Review

Photomorphogenic Responses to Ultraviolet-B light

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ABSTRACT

Exposure to UV-B light regulates numerous aspects of plant metabolism, morphology and physiology through the differential expression of hundreds of genes. Photomorphogenic responses to UV-B are mediated by the photoreceptor UV RESISTANCE LOCUS8 (UVR8). Considerable progress has been made in understanding UVR8 action: the structural basis of photoreceptor function, how interaction with CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) initiates signaling and how REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUP) proteins negatively regulate UVR8 action. In addition, recent research shows that UVR8 mediates several responses through interaction with other signaling pathways, in particular auxin signaling. Nevertheless, many aspects of UVR8 action remain poorly understood. Most research to date has been undertaken with Arabidopsis, and it is important to explore the functions and regulation of UVR8 in diverse plant species. Furthermore, it is essential to understand how UVR8, and UV-B signaling in general, regulates processes under natural growth conditions. UV-B regulates the expression of many genes through UVR8-independent pathways, but the activity and importance of these pathways in plants growing in sunlight are poorly understood.

Key-words: UV-B**;** UVR8; photomorphogenesis; photoreceptor; RUP proteins; COP1; auxin signaling

Short summary

Exposure to UV-B light regulates plant metabolism, morphology and physiology through differential gene expression. This review summarises current understanding of the role of the photoreceptor UVR8 in mediating photomorphogenic responses to UV-B, including its interactions with other signaling pathways and its action under natural growth conditions.

INTRODUCTION

Ultraviolet B (UV-B) wavelengths (280-315 nm) are a minor component of sunlight, but they have a major regulatory impact on plant growth and development. Much of the UV-B radiation and all the highly damaging UV-C radiation that impinges on our planet is absorbed by the stratospheric ozone layer, whereas UV-A wavelengths are transmitted. Only wavelengths above approximately 295 nm are present in the daylight spectrum and the UV-B component is normally less than 1% of incident light. The relative level of UV-B in sunlight is very variable because it is strongly affected by diurnal, seasonal and meteorological factors and also by latitude, altitude and atmospheric pollution (Paul & Gwynne-Jones 2003; Bais *et al.* 2015). In addition, since the ozone layer varies in thickness over the earth, some regions have higher relative levels of UV-B than others. Furthermore, UV-B radiation has complex interactions with environmental factors associated with climate change (Williamson *et al.* 2014), which has important implications for plant distribution.

Although UV-B wavelengths have the potential to damage molecules such as DNA and to impair cellular processes, plants growing in sunlight rarely show signs of UV-damage because they have evolved efficient means of protection. It is well established that UV-B exposure induces an acclimation response that both minimises UV-B penetration into tissues and repairs potential damage by high levels of UV-B (Jansen *et al.* 1998; Frohnmeyer & Staiger 2003; Jenkins 2009). This response includes the well documented synthesis of phenolic compounds that are deposited in epidermal tissues and act as a UV-absorbing sunscreen (Caldwell *et al.* 1983; Li *et al.* 1993; Stapleton & Walbot 1994; Landry *et al.* 1995; Rozema *et al.* 1997). Moreover, the effects of UV-B on metabolism and biochemical content promote defence against pests and enhance nutritional qualities of many plants harvested for food (Ballaré *et al.* 2012; Schreiner *et al.* 2012). In addition, many regulatory effects of UV-B on plant morphology and development have been reported (Klein 1978; Tevini & Teramura

1989; Jordan 1996; Jansen 2002; Robson *et al.* 2015); for instance, UV-B exposure generally reduces extension growth and leaf expansion and increases leaf thickness and axillary branching. Thus, ambient UV-B should be regarded as a key regulatory stimulus that modulates plant metabolism and development and actively promotes viability in diverse ecosystems.

Research in the 1970's demonstrated that low doses of UV-B elicit photomorphogenic responses in plants, which include the inhibition of hypocotyl extension, promotion of cotyledon expansion and stimulation of flavonoid biosynthesis (Wellmann, 1976, 1983; Ballaré *et al.* 1995; Boccalandro *et al.* 2001; Suesslin & Frohnmeyer, 2003; Ryan *et al.* 2001). These observations suggested the existence of a specific UV-B photoreceptor, but little progress was made in its identification for several decades. The application of a genetic approach in Arabidopsis was key, and led to the discovery that mutants in *UV RESISTANCE LOCUS 8* (*UVR8*) are defective specifically in photomorphogenic UV-B responses (Kliebenstein *et al.* 2002; Brown *et al.* 2005; Favory *et al.* 2009). Subsequent functional and structural characterization revealed that UVR8 is a UV-B photoreceptor that mediates photomorphogenic responses and has a unique mechanism of photoreception (Rizzini *et al.* 2011; Christie *et al.* 2012; Wu *et al.* 2012).

There is presently considerable interest in UV-B-mediated photomorphogenesis following the discovery of UVR8. Nevertheless, much remains to be learnt about how UVR8, and UV-B in general, regulates plant processes. The purpose of this article is to summarise current understanding of the regulatory effects of UV-B on plants, highlighting both mechanistic aspects and relevance to plants growing in natural conditions. Further discussion of the topic can be found in several reviews, including: Jenkins (2009; 2014a, b), Heijde and Ulm (2012), Tilbrook *et al.* (2013), Hideg *et al.* (2013), Li *et al.* (2013); Robson *et al.* (2015), Ulm & Jenkins (2015) and Vanhaelewyn *et al.* (2016a).

MULTIPLE MOLECULAR PATHWAYS ARE INVOLVED IN PLANT RESPONSES TO UV-B

Plant responses to UV-B are achieved through the regulation of gene expression (Brosché & Strid 2003, Ulm & Nagy 2005; Jenkins 2009). Transcriptome analyses, in particular with maize (Casati & Walbot 2003, 2004; Casati *et al.* 2006; Casati *et al.* 2011a, b) and Arabidopsis (Brosché *et al.* 2002; Ulm *et al.* 2004, Brown *et al.* 2005, Kilian *et al.* 2007, Oravecz *et al.* 2006; Hectors *et al.* 2007; Brown & Jenkins 2008; Favory *et al.* 2009; Morales *et al.* 2013) have demonstrated that exposure to UV-B differentially regulates the expression of hundreds of genes in diverse functional categories. Nevertheless, the gene lists vary considerably, depending on the age and growth conditions of the plants, the UV-B dose (fluence rate and duration) and the spectral quality of the UV-B source. Low doses of longer wavelength UV-B activate expression principally via UVR8 (Fig. 1). For instance, Favory *et al.* (2009) found that nearly all the genes regulated by exposure of Arabidopsis seedlings to 1 or 6 hours of narrowband UV-B (λ_{max} 312 nm) were under UVR8 control. In contrast, shorter wavelengths and higher doses of UV-B induce additional sets of genes, many of which are in common with those induced by various stress treatments (Ulm *et al.* 2004, Kilian *et al.* 2007, Brown & Jenkins 2008; Fig. 1).

The above studies show that UV-B can regulate gene expression both by UV-B specific, UVR8 photoreceptor mediated signaling, and by activation of pathways that are not specific to UV-B (Brosché & Strid 2003; Jenkins & Brown 2007; Jenkins 2009; Fig. 1). The latter include DNA damage signaling and defence and wound signaling pathways; MAP kinase activity, reactive oxygen species (ROS), salicylic acid, nitric oxide, ethylene and jasmonic acid have all been implicated in UV-B induced gene expression responses (A-H-Mackerness 2000, A-H-Mackerness *et al.* 2001; Jenkins & Brown 2007; Jenkins 2009; Gonzalez Besteiro *et al.* 2011; Tossi *et al.* 2011; Hideg *et al.* 2013; Vanhaelewyn *et al.* 2016a). Moreover, several plant hormone signaling

pathways are involved in both UVR8 mediated and non-specific UV-B responses (Vanhaelewyn *et al.* 2016a).

Clearly it is important to determine which pathways mediate plant UV-B responses in natural growth environments. Do non-specific UV-B pathways regulate gene expression under ambient conditions or are they only activated by non-physiological laboratory treatments? If both UVR8-mediated and non-specific UV-B pathways operate, under what conditions are they active and what is their relative importance to the plant? The answers to these questions are not clear, but some information is available. Undoubtedly UVR8 is a key regulator of gene expression in natural environments because it will mediate acclimation to changing levels of ambient UV-B, both to prevent UV-damage through sunscreen biosynthesis and to repair UV-damage. Moreover, expression of antioxidant genes, mediated substantially by UVR8, primes plants to deal with oxidative stress if they become exposed to high levels of UV-B (Hideg *et al.* 2013). In addition, UVR8 mediates expression of genes concerned with morphological responses and defence. Nevertheless, there is evidence that UVR8 action does not account for all UV-B induced gene expression in plants exposed to sunlight (Morales *et al.* 2013). Gene expression mediated by non-UVR8 pathways is likely to occur in natural conditions in response to fluctuations in levels of UV-B. When plants acclimated to a particular level of UV-B are exposed to a significantly higher level, non-specific UV-B signaling pathways may be activated, leading to altered gene expression. Mild UV-B stress conditions are unlikely to cause damage and may be beneficial if they stimulate the plant to ‘re-assess’ its acclimation status. Robson *et al.* (2015) suggest that both UVR8 and non-UVR8 signaling pathways may regulate morphogenesis in natural conditions. In addition, other processes may be regulated by a combination of UV-B pathways. Various genes, including those encoding the cyclobutane pyrimidine dimer photolyase PHR1 (Li *et al.* 2015) and the E3 ubiquitin ligase ARIADNE12 (Xie *et al.* 2015) are regulated by both UVR8 and non-UVR8 pathways, depending on the spectral quality and fluence rate.

It is important to consider how responses to diurnal fluctuations in ambient UV-B are integrated with underlying circadian regulation. The UV-B induction of many genes is 'gated' by the circadian clock to ensure they are expressed at the appropriate times of day (Feher *et al.* 2011; Takeuchi *et al.* 2014). UVR8 mediates the ability of low fluence rates of UV-B to entrain the Arabidopsis circadian clock and this involves the regulation of genes encoding clock components (Feher *et al.* 2011). Interestingly, Takeuchi *et al.* (2014) observed that in Arabidopsis, sensitivity to a brief UV-B stress treatment is greater during the night than the day and that the circadian clock regulates this sensitivity. However, *uvr8* mutant plants were not altered in their sensitivity to the short stress-inducing UV-B treatment (Horak & Farré 2015). Thus, both UVR8-dependent and UVR8-independent UV-B signaling pathways may operate at particular times of day in response to different fluence rates of UV-B to regulate acclimation and stress protection in natural growth conditions. The balance of pathway activation will depend on several factors: the adaptation of a particular genotype to its UV-B environment, the extent of acclimation to the ambient level of UV-B, the type and timing of UV-B exposure (in particular the fluence rate and duration in natural conditions) and the presence of other abiotic and biotic factors (Jenkins 2009; Fig. 1).

MOLECULAR BASIS OF UVR8 ACTION

Initial characterization of UVR8 mediated responses: UVR8 functions with COP1 and HY5

UVR8 was originally identified in a genetic screen for Arabidopsis mutants hypersensitive to UV-B (Kliebenstein *et al.* 2002). Further alleles were obtained in screens for mutants impaired in UV-B induced luciferase expression driven by the promoters of either the *CHALCONE SYNTHASE* (*CHS*; Brown *et al.* 2005) or *ELONGATED HYPOCOTYL 5* (*HY5*; Favory *et al.* 2009) genes. Initial characterization of *uvr8* mutant plants showed that they are altered specifically in

responses to UV-B (Brown *et al.* 2005). In addition, transcriptome analysis explained why *uvr8* has reduced viability under UV-B illumination; the mutant fails to induce genes concerned with UV-protection, including flavonoid biosynthesis, DNA repair and antioxidant activity. Another important finding was that UVR8 mediates the rapid, UV-B induced expression of the HY5 transcription factor and the closely related HY5 HOMOLOG (HYH) (Brown *et al.* 2005). HY5 is the major effector of UVR8 action, in that it regulates transcription of numerous downstream target genes, but in several cases it functions redundantly with HYH (Brown & Jenkins 2008). A further important discovery was that CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1) acts as a positive regulator of responses to UV-B mediated by UVR8 (Oravecz *et al.* 2006). Moreover, transcriptome analysis revealed extensive overlap in the sets of genes regulated by UVR8 and COP1, with both proteins required for *HY5* and *HYH* expression (Favory *et al.* 2009). Thus, the model of UVR8 action that emerged from these initial studies was that UVR8 and COP1 function together in the same pathway upstream of the transcriptional effectors HY5 and HYH (Brown *et al.* 2005; Oravecz *et al.* 2006; Brown & Jenkins, 2008; Favory *et al.* 2009). Furthermore, Favory *et al.* (2009) made the key observation that UV-B exposure induces a physical interaction between UVR8 and COP1. Current models of UVR8 action (see Fig. 2) are based on these initial findings.

Photoreception by UVR8

The structure of UVR8 and its mechanism of photoreception have been discussed in detail in several previous reviews (Tilbrook *et al.* 2013; Jenkins 2014a, b; Yang *et al.* 2015) and will only be summarized here. X-ray crystallography (Christie *et al.* 2012; Wu *et al.* 2012) revealed that UVR8 is a 7-bladed β-propeller protein, as expected from the gene sequence obtained by Kliebenstein *et al.* (2002). UVR8 exists as a homo-dimeric protein in the absence of UV-B (Rizzini *et al.* 2011; Christie *et al.* 2012; Wu *et al.* 2012). The dimer is held together by salt-bridge interactions between

charged amino acids across the dimer interface. Mutational analysis identified particular amino acids that are key to maintaining the dimer structure, notably arginines R286 and R338, which interact with specific aspartate and glutamate residues on the opposing monomer (Christie *et al.* 2012; Wu *et al.* 2012; Heilmann *et al.* 2016). UV-B photoreception results in neutralization of these interactions, leading to dissociation of the dimer into monomers (Fig. 2). As discussed further below, this process is crucial because monomeric UVR8 initiates signaling.

UVR8 is unique among photoreceptors in that it does not use an attached chromophore for the absorption of specific wavelengths. Instead, tryptophan amino acids in the primary sequence of UVR8 absorb UV-B, essentially acting as intrinsic chromophores. A cluster of tryptophans located at the dimer interface is crucially important in photoreception. In particular, mutation of either tryptophan W233 or W285 to phenylalanine essentially prevents UVR8 functioning as a UV-B photoreceptor (Rizzini *et al.* 2011; Christie *et al.* 2012; Wu *et al.* 2012; Liu *et al.* 2014; Miyamori *et al.* 2015; Zeng *et al.* 2015). Similarly, responses to UV-B are strongly impaired in plants expressing these UVR8 mutants (O'Hara & Jenkins 2012; Heijde *et al.* 2013; Huang *et al.* 2013, 2014). Hence W233 and W285 have essential, non-redundant functions in UVR8 photoreception. These tryptophans are closely associated with key salt-bridge amino acids in the dimer interface (Christie *et al.* 2012; Wu *et al.* 2012). Computational modeling (Wu *et al.* 2014; Li *et al.* 2014; Voityuk *et al.* 2014) and experimental studies (Liu *et al.* 2014; Mathes *et al.* 2015) indicate that proton coupled electron transfer (PCET) from chromophore tryptophans to adjacent charged amino acids neutralises key cross-dimer salt-bridges. Mathes *et al.* (2015) used time-resolved absorption and fluorescence spectroscopy to monitor sub-second photochemical processes following UV-B absorption by UVR8, and proposed that PCET from W285 neutralises the salt bridges involving R286 and aspartates D96 and D107. In addition, low temperature dynamic crystallography revealed that photoreception causes reorientation of the indole rings of W233 and W285, resulting

in the ejection of a water molecule involved in formation of hydrogen bonds between W285, R286 and D96, weakening the network of interactions that maintain the dimer (Zeng *et al.* 2015).

UVR8 signal transduction

Importantly, Rizzini *et al.* (2011) found that UVR8 monomers generated by UV-B photoreception are able to interact with COP1 to initiate signaling (Fig. 2). Several studies provide evidence that conformational changes accompany UVR8 monomer formation (Rizzini *et al.* 2011, Heilmann *et al.* 2014, Miyamori *et al.* 2015, Zeng *et al.* 2015) and likely facilitate the binding of COP1. A key region of UVR8 involved in the UV-B dependent interaction with COP1 lies towards the C-terminus of the protein. This 27 amino acid region, termed C27, interacts with the WD40 domain of COP1 and is required for UVR8 function *in vivo* (Cloix *et al.* 2012, Yin *et al.* 2015). However, the mechanism of interaction of UVR8 and COP1 is not fully understood (see below).

The finding that COP1 has a positive role in UV-B responses (Oravecz *et al.* 2006) was unexpected because it is a well known repressor of photomorphogenesis (Lau & Deng 2012; Huang *et al.* 2014a). COP1, bound to a SUPPRESSOR OF PHYA-105 (SPA) protein, acts as a substrate receptor for E3 ubiquitin-ligase complexes that degrade positive regulators of light responses, including HY5 (Osterlund *et al.* 2000; Zhu *et al.* 2008; Lau & Deng, 2012; Huang *et al.* 2014a). The binding of COP1-SPA to UVR8 reduces its association with CUL4-DDB1 (Huang *et al.* 2013; Fig. 2). Hence, the sequestration of COP1 reduces its ability to mediate targeted proteolysis, and consequently HY5 is stabilized following UV-B exposure (Favory *et al.* 2009; Huang *et al.* 2013). Whether this mechanism entirely explains the accumulation of HY5 under UV-B illumination is not clear, because there is evidence that COP1 is required to prevent HY5 degradation by an unidentified proteolytic activity under UV-B exposure (Huang *et al.* 2013).

The stabilization of HY5 under UV-B conditions in wild-type plants stimulates further HY5 accumulation because the protein positively regulates its own transcription (Binkert *et al.* 2014). In addition, HY5 is involved in the stimulation of *COP1* transcription (Huang *et al.* 2012) and COP1 protein is stabilized following UV-B exposure (Favory *et al.* 2009; Heijde *et al.* 2013; Huang *et al.* 2014b). Together these elements of regulation generate a positive feedback loop on HY5 expression, facilitating the transcription of UVR8 target genes (Fig. 2). Some mutant forms of UVR8 are altered in COP1 binding, which may have functional consequences. This is seen with alanine mutants of either tryptophan W285 or arginine R338: constitutive interaction of $UVR8^{W285A}$ and $UVR8^{R338A}$ with COP1 causes COP1 sequestration and HY5 stabilization, resulting in plants with a partial *cop* mutant phenotype (short hypocotyls and open cotyledons in seedlings) (Heijde *et al.* 2013; Huang *et al.* 2014b). This phenotype is exaggerated when the $UVR8^{W285A}$ mutant is over-expressed (Heijde *et al.* 2013).

UVR8 is localized principally in the cytoplasm in plants that have never been exposed to UV-B, but rapidly accumulates in the nucleus following treatment with low doses of UV-B (Kaiserli & Jenkins 2007). In principle, nuclear accumulation could be achieved through translocation into the nucleus, but also by retention in the nucleus if there is cycling between the nucleus and cytoplasm. UVR8 does not possess an obvious nuclear localization signal (NLS) and therefore translocation into the nucleus would require interaction with another protein containing a NLS. Yin *et al.* (2016) recently reported that COP1 is required for nuclear accumulation of UVR8 and proposed that COP1, which has a NLS, is directly involved in mediating nuclear translocation of UVR8 through a co-import mechanism. It is also possible that UVR8 is retained in the nucleus when it is active in signaling in association with COP1. Moreover, since it takes many hours for the photoreceptor to re-accumulate in the cytoplasm when UV-B treated plants are returned to darkness (Kaiserli & Jenkins

2007), it is likely that UVR8 remains in the nucleus in plants growing under standard photoperiodic conditions.

Negative regulation of UVR8

Photoreception initiates the positive pathway of UVR8 action entailing monomer formation, binding to COP1 and induction of downstream transcriptional responses. However, a negative feedback mechanism is in place to constrain UVR8 action and thus prevent hyper-activation of responses (Fig. 2). Such hyper-activation is seen in transgenic plants that over-express UVR8 and consequently display increased hypocotyl growth suppression and gene expression under UV-B conditions (Favory *et al.* 2009; Heijde *et al.* 2013). Negative regulation of UVR8 is mediated by the REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUP) proteins, RUP1 and RUP2 (Gruber *et al.* 2010). A *rup1rup2* double mutant displays similar hyper-activation of UVR8 signaling to that seen in plants over-expressing UVR8. *RUP* gene expression is stimulated by UV-B exposure via UVR8 signaling (Gruber *et al.* 2010). Thus, UVR8 initiates the negative feedback mechanism that limits its own action.

The RUP proteins bind to the same C-terminal region of UVR8 as COP1 and impair COP1 binding (Cloix *et al.* 2012; Heijde & Ulm 2013). There is no information on the relative affinities of COP1 and RUP proteins for binding to UVR8 and it may be that a relative increase in amount of RUPs following UV-B exposure leads to their increased association with UVR8 and the displacement of COP1. In addition, the RUP proteins mediate re-dimerisation of UVR8 monomers (Heijde & Ulm 2013; Fig. 2). Monomers of purified UVR8 re-associate very slowly *in vitro*, taking 24 to 48 hours for completion, whereas the process occurs within less than an hour *in vivo* (Heilmann & Jenkins 2013). Re-dimerisation is much slower in *rup1rup2* mutant plants than in wild-type (Heijde & Ulm 2013; Findlay & Jenkins 2016). Thus, the RUPs constrain UVR8 action through a combination of COP1 displacement and reversion of the signaling active monomers to the dimeric form.

Some questions regarding UVR8 action

Important questions remain to be addressed regarding virtually every aspect of the molecular mechanisms of UVR8 action: UV-B absorption and primary photochemistry, conformational changes, interactions between proteins, nuclear localization, HY5 stabilization and regulation, and downstream transcriptional control. Some of these issues are discussed below.

One question concerns the role of the UVR8 tryptophans. The 14 tryptophans in UVR8 are highly conserved in number and position in sequences from diverse species (Wu *et al.* 2011; Rizzini *et al.* 2011; Fernández *et al.* 2016), but their functions are not entirely clear. There is evidence both from the crystal structure and mutant characterization that the ring of 6 tryptophans in the core of the protein helps to maintain the β-propeller structure (O'Hara & Jenkins 2012). In addition, as outlined above, it is evident that W233 and W285 are of crucial importance in the mechanism of photoreception. However, mutational studies of other tryptophans in the dimer interface have provided little insight into their roles in plants (O'Hara & Jenkins, 2012). For example, W94 and W337 are very closely associated with the chromophore tryptophans W233 and W285, but mutation of these tryptophans has relatively little effect on UVR8 function (Christie *et al.* 2012; Wu *et al.* 2012; O'Hara & Jenkins, 2012). Based on calculations of the absorption spectra of individual tryptophans, a light-harvesting hypothesis has been proposed, whereby peripheral tryptophans excited by UV-B would transfer exciton energy principally to W233 (Wu *et al.* 2015; Yang *et al.* 2015). Such a mechanism could potentially increase the photoreception quantum efficiency of UVR8 and broaden the range of UV-B wavelengths over which it functions. Some biophysical experiments with purified UVR8 protein provide support for this hypothesis (Liu *et al.* 2014) whereas others do not (Mathes *et al.* 2015), but the key question is whether the mechanism operates *in vivo*. In this respect, it is irrelevant whether UVR8 *in vitro* can absorb UV-B below 295 nm, because only

wavelengths above approximately 295 nm are present in the daylight spectrum. Action spectroscopy shows that UVR8 can function at 310 nm in plants (Brown *et al.* 2009) and some activity at longer wavelengths is feasible. Hence, detailed photobiological studies with selected single and multiple tryptophan mutants will be required to determine the roles of the tryptophans in UVR8 action *in vivo*.

A second important question concerns the *in vivo* significance of UVR8 monomer photoreception. In principle, monomeric UVR8 should be capable of photoreception, in that it contains the necessary tryptophans for UV-B absorbance. Indeed, constitutively monomeric mutants of UVR8 exhibit UV-B induced spectroscopic signals similar to the wild-type *in vitro* (Heilmann *et al.*, 2014; Mathes *et al.*, 2015; Miyamori *et al.*, 2015). Furthermore, there is now evidence that a mutant UVR8 protein that is strongly impaired in dimer formation is able to mediate UV-B responses *in vivo* similarly to wild-type UVR8 (Heilmann *et al.* 2016). Aspartates D96 and D107 form salt-bridges with R286 that are crucial in dimer formation, and conservative mutation of these amino acids to asparagine makes the UVR8 protein constitutively monomeric *in vitro*. Similarly, when expressed in plants, only monomeric UVR8^{D96N,D107N} protein can be detected in extracts, and any dimer formation in cells is likely to be weak and transient. Nevertheless, the UVR8^{D96N,D107N} mutant mediates UV-B induced gene expression and hypocotyl growth suppression with similar dose-response efficiency to wild-type UVR8 (Heilmann *et al.* 2016). These findings raise the question of whether monomeric UVR8 in wild-type plants could also act in photoreception to mediate responses, or whether only monomer formed by dimer photoreception is active. This will be a difficult question to answer if there is no physiological difference between the activity of the monomeric forms derived from dimer and monomer photoreception, but further examination of monomeric mutants may help to address this point.

Another key question is: how does UVR8 interact with COP1? UVR8 interacts with the COP1 WD40 domain via its C27 region (Cloix *et al.* 2012; Yin *et al.* 2015), as

mentioned above, and amino acids valine V410 and proline P411 are essential for this interaction (Yin *et al.* 2015). There is evidence that COP1 can also interact with the β-propeller core of UVR8: UVR8 either lacking the C27 region or with both V410 and P411 mutated to alanine can still interact with COP1 (Yin *et al.* 2015). It would not be surprising if a large protein such as COP1 made physical contact with UVR8 in more than one position, but the extent of interaction with the core appears to be relatively weak in plants when the C-terminal region is absent (Yin *et al.* 2015). Moreover, interaction with the core is not detectable under high stringency conditions (Cloix *et al.* 2012). Yin *et al.* (2015) speculated that the WD40 domain of COP1 may interact with the dimerization surface of UVR8 exposed following monomerisation and that the C-terminus facilitates this interaction. While this is an attractive model, it is not yet clear whether the C-terminus can fulfill this role. The position of the C-terminus is unknown because it is not represented in the crystal structures of UVR8. Small-angle X-ray scattering data suggest that the C-terminus may be located distal to the interaction surface in the dimer (Christie *et al.* 2012). In addition, it has been suggested that the C-terminus could interact with the N-terminus of UVR8 in the dimer to form a β-sheet structure that impairs interaction with COP1 (Yang *et al.* 2015). It is not known how structural changes associated with photoreception and monomerisation, some of which may be substantial (Zeng *et al.* 2015), might affect the location of the C-terminus. Structural changes to the β-propeller core of UVR8 following photoreception are observed in mutants lacking the C-terminal region (Heilmann *et al.* 2014; Miyamori *et al.* 2015), but in the context of the intact protein such changes could influence the position of the C-terminus and its availability for binding to COP1. There is evidence from antibody binding (Rizzini *et al.* 2011) and limited proteolysis experiments (Heilmann *et al.* 2014) that the C-terminus becomes more accessible after monomerisation. Clearly, further information, both on the location of the C-terminus

and on conformational changes to UVR8 following monomerisation, is required to reveal how the protein interacts with COP1.

A further unresolved question is: how does UVR8 regulate transcription? *In vitro* experiments showed that UVR8 binds quite strongly to histones immobilized on agarose beads (Brown *et al.* 2005), which prompted investigation of *in vivo* chromatin association. Chromatin immunoprecipitation (ChIP) experiments provided evidence that UVR8 associates with genomic sequences of some genes it regulates, such as *HY5* (Brown *et al.* 2005; Kaiserli & Jenkins 2007; Cloix & Jenkins 2008; Cloix *et al.* 2012), but not others (Cloix & Jenkins 2008), raising uncertainty about the specificity of the interaction (Jenkins 2014a). The UVR8 ChIP signal is weak and at the limits of detection in standard ChIP experiments, and it is therefore difficult to know whether it represents specific or non-specific binding. Binkert *et al.* (2016) questioned whether UVR8 has any association with chromatin; in ChIP experiments with wild-type Arabidopsis and a line over-expressing UVR8 they did not detect a significant association of UVR8 with *HY5* and *MYB12* target sequences. They showed that HY5 gives a much stronger ChIP signal than UVR8, which is consistent with the strong binding of transcription factors to DNA compared to the relatively weak interaction of proteins with histones. It would therefore be interesting to assess UVR8 association with chromatin under conditions that permit the detection of mild interactions. Thus, it remains unclear whether the weak association of UVR8 with chromatin observed in previous reports represents a non-specific association or a biologically meaningful interaction. Interestingly, there is evidence that regulation of transcription mediated by UVR8 involves a specific histone modification. It was reported that UV-B exposure increases acetylation of lysines K9 and/or K14 of histone H3 associated with genes regulated by UVR8 (Cloix & Jenkins 2008). Velanis *et al.* (2016) found that this increase in acetylation is dependent on UVR8. Furthermore, ChIP sequencing showed that all UV-B induced enrichment of H3K9,K14 in the genome is dependent on UVR8. While this study provides information on the processes through which UVR8 regulates

transcription, it does not explain how UVR8 influences histone acetylation. The identity of the histone acetyltransferase(s) involved in UVR8 mediated transcription is unknown. Histone acetyltransferases are components of protein complexes associated with chromatin (Lee & Workman 2007), but how UVR8 might affect the activity of such a complex is an open question.

RESPONSES MEDIATED BY UVR8: INTEGRATION WITH OTHER PATHWAYS

Following the discovery of UVR8, researchers started to use *uvr8* mutant plants to investigate whether the photoreceptor is involved in a variety of UV-B responses. Initial studies demonstrated that UVR8 mediates the suppression of hypocotyl extension by low fluence rates of UV-B (Favory *et al.* 2009) and plays a role in the regulation of leaf expansion by UV-B (Wargent *et al.* 2009). Subsequent studies extended the list of responses mediated by UVR8 (Table 1) and the number will likely continue to grow.

Interestingly, evidence is emerging of a role for UVR8 in plant defence. UV-B exposure of plants increases resistance to attack by various pests and pathogens (Roberts and Paul 2006; Ballaré *et al.* 2012), and this is principally due to biochemical changes, in particular the synthesis of inhibitory or unpalatable compounds. UVR8 action contributes to some of these protective responses. Morales *et al.* (2013) found that UVR8 mediates the UV-B induced expression of several genes concerned with countering attack by herbivorous pests in Arabidopsis. In addition, the ability of UV-B exposure to reduce infection by the fungus *Botrytis cineria* is diminished in *uvr8* mutant plants (Demkura & Ballaré 2012). This protection is likely due to UVR8-induced synthesis of sinapic acid derivatives, because the protective response is also absent in plants defective in sinapate biosynthesis. A recent report suggests that UVR8 may be involved in systemic acquired resistance (SAR) in Arabidopsis (Carella *et al.* 2016). The abundance of UVR8 in phloem exudates of leaves decreased

following infection with strains of *Pseudomonas syringae* that induce SAR, relative to controls. Both *uvr8* mutant and UVR8 over-expression lines showed reduced SAR compared to wild-type, and the authors suggested that UVR8 might have distinct positive and negative regulatory roles in SAR. However, the experiments were reportedly undertaken in light conditions lacking UV-B, so it is not clear how UVR8 might be acting in this response.

A recurrent theme in recent research is that UVR8 often functions through interaction with other signaling pathways. In particular, several studies highlight an interaction between UVR8 and the hormonal pathways that regulate extension growth. One example is the role of UVR8 in suppressing the shade avoidance response. Many plant species respond to the presence of neighbouring vegetation by stimulating extension growth as a result of increased auxin biosynthesis. Leaves absorb red light but reflect far-red light, and therefore shading by vegetation leads to a relative decrease in the ratio of ambient red:far-red light, which is detected by phytochrome, causing a decrease in Pfr relative to Pr (Casal 2013; Fraser *et al.* 2016). In turn, the decrease in Pfr/Pr leads to an increase in stability and activity of several PHYTOCHROME INTERACTING FACTOR (PIF) transcription factors, notably PIFs 4, 5 and 7, which stimulate expression of auxin biosynthesis genes, leading to extension growth (Hornitschek *et al.* 2012; Li *et al.* 2012). Hayes *et al.* (2014) showed that UV-B antagonizes shade avoidance responses in Arabidopsis elicited by low red:far-red light, and the UV-B effect was strongly impaired in *uvr8* mutant plants. UV-B, detected by UVR8, inhibited the increase in expression of auxin biosynthesis and signaling genes promoted by reduced red:far-red light. Furthermore, UVR8 signaling stimulated *GA2OXIDASE1* expression, which causes reduced levels of gibberellic acid and consequent stabilization of DELLA proteins, which antagonize PIF activity (De Lucas *et al.* 2008; Feng *et al.* 2008). Whereas the effect of UV-B on *GA2OXIDASE1* expression required HY5/HYH, that on the auxin related genes did not. The experiments further showed that UV-B elicited destruction of PIFs 4 and 5 and the

stabilization of DELLA proteins, although it remains to be established directly whether the effects on these proteins are mediated by UVR8. Thus, UV-B, detected by UVR8, signals to plants that they are in sunlight and negates shade-induced extension growth by antagonizing PIF action and auxin biosynthesis.

UV-B also inhibits the morphogenic responses caused by exposure to elevated temperature, which include hypocotyl extension in seedlings and petiole extension and leaf elevation in mature plants; again the effect of UV-B is substantially mediated by UVR8 (Hayes *et al.* 2016). However, in contrast to the action of UV-B in suppressing shade avoidance, UV-B inhibition of thermomorphogenesis does not involve either PIF destruction or an effect on DELLA proteins. PIF4 is a key regulator of thermomorphogenesis, promoting expression of genes concerned with auxin biosynthesis and signaling. UV-B inhibits *PIF4* transcript accumulation, consequently preventing an increase in PIF4 protein, and also stabilizes the LONG HYPOCOTYL IN FAR-RED 1 (HFR1) transcription factor, which binds to PIF4, impairing its ability to bind to DNA. Together these mechanisms block the accumulation and activity of PIF4 at elevated temperature (Hayes *et al.* 2016). The inhibition of thermomorphogenesis by UV-B is likely to be advantageous for plants, as it will prevent detrimental extension growth under natural conditions where elevated temperature is often accompanied by exposure to relatively high levels of UV-B.

Another auxin-regulated growth response is phototropism. It is well established that phototropism in response to unilateral UV-A/blue light is mediated by phototropins, which direct accumulation of auxin on the non-illuminated side of the stem, causing localized extension and hence bending towards the light source (Christie & Murphy 2013). Vandenbussche *et al.* (2014) reported that UV-B can also induce phototropic bending, and that the UV-B response in *phot1phot2* mutant plants requires UVR8. However, UV-B induced bending is slower in *phot1phot2* than in wild-type, indicating that phototropin action is involved in the wild-type UV-B response, and that the phototropin mediated response is faster than that mediated by UVR8

(Vandenbussche *et al.* 2014; Vandenbussche & Van Der Straeten 2014). Moreover, the response mediated by phototropin is initiated at lower fluence rates than that mediated by UVR8 (Vanhaelewyn *et al.* 2016b). The UV-B induced phototropic response involves the establishment of an auxin gradient across the hypocotyl, as in the UV-A/blue light response, but formation of the gradient in UV-B does not require phototropins and involves some different auxin signaling components to phototropism mediated by UV-A/blue light (Vandenbussche *et al.* 2014). UVR8 mediates repression of genes involved in auxin biosynthesis and signaling, which likely contributes to the generation of the auxin gradient across the hypocotyl. Vandenbussche & Van Der Straeten (2014) showed that the accumulation of HY5 on the UV-B exposed side of the hypocotyl (demonstrated using a HY5-YFP fusion) correlated with UVR8 response kinetics, and is likely to mediate the repression of auxin biosynthesis genes on the illuminated side.

A further response involving UVR8 and auxin signaling is leaf epinasty, which is the downward curling of leaf edges away from incident light. Epinasty is stimulated by UV-B exposure (Wilson & Greenberg 1993; Jansen 2002) and also by the action of phyB, whereas phototropins promote leaf flattening (Kozuka *et al.* 2013). Fierro *et al.* (2015) showed that the epinastic response to UV-B in Arabidopsis is mediated by UVR8, most likely through the regulation of auxin transport. Moreover, they found considerable overlap in the sets of genes regulated by UVR8 and phyB, notably in the repression of genes involved in auxin action. The phyB action in epinasty involves the regulation of specific PIFs (Johansson & Hughes 2014), and there is evidence that PIFs are required for the UV-B induced response (Fierro *et al.* 2015). A possible scenario is that UV-B de-stabilises PIFs, as in the inhibition of shade avoidance, causing the repression of auxin response genes and consequently initiating the changes in auxin transport associated with the epinastic response.

Fasano *et al.* (2014) highlighted the potential interactions between UVR8 and abiotic stress signaling pathways and proposed that the cross-talk may involve auxin

signaling. They reported that high salt and osmotic stress stimulate UVR8 expression and that a *uvr8* mutant has increased salt tolerance under UV-B conditions. In addition, the reduced extension growth of plants over-expressing UVR8, previously observed by Favory *et al.* (2009), was enhanced under osmotic stress. Fasano *et al.* (2014) found that the UVR8 over-expression phenotype is due to reduced cell expansion and suggested that the phenotype could be explained by altered auxin signaling. Abiotic stresses such as drought, salinity and high temperature will often be accompanied by relatively high fluence rates of UV-B in nature, and the interplay between UVR8 signaling and auxin signaling could be modulated under such conditions to regulate growth and promote survival.

The stimulation of stomatal closure by UV-B involves interaction of UVR8 with different signaling pathways to those that regulate growth responses. In species such as *Vicia faba* (Jansen & Noort 2000) and Arabidopsis (Eisinger *et al.* 2003; He *et al.* 2013; Tossi *et al.* 2014), low fluence rates of UV-B stimulate stomatal opening whereas higher fluence rates promote closure. He *et al.* (2013) showed that the closure response in Arabidopsis is mediated by an increase in H_2O_2, generated through NADPH oxidase activity. UV-B induced cytosolic alkalinisation is involved in mediating the increase in H_2O_2 production (Zhu *et al.* 2014). In turn H_2O_2 stimulates nitric oxide (NO) production (He *et al.* 2013). Inhibition of endogenous NO accumulation prevents closure even under conditions where H_2O_2 remains high (Tossi *et al.* 2014). Tossi *et al.* (2014) found that UV-B induced stomatal closure is impaired in *uvr8*, with a concomitant reduction in H_2O_2 and NO accumulation in the guard cells. Nevertheless, the mutant stomata were viable and they closed when either a NO donor or abscisic acid was added. It is likely that UVR8 acts to promote H_2O_2 and hence NO accumulation, but it is not clear how it does so. The UVR8 action likely involves gene expression, because a mutant lacking the HY5/HYH transcription factors is impaired in the closure response (Tossi *et al.* 2014), but the relevant target genes are not known.

The ability of UVR8 to influence auxin and gibberellic acid signaling, as well as redox signaling, is likely to affect a larger number of physiological processes than reported to date. Furthermore, it is likely that interactions between UVR8 and additional signaling pathways will be discovered. UVR8 photoreception leads to sequestration of COP1 and stimulation of HY5 accumulation, and both these proteins participate in a range of cellular processes (Lau & Deng 2012; Huang *et al.* 2014a; Gangappa & Botto 2016). For instance, COP1 is involved in controlling abundance of the flowering time regulator CONSTANS (Jang et al. 2008; Liu et al. 2008; Sarid-Krebs et al. 2015) and hence UVR8 activation might influence flowering time, as suggested in some studies (Morales *et al.* 2013; Fasano *et al.* 2014). HY5 binds to over 9000 genomic loci in Arabidopsis (Zhang *et al.* 2011) and regulates genes in numerous processes (Gangappa & Botto 2016). Thus, regulation of HY5 provides a potential mechanism for UVR8 to influence several aspects of plant physiology. Fig. 3 illustrates some of the known and potential interactions involving UVR8.

HOW DOES UVR8 FUNCTION IN NATURAL GROWTH CONDITIONS?

To date, most research on UVR8 has been undertaken with either the purified photoreceptor protein or Arabidopsis plants grown and treated in rather artificial conditions. Clearly, it is important to understand how UVR8 works in natural growth environments, where plants grow under photoperiodic cycles usually with much higher levels of UV-A and photosynthetically active radiation (PAR) than in growth chambers. One of the first steps in this direction was taken by Morales *et al.* (2013), who examined transcriptome profiles and metabolite accumulation in greenhouse-grown wild-type and *uvr8* mutant plants transferred to sunlight, using filters to prevent some plants being exposed to UV-B, or both UV-B and UV-A, in the daylight spectrum. Several of the gene expression and metabolite accumulation responses mediated by UVR8 were similar to those reported previously in experiments in growth cabinets, but

there were some notable differences, including increased expression of some UV-regulated genes in the *uvr8* mutant. The experiments indicate that some UV-B action is mediated by non-UVR8 pathways and also that the presence of UV-A and/or PAR can modulate UV-B responses. In addition, evidence was presented that UVR8 can modify gene expression and accumulation of particular metabolites mediated by UV-A, suggesting cross-talk between UVR8 and cryptochrome signaling. The findings reveal complexity in responses to UV light in natural sunlight that likely arise from interactions between different photoreceptor signaling pathways and demonstrate that further studies are needed to explore UVR8 action in natural conditions.

To understand how UVR8 functions in natural growth conditions it is important to know how the amount of the signaling-active monomer is regulated. If UVR8 acts as a simple dimer-to-monomer UV-B switch, one might expect that the photoreceptor would rapidly be converted to the monomeric form when plants are first exposed to UV-B in sunlight at the start of the photoperiod, and then would revert to the dimer overnight. However, this pattern was not seen in Arabidopsis plants growing for several weeks under photoperiodic cycles with supplementary UV-B (Findlay & Jenkins 2016). Instead, UVR8 was present as a mixture of dimer and monomer throughout the diurnal period, with approximately 75% of total UVR8 in the dimeric form in plants exposed to a range of supplementary UV-B fluence rates. These experiments show that UVR8 does not operate as a simple UV-B switch under photoperiodic conditions but exists in a dynamic photoequilibrium, dependent on the relative rates of monomerisation, resulting from dimer photoreception, and re-dimerisation. The RUP proteins are crucial in maintaining the photoequilibrium, as they mediate re-dimerisation; *rup1rup2* mutant plants failed to establish a stable photoequilibrium and the relative amount of UVR8 monomer increased during the photoperiod, reaching 80% of total UVR8.

A mixture of dimer and monomer was also found in plants growing in natural daylight (Findlay & Jenkins 2016), and a correlation was seen between the formation

of monomer and the level of ambient UV-B at low fluence rates. However, the relative amounts of dimer and monomer were quite variable and did not always follow fluctuations in the amount of ambient UV-B, suggesting that non-UV-B factors could influence the photoequilibrium. Consistent with this notion, evidence was presented that temperature could influence the rate of UVR8 re-dimerisation, and the increased rate of dimer formation at higher temperatures was dependent on the presence of the RUP proteins. It has been reported that *RUP* gene expression can be regulated by different light qualities (Gruber *et al.* 2010; Wang *et al.* 2011; Morales *et al.* 2013) and is also subject to a circadian rhythm (Wang *et al.* 2011); whether other factors also influence *RUP* expression is unknown. Thus, the control of *RUP* gene expression provides an important potential mechanism for regulating UV-B signaling by non-UV-B factors.

Fig. 4 shows a model for UVR8 action in light-grown plants. The relative amounts of dimer and monomer are dependent on the rates of monomerisation through photoreception, and re-dimerisation mediated by RUP proteins. Monomeric UVR8 initiates gene expression responses, but the relationship between the amount of monomer and transcriptional activity may not be simple. For example, it is known that many UVR8-regulated genes are subject to circadian regulation, such as those involved in flavonoid biosynthesis (Harmer *et al.* 2000; Feher *et al.* 2011; Takeuchi *et al.* 2014), but the amount of monomer does not appear to be under circadian regulation, either in growth room conditions or in natural sunlight (Findlay & Jenkins 2016). Hence the mechanism through which UVR8 monomer is coupled to transcription needs further investigation.

The photoequilibrium mechanism might be advantageous to the plant in that there is always a pool of dimer to generate signaling-active monomer; so, for instance, if the fluence rate of UV-B suddenly increases, additional monomer could be formed to initiate the appropriate response. However, the potential for monomer photoreception (Heilmann *et al.* 2016; see above) suggests that plants may not need dimeric UVR8 to

respond rapidly to fluctuations in ambient UV-B, although the extent to which monomer photoreception occurs under natural conditions is unknown. One advantage to having a population of dimeric UVR8 is that it limits interaction with COP1. But is there some other advantage to possessing a dimer and maintaining a dimer-monomer photoequilibrium? One possibility is that the photoequilibrium may provide a mechanism for cross-talk with other signaling pathways. Thus, non-UV-B pathways could modulate the extent of monomer formation, and hence UVR8 action, through influencing the rate of re-dimerisation, as reported for the effect of temperature (Findlay & Jenkins 2016). The influence of environmental factors on UVR8 action is likely achieved through regulation of the expression, and possibly activity, of RUP proteins (Fig 4).

UVR8 FUNCTION IN DIVERSE PLANT SPECIES

Examination of databases of genomic sequences of diverse plant species reveals numerous putative homologs of UVR8 with key functional amino acids highly conserved. Sequences encoding proteins with high percentage similarity to Arabidopsis UVR8 are present in algae (e.g. *Chlamydomonas reinhardtii*), bryophytes (e.g. *Physcomitrella patens*), lycophytes (e.g. the spike moss *Selaginella moellendorffii*), and both monocot and dicot Angiosperms (Rizzini *et al.* 2011; Wu *et al.* 2011; Fernández *et al.* 2016). A study of the genome of the marine Angiosperm *Zostera marina* claims that UVR8 is not present (Olsen *et al.* 2016), and this merits investigation at the protein level. Nevertheless, the early appearance of UVR8 in plant evolution and the high degree of sequence conservation in the plant kingdom emphasize the protein's functional importance. One can speculate that UVR8 evolved to help early photosynthetic plants protect themselves against the high levels of UV-B impinging on the earth in that era, prior to the formation of a mature ozone layer (Rozema *et al.* 1997). However, little is known about the functions of UVR8 in such

species. Recently it was shown that *Chlamydomonas reinhardtii* UVR8 monomerizes upon exposure to UV-B, interacting with *Chlamydomonas* COP1 to induce changes in gene expression associated with acclimation to UV-B in the algal cells (Tilbrook *et al.* 2016). Moreover, it was reported that *Chlamydomonas* UVR8 functionally complements an Arabidopsis *uvr8* mutant, indicating that the mechanism of UVR8 action is conserved through evolution (Tilbrook *et al.* 2016). It is important to further explore the functions of UVR8 in early plant species, including in early land plants to discover when the role of UVR8 in regulating aspects of morphogenesis first appeared.

Arabidopsis has one *UVR8* gene that is constitutively expressed, both spatially and in different light environments (Rizzini *et al.* 2011; Kaiserli *et al.* 2007). However, there is evidence that *UVR8* expression can be affected by various factors in other species. In maize, *UVR8* shows transient changes in expression following UV-B exposure (Casati *et al.* 2011a, b). *UVR8* expression is subject to developmental regulation and inhibition by shading and elevated temperature in *Vitis vinifera* (grape) berries (Liu *et al.* 2015; Loyola *et al.* 2016, who refer to VvUVR8 as UVR1), and is stimulated by UV-B in apple fruits (Zhao *et al.* 2016) and sunlight in litchi fruits (Zhang *et al.* 2016). In radish (*Raphanus sativus*), UV-B exposure and abiotic stresses stimulate UVR8 expression, and evidence was presented that these responses involve hydrogen peroxide and nitric oxide signaling (Wu *et al.* 2016). Furthermore, examination of genomic data reveals that some plant species have several *UVR8* genes, raising the possibility of differential expression. There is some evidence of this in litchi fruits (Zhang *et al.* 2016), and it will be interesting to see whether it is a common feature in other species.

FUTURE PERSPECTIVE

Considerable progress has been made in understanding how plants detect and respond to UV-B light, particularly with the discovery of the previously elusive UV-B photoreceptor UVR8. The structural analysis of UVR8 has revealed a novel type of photoreceptor that does not depend on an attached chromophore for wavelength specificity but uses a tryptophan based photoreception mechanism. Moreover, the functional characterisation of UVR8 has added an important new dimension to research in photomorphogenesis. Whereas UV-B signaling has traditionally been associated with such processes as sunscreen biosynthesis, it is now clear that it impacts on diverse aspects of plant growth and development, including morphogenesis, defence, circadian rhythmicity, phototropism and stomatal regulation. To achieve many of its effects, UV-B signaling interacts with other signaling pathways in plant cells, including hormonal and redox signaling. The interaction of UV-B signaling with auxin signaling likely underpins many morphological responses to UV-B exposure.

Nevertheless, much remains to be learnt about how plants perceive and respond to UV-B. As discussed in earlier sections, many aspects of UVR8 function and regulation are not fully understood and detailed information on how UVR8 acts in a range of responses is lacking. Furthermore, knowledge of UVR8 has been obtained mainly from research with Arabidopsis, often using artificial growth environments, and it is important to extend the research both to diverse species and to agricultural and natural ecosystems. Some progress is being made in these directions but much remains to be done. The concept that UVR8 operates as a simple dimer-to-monomer UV-B on/off switch appears too simple to explain how the photoreceptor functions in natural growth conditions. It is therefore important to further investigate the control of UVR8 action by non-UV-B factors and the role of RUP proteins in this mechanism, and also to assess the significance of monomer photoreception in plants growing in sunlight.

There is also a lack of knowledge regarding the activity and importance of UVR8-independent UV-B signaling pathways in natural growth environments. It is likely that such pathways are stimulated by fluctuations in ambient UV-B, depending on the acclimation status of the plant and other environmental conditions; but which pathways are stimulated and how they influence the growth and development of the plant are not clear. Hence, research is needed to investigate how the different UV-B pathways act in conjunction with UVR8 to regulate gene expression and physiological responses under natural growth conditions.

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Table 1. Responses to UV-B mediated by UVR8

Response	References [a]
Gene regulation	1-10; 13-16, 18-31
UV-B tolerance	1-4; 15, 16, 27
Flavonoid biosynthesis	1, 4, 6, 10, 12, 14-16, 18
Hypocotyl growth suppression	4, 13-16, 18, 20, 26, 29, 30
Leaf/epidermal cell expansion	4, 10, 11, 16, 20
Endoreduplication in epidermal cells	11
Stomata per epidermal cell	11
Entrainment of circadian clock	7
Increased photosynthetic efficiency	8
Photoprotection of photosynthesis	31
Tolerance of *Botrytis* infection	12
Response to osmotic stress	16
Stomatal closure	17
UV-B induced phototropism	19
Inhibition of shade avoidance	20, 23
Leaf epinasty	22
Inhibition of thermomorphogenesis	30

[a] 1, Kliebenstein *et al.* (2002); 2, Brown *et al.* (2005); 3, Brown & Jenkins (2008); 4, Favory *et al.* (2009); 5, Brown *et al.* (2009); 6, Grüber *et al.* (2010); 7, Feher *et al.* (2011); 8, Davey *et al.* (2012); 9, Lang-Mladek *et al.* (2012); 10, Morales *et al.* (2013); 11, Wargent *et al.* (2009); 12, Demkura & Ballaré (2012); 13, Cloix *et al.* (2012); 14, Huang *et al.* (2013); 15, Heijde *et al.* (2013); 16, Fasano *et al.* (2014); 17, Tossi *et al.* (2014); 18, Huang *et al.* (2014); 19, Vandenbussche *et al.* (2014); 20, Hayes *et al.* (2014); 21, Binkert *et al.* (2014); 22, Fierro *et al.* (2015); 23, Mazza & Ballaré; 24, Xie *et al.* (2015); 25, Li *et al.* (2015); 26, Yin *et al.* (2015); 27, Tilbrook *et al.* (2016); 28, Velanis *et al.* (2016); 29, Heilmann *et al.* (2016). 30, Hayes *et al.* (2016). 31. Allorent *et al.* (2016)

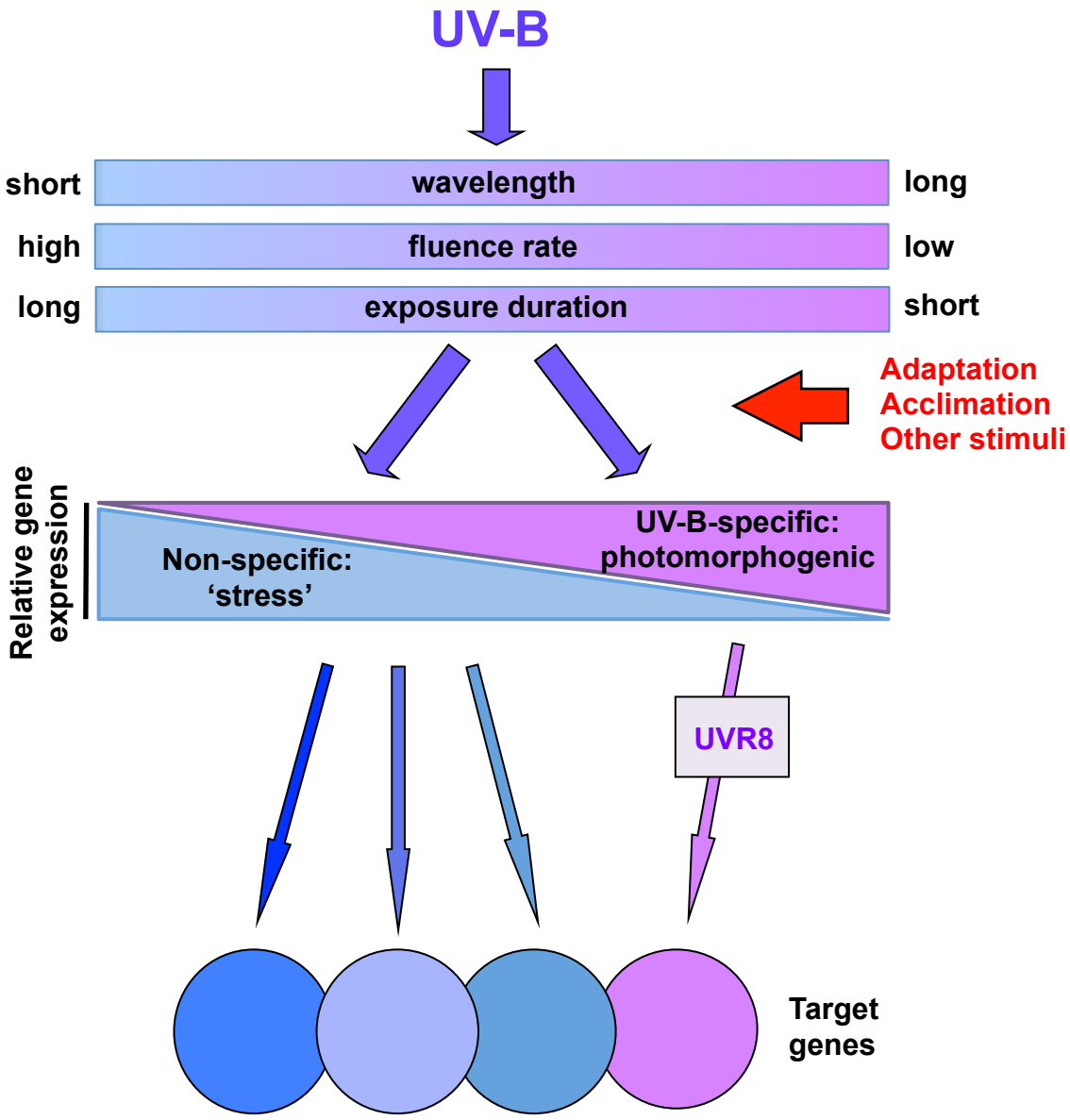
FIGURE LEGENDS

Figure 1. UV-B signaling pathways. Long wavelength UV-B at low fluence rates and short duration stimulates principally UV-B-specific, photomorphogenic signaling mediated by UVR8 (shown in magenta). UVR8 is also active in short wavelength, high fluence rate, long duration UV-B exposure, but additional 'stress' signaling pathways (shown in shades of blue) are activated that are not specific to UV-B (e.g. DNA damage, wound and defence signaling pathways). The different signaling pathways regulate particular sets of target genes, which partly overlap. The nature of the pathways activated will be dependent on the extent to which the plant is adapted and acclimated to UV-B exposure, together with the influence of non-UV-B stimuli (shown by red arrow). Modified from Jenkins & Brown (2007) and Jenkins (2009).

Figure 2. A model of UVR8 action. UV-B stimulated processes are shown by pink arrows, and negative regulation by the dark purple arrows and negative regulation symbol. 1. UV-B exposure causes dissociation of the UVR8 homo-dimer, yielding monomers. 2. Monomeric UVR8 binds to COP1-SPA via the C27 region and WD40 domain (see text). 3. Following UV-B exposure COP1-SPA dissociates from the CUL4-DDB1 E3 ubiquitin ligase complex that promotes degradation of the HY5 transcription factor. 4. UVR8-COP1-SPA regulates transcription of target genes leading to downstream responses. HY5, sometimes acting redundantly with HYH, mediates many of these transcriptional responses. HY5/HYH accumulate rapidly following UV-B exposure through transcription (requiring UVR8, COP1 and HY5) and post-translational stabilization. In addition, *COP1* transcription is stimulated by UV-B. *RUP1* and *RUP2* transcription is stimulated by UV-B, mediated by UVR8, COP1 and HY5. 5. The RUP proteins negatively regulate UVR8 by binding to the C27 region, displacing COP1, and (6.) by promoting re-dimerisation of the photoreceptor. Modified from Jenkins (2014b).

Figure 3. Integration of UVR8 into the cellular signaling network. Pink arrows: UV-B exposure leads to dissociation of the UVR8 homo-dimer, association of COP1-SPA with monomeric UVR8 rather than the DDB1/CUL4 E3 ubiquitin ligase complex that degrades many proteins, including the flowering regulator CO, and stimulation of HY5/HYH accumulation. Black arrows: illustrate the range of processes affected by UVR8 signaling; HY5/HYH regulate transcription of genes involved in numerous signaling pathways, only some of which are shown. Red arrows: the processes shown could potentially be regulated by UVR8 but further evidence is needed. Abbreviations: CO: CONSTANS; GA: gibberellic acid; GA2OX1: gibberellic acid 2-oxidase 1; NO: nitric oxide.

Figure 4. UVR8 dynamics in vivo. UV-B photoreception by the UVR8 dimer induces monomerisation. In parallel, RUP proteins mediate reversion from monomer to dimer. The rates of monomerisation (K_p) and reversion (K_r) are balanced, producing a dimer/monomer photoequilibrium. The photoequilibrium can be influenced by UV-B and potentially other environmental factors through the regulation of *RUP* gene expression and/or RUP activity. The potential for monomer photoreception is also indicated, although the extent to which this occurs in wild-type plants is unknown. Monomeric UVR8 initiates changes in gene transcription, including of the *RUP* genes, and hence a range of responses. Modified from Findlay & Jenkins (2016).


UV-B
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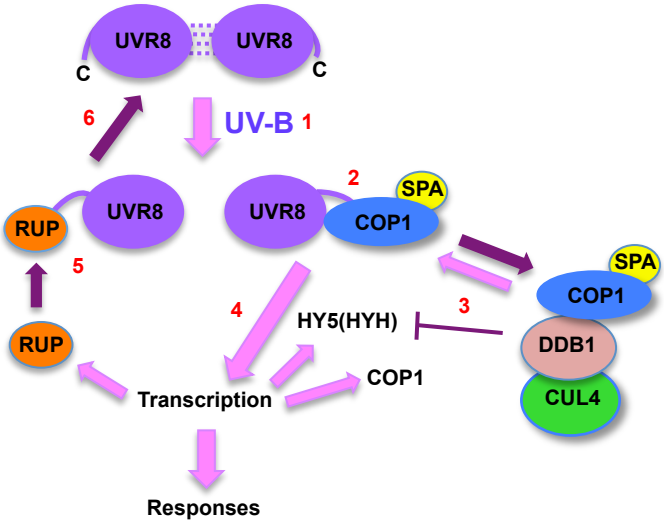
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COP1
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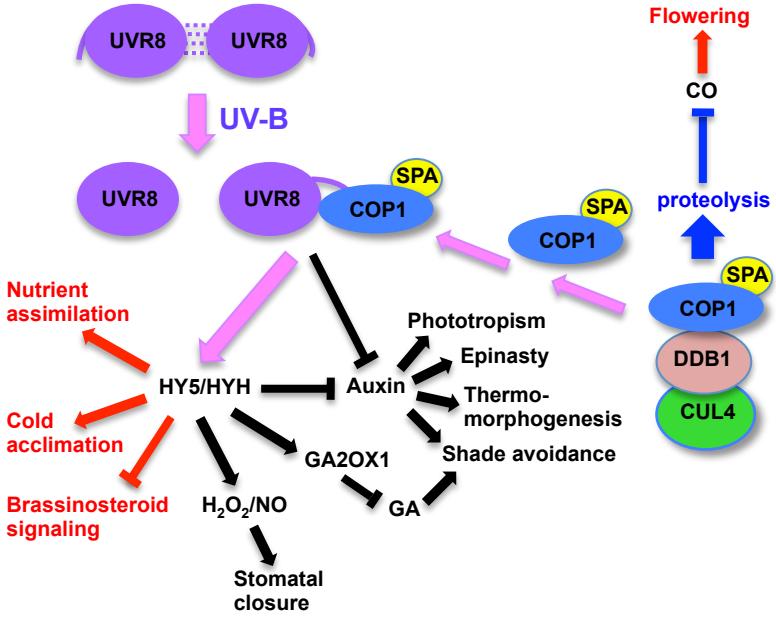
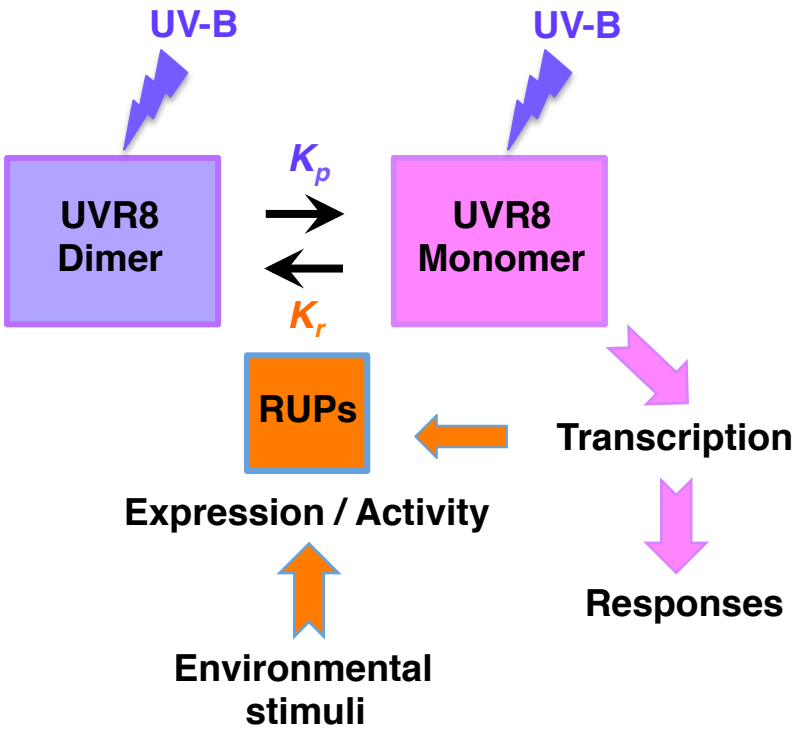
DDB1
RUP
COP1
CUL4
Transcription
Responses


UVR8
UVR8
UV-B
UVR8
UVR8
SPA
COP1
SPA
COP1
SPA
COP1
DDB1
CUL4
Flowering
CO
proteolysis
Nutrient assimilation
HY5/HYH
Auxin
Phototropism
Epinasty
Thermo-morphogenesis
Shade avoidance
Cold acclimation
Brassinosteroid signaling
GA2OX1
GA
H2O2/NO
Stomatal closure


UV-B
UV-B
UVR8 Dimer
K_p
K_r
UVR8 Monomer
RUPs
Transcription
Expression / Activity
Responses
Environmental stimuli