythmicity, at least in Arabidopsis, the phytochromes phyA, phyB, phyD, and phyE as well as cryptochrome 1 and 2 convey light input to the circadian clock to synchronize the endogenous timekeeper to local time each day (Devlin, 2002; Fankhauser and Staiger, 2002). In contrast, no systematic study has been reported yet to investigate a potential influence of UV-B on the clock.

UV-B Signal Perception

The existence of UV-B receptors has been questioned for decades, although the effect of UV-B on anthocyanin biosynthesis has long been known (Arthur, 1936). Rather, the perception of UV-B radiation has been either connected to the action of phytochromes and cryptochromes, as they partially absorb UV-B, or attributed to DNA, aromatic amino acids, and phospholipids (Beggs et al., 1986). High doses of UV-B or UV-C are damaging to cellular components, and the energy of the radiation is sufficient to cause photochemical changes to a certain set of molecules. This does not involve specific cellular receptors and the deleterious effects of such radiation stimulate general stress responses such as wound signaling (Conconi et al., 1996) or repair mechanisms, i.e. homologous recombination to remove genotoxic substrates (Ries et al., 2000a). Especially the DNA molecule itself has been considered an attractive candidate for a UV-B receptor, and a number of responses in plants and animals were related to UV-B absorption by DNA, because they were maximally stimulated by wavelengths between 250 and 280 nm (Herrlich et al., 1997). However, action spectra of UV-B responses in plants revealed their maximal stimulation between 290 and 310 nm, whereas wavelengths below 290 nm inhibited these responses (Herrlich et al., 1997). In addition, a lack of correlation between the increase of DNA damage (finally caused by UV-B impinging on DNA) and UV-B-elicited changes in transcript profiles contradicts the theory that damaged DNA serves as a UV-B receptor (Kim et al., 1998; Frohnmeyer et al., 1999; Kalbin et al., 2001).

The hypothesis that phytochromes and cryptochromes serve as putative UV-B receptors has also been disproven for most light responses. For example, the hypocotyl elongation response has been exclusively attributed to phytochrome- or cryptochrome action in plants (Mohr and Schäfer, 1983). Studies with mutants devoid of these photoreceptors now demonstrate that UV-B radiation independently affects the hypocotyl elongation response (Kim et al., 1998; Suesslin and Frohnmeyer, 2003). Because some UV-B responses, e.g. chalcone synthase (*CHS*) expression, can be modulated by blue or red light, there is evidence that a complex web exists between phytochrome-, cryptochrome-, and UV-B-signaling chains in cell cultures (Ohl et al., 1989) and plants (Boccalandro et al., 2001; Wade et al., 2001).

The nature of UV-B receptors, however, has been not elucidated so far. In animal cells, a putative receptor seems to be located in the cytosol and might also be attached to membranes (Devary et al., 1993), which is consistent with pharmacological studies in Arabidopsis (Long and Jenkins, 1998). There is large agreement that a UV-B receptor consists of a protein with a bound pterin or flavin as chromophores (Galland and Senger, 1988; Ensminger and Schäfer, 1992). Feeding of parsley cell cultures with radioactively labeled flavins enhanced the amount of UV-B-induced flavonoid end products and has been taken

as a hint that flavins might represent the chromophore of a UV-B receptor in this system (Ensminger and Schäfer, 1992).

Taken together, biochemical or genetic approaches will be useful tools for the isolation of UV-B photoreceptors. However, two premises are necessary to succeed: First, a given response should be specifically stimulated by UV-B to omit possible interaction with phytochrome- or cryptochrome-mediated signaling networks. Second, only low doses of UV-B that generate no or only negligible amounts of DNA damage should be considered to exclude responses unrelated to UV-B photoreceptor action.

Transduction of UV-B Signals

Information on light-mediated signal transduction intermediates has emerged by a combination of cell physiological, biochemical, and genetic approaches. The phytochrome signal transduction pathway regulating *CHS* expression in tomato (*Lycopersicon esculentum*) seedlings and soybean (*Glycine max*) cell cultures has been studied by microinjection and pharmacological agents (Neuhaus et al., 1993; Bowler et al., 1994). These studies indicated that activated phyA transduces the light signal to a trimeric G protein and that second messengers of this pathway include cGMP and Tyr kinases. However, although molecular approaches led to the isolation of several components involved in phyA signaling (Quail, 2002), these molecules are not obviously related with the second messengers found by microinjection studies.

In contrast to the numerous phytochrome- and cryptochrome-signaling components described within the last decade, our knowledge about UV-B-mediated signal transduction is rather limited. One approach to identify such UV-B-signaling components paralleled the early phytochrome studies by using pharmacological agents in cell cultures. Parsley and Arabidopsis cell cultures strongly express *CHS* transcripts within a few hours after UV-B irradiation. The response is much less stimulated by blue light and is completely insensitive to red and far-red irradiation, excluding a preferential action of other photoreceptors during UV-B stimulation (Christie and Jenkins, 1996; Frohnmeyer et al., 1997). In contrast to the proposed components of phytochrome-signaling pathways to *CHS*, cGMP and modulators of Tyr kinases did not affect UV-B-induced *CHS* expression. Moreover, antagonists of calcium, calmodulin, and Ser kinases strongly affected UV-B-mediated *CHS* transcription (Christie and Jenkins, 1996; Frohnmeyer et al., 1997), whereas they did not inhibit phytochrome-mediated *CHS* expression (Bowler et al., 1994). These results were confirmed in soybean cell cultures that exhibit phytochrome and UV-B sensitivity with respect to *CHS* expression and showed that both pathways act independently within a single cell (Frohnmeyer et al., 1998).

The involvement of calcium in UV-B signaling was further addressed in parsley cell cultures. Millisecond UV-B pulses caused an immediate rise of cytosolic calcium lasting for more than 20 min. Increased calcium levels correlated with the subsequent stimulation of *CHS* expression (Frohnmeyer et al., 1999). A target for calcium remains elusive so far, but a possible candidate encoding a calcium-binding protein has been found by screening for early UV-B-induced genes (Loyall et al., 2000).

The participation of Ser kinases during light signal transduction in parsley cell cultures has been shown by different approaches. Irradiation of purified cytosol and membrane fractions stimulates a change of phosphorylation patterns within seconds (Harter et al., 1994a). Such a light-regulated kinase could be involved in the regulation of transcription factor activities because their transfer from the cytosol into the nucleus and their binding affinity to the light-responsive unit of the parsley *CHS*-promoter depend on the phosphorylation status (Harter et al., 1994b). A recent description of UV-B-induced mitogen-activated protein kinase activity in tomato cell cultures (Holley et al., 2003) can be taken as a further proof that kinases play a crucial role during UV-B-mediated signaling.

The second messenger nitric oxide has also been implicated in UV-B-induced *CHS* expression in Arabidopsis (Mackerness et al., 2001). However, the participation of this compound is currently a matter of debate, and definite proof may require further studies (Brosché and Strid, 2003).

Investigations of other UV-B-induced events indicated that reactive oxygen species (ROS) serve as signaling components. UV-B irradiation of plant tissue itself causes the generation of ROS such as singlet oxygen, and Green and Fluhr (1995) demonstrated that the expression of pathogenesis-related proteins (PR-1) is mediated by ROS in tobacco (*Nicotiana tabacum*) leaves. In addition, more UV-B-inducible genes whose expression can be modulated by ROS scavenging have been found in Arabidopsis (Mackerness et al., 2001). Interestingly, ROS is not linked to *CHS* expression (Green and Fluhr, 1995; Frohnmeyer et al., 1997), indicating that at least two different signaling pathways mediate UV-B-induced responses (Fig. 2). The correlation between ROS generation and UV-B-stimulated gene expression is not limited to plants. Pioneer studies in mammalian cell cultures revealed that ROS substitutes UV-B radiation as a stimulus for genes related to cancer proliferation (Devary et al., 1991; Herrlich et al., 1997).

Besides the ROS-responsive pathway and calcium-sensitive pathways, a third nonspecific pathway, activated by high doses of UV-B and/or UV-C, has been proposed (Brosché and Strid, 2003). This pathway might be activated by deleterious effects of high-energy radiation and is possibly linked to wound signal transduction in plants (Conconi et al., 1996).

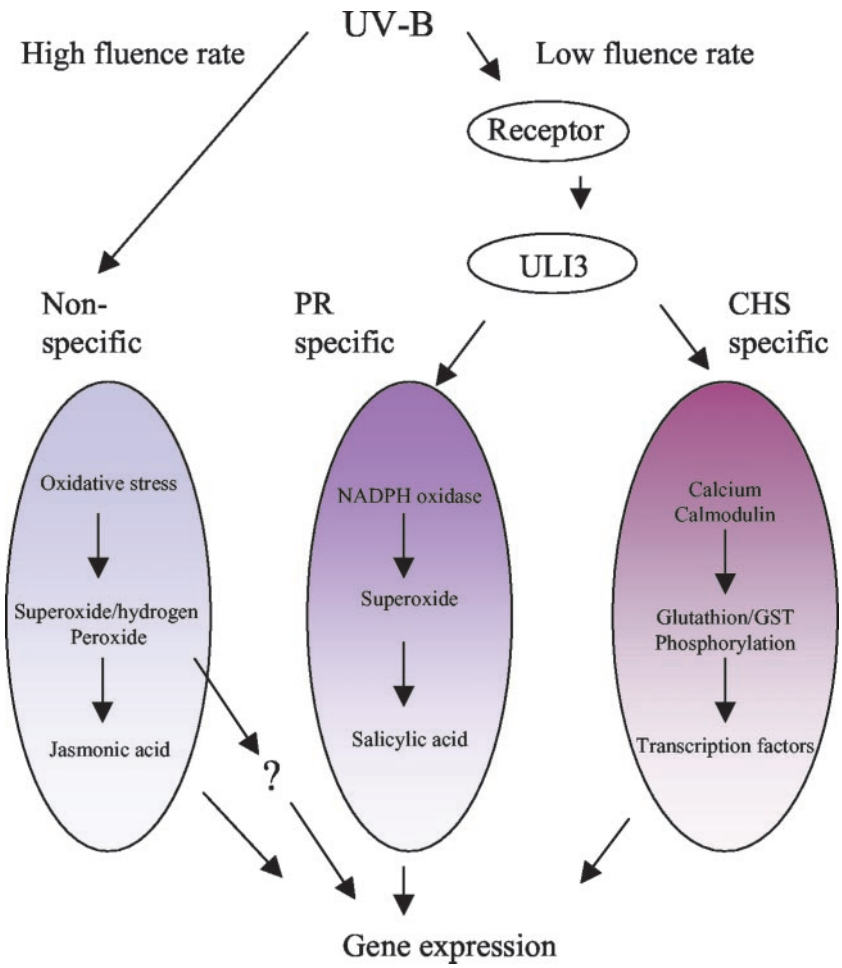

Figure 2. Proposed model for UV-B-mediated signal transduction. The model is modified from Brosché and Strid (2003). PR, Pathogenesis-related protein; GST, glutathione *S*-transferase; ULI3, protein isolated from a UV-light-insensitive Arabidopsis mutant.

Because the response was connected to UV, this pathway was included in the proposed model of UV-B-mediated signal transduction pathways (Fig. 2). However, the demarcation line between a nonspecific pathway stimulated by high doses of UV-B and a PR-1-specific pathway stimulated by low doses of UV-B is not clearly defined so far.

Taken together, at least two independently acting UV-B-specific signal transduction cascades are present in plants that activate different sets of genes (Fig. 2). As will be discussed in the section on genetic approaches, photoreceptor or early signal transduction mutants should be therefore impaired in both of these responses.

TRANSCRIPTOME CHANGES TRIGGERED BY UV-B RADIATION

Changes in gene expression triggered by UV-B largely depend on the dose, as observed for phytochrome- and cryptochrome-mediated responses. An increasing number of studies have investigated UV-B-mediated transcriptome changes associated with the repair of DNA (Ries et al., 2000a), cell cycle control (Logemann et al., 1995), detoxification of ROS (Willekens et al., 1994), adaptation of photosynthetic capacity (for summary, see Jordan, 1996), senescence (John et al., 2001), and the production of protective pigments of phenylpropanoid origin (Beggs et al., 1986; Hahlbrock and Scheel, 1989). Alternatively, transcriptome changes were systematically monitored from plants grown in a UV-B environment in the laboratory (Brosché et al., 2002) or in the field (Casati and Walbot, 2003) using gene profiling assays.

UV-B has also been shown to stimulate a complete biosynthetic pathway consisting of more than a dozen genes. Synchronous transcriptome changes of flavonoid biosynthetic genes have been first described in parsley cell cultures. Early components of this metabolic pathway are transcriptionally activated in a timely coordinated manner within a few hours (Hahlbrock and Scheel, 1989; Ohl et al., 1989; Loyall et al., 2000). Several light-responsive promoters of this pathway were analyzed (Logemann and Hahlbrock, 2002) and a number of conserved cis-acting elements were described that confer light responsiveness. Some of these elements also function as light-responsive units in promoters that control the expression of homologous genes in other species

(Kaulen et al., 1986; Schulze-Lefert et al., 1989; Staiger et al., 1991; Arguello-Astorga and Herrera-Estrella, 1996). A minimal light-responsive unit of the parsley *CHS* promoter consists of one ACGT element that binds basic region/zipper domain proteins and of one motif encoding the recognition sequence of mammalian MYB factors (Weisshaar et al., 1991; Feldbruegge et al., 1997). This unit confers sensitivity to light but is not specific to UV-B. Further studies in Arabidopsis seedlings carrying reporter genes under the control of such a light-responsive unit showed increased reporter gene activity also under phytochrome- or cryptochrome-stimulating light conditions (Batschauer et al., 1996). Therefore, the specificity of responses stimulated by a certain wavelength might be determined by the presence of signaling compounds or the combination of transcription factors available in the cell of interest in addition to the architecture of light-dependent promoters. However, the large multigene families of basic region/zipper domain proteins and MYB proteins comprising about 80 and more than 100 members, respectively, in the Arabidopsis genome, make it difficult to identify the ultimate transcription factors for the regulation of specific phenylpropan and flavonoid biosynthetic enzymes by biochemical approaches.

A way out of these limitations came from genetic approaches originally described from maize and subsequently adapted to Arabidopsis that were designed to find mutants with altered phenylpropan biosynthesis. A few examples of these mutants are discussed below to illustrate the complex regulatory network of transcriptome changes.

While transcription factors binding to light-responsive elements are generally thought to function as activators, at least one MYB-type factor acts as a repressor in snapdragon (*Antirrhinum majus*) and Arabidopsis: Overexpression of Antirrhinum AmMYB308 in tobacco caused the repression of the phenylpropanoid biosynthetic genes cinnamate 4-hydroxylase (C4H) and 4-coumaric acid ligase (Tamagnone et al., 1998). The identification of an Arabidopsis mutant deficient in the AmMYB308 ortholog AtMYB4 resolved the phenomenon. The mutant contains elevated levels of sinapoyl malate due to overexpression of C4H (Jin et al., 2000). As a consequence, the plant is more tolerant to UV-B. In wild-type plants, AtMYB308 transcripts are present in white light but rapidly decline upon UV-B irradiation. The concomitant de-repression of C4H seems to be an important mechanism for acclimation to UV-B.

GENETIC APPROACHES TO STUDY UV-B SIGNALLING

Screens for Arabidopsis mutants with altered sensitivity to a given wavelength of the solar spectrum have been powerful approaches to understand detailed aspects of photomorphogenesis. Such mutants turned out to be either defective in the corresponding phytochromes and blue/UV-A photoreceptors or in the cognate signal transduction compounds (Batschauer, 1999; Quail, 2002).

In the UV-B range, genetic screens were mainly designed to identify hypersensitive mutants with reduced tolerance to UV-B by focusing on the identification of plants defective in the biosynthesis of phenolic sunscreens or DNA repair. The *uvr2-1* mutant is impaired in the CPD photolyase gene *PHR1*, and the *uvr-3* mutant has a nonsense mutation in the 6-4 photolyase gene and is defective in photoreactivation of 6-4 PPs. Notably, both of these mutants are hypersensitive to high doses of UV-B (Landry et al., 1997; Nakajima et al., 1998). More recently, genes involved in nucleotide excision repair were identified, and the combined action of these components provides a detailed picture of the mechanisms underlying DNA repair (Gallego et al., 2000; Liu et al., 2000; Britt and May, 2003).

In contrast, mutants resistant to UV-B (hyposensitive or insensitive) have rarely been described so far. Among these, the *UV-B insensitive 1* mutant was identified by virtue of its rapid growth under UV-B (Tanaka et al., 2002). The increased resistance correlated strongly with elevated photoreactivation of CPDs and elevated dark repair of 6-4 PPs. The *PHR1* transcript encoding CPD photolyase was present at higher levels than in wild type both under white-light conditions and after exposure to UV-B. Although the mutation has not yet been linked to the gene, it is predicted to represent a negative regulator of the two DNA repair pathways (Tanaka et al., 2002).

The high UV-B tolerance of another mutant with a resistant phenotype under elevated UV-B, *UV tolerant 1*, was based on increased basal levels of UV-absorbing flavonoids and sinapate esters. The elevated accumulation of phenolic sunscreens may at least partly be caused by a constitutively elevated *CHS* transcript level (Bieza and Lois, 2001). Although the mutation has not been located yet, the correlation between increased levels of UV-absorbing pigments and UV-B resistance has been proven again.

In contrast to the mutants discussed above that prove the importance of phenolic compounds or of an intact DNA repair system for protection against damaging UV-B, no mutant deficient in a UV-B receptor has been identified so far. The failure to recover such mutants may be due to the choice of light conditions. Screens carried out under UV-B-supplemented white light, which is absorbed by all photoreceptors, may lead to masking of a true UV-B response by other light responses, and high fluence rates of UV-B cause DNA damage that may negatively affect the response of interest. In contrast, low UV-B doses are not affecting other photoreceptors and cause negligible amounts of DNA damage. Physiological studies with several plant species proved

that these low UV-B doses are sufficient to confer photomorphogenesis, i.e. the inhibition of hypocotyl elongation or apical hook opening in etiolated seedlings (Ballaré et al., 1991; Kim et al., 1998; Suesslin and Frohnmeyer, 2003). Such low doses of UV-B were used to identify mutants with defects in UV-B perception or signal transduction, and the results are summarized below (Suesslin and Frohnmeyer, 2003).

Dark-grown seedlings were irradiated with UV-B for 5 min d^{-1}, at a fluence rate that was sufficient to inhibit hypocotyl elongation but was too weak to stimulate flavonoid biosynthesis or increased DNA damage. To ensure that elevated levels of pyrimidine dimers are excluded by this treatment, the hypocotyl elongation was also determined in photolyase-deficient mutants that strongly respond to DNA-damaging irradiation (Kim et al., 1998). Under our screening conditions, no phenotype appeared in the *uvr2-1* mutant.

Several UV-B hyposensitive *uli* mutants were identified from T-DNA collections that exhibited a 50% longer hypocotyl compared with wild-type seedlings. The defect was specific to UV-B and was not attributable to phytochrome or cryptochrome action, because all *uli* mutants were indistinguishable from the wild type after far-red, red, blue, or UV-A treatment. *Uli3* was not only affected in its hypocotyl elongation but was also impaired in *CHS* and *PR-1* gene expression after irradiation with continuous UV-B. The *ULI3* gene is predicted to encode an 80-kD protein with 27% homology to human diacyl glycerol kinases. However, although a conserved 50-amino acid diacyl glycerol-binding domain is present in ULI3, no obvious conserved kinase domains were found. *ULI3* mRNA is already present at low levels in darkness and strongly stimulated by UV wavelength in seedlings. The protein is located in the outer cell layers of cotyledons and hypocotyls but not in roots. Within the cells, it was preferentially localized in the cytosol. Small amounts were attached to membranes. Overall, the phenotypes of *uli3* mutants in combination with the spatial and temporal expression pattern fit the hypothesis that ULI3 is a component of a UV-B-specific signaling pathway. Although *PR-1* and *CHS* expression are mediated by different signal transduction pathways, both are affected in *uli3* mutants. We therefore propose that ULI3 must be an early component of a signaling cascade and might be closely linked to a UV-B receptor (Fig. 2).

CONCLUSIONS

UV-B radiation causes a multitude of responses that are summarized as low- and high-fluence responses similar to phytochrome responses (Kim et al., 1998). Because five different phytochromes and several blue/UV-A-light receptors are present in Arabidopsis that confer light intensity-dependent responses, the question arises whether one hypothesized UV-B photoreceptor is sufficient to mediate all responses. In analogy to phytochromes and cryptochromes, distinct low- and high-fluence responses could be also sensed by different UV-B receptors. A hint comes from the residual sensitivity to UV-B in *uli3* mutants. More mutants with specific defects in UV-B perception and signal transduction are needed to address this question. Generally, the strong relationship of photomorphogenetic responses, DNA damage, and UV-B radiation should be kept in mind. Our increasing knowledge about UV-B-related responses in Arabidopsis (Boccalandro et al., 2001) will enable us to design new screens to isolate mutants with a defective UV-B receptor.

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