Abstract of Ph.D. thesis

Tissue-dependent and independent signalling events mediated by the UV RESISTANCE LOCUS 8 (UVR8) UV-B photoreceptor

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Introduction

Plants are exposed to the ever-changing environmental cues. Light is one of the most important environmental factor for plants: it is essential not only for producing energy by photosynthesis, but also as a signal, which helps plants to optimize their growth and development in the changing environment. On the other hand, sunlight can cause serious damages due to its high energy content. There are several processes which are influenced by light, for example germination, development of seedlings, phototropism, shade avoidance, chloroplast movements, opening of stomas and most of the rhythmic processes during the whole lifecycle. Thus, plants have evolved various photoreceptors in order to monitor the duration, the wavelength, the intensity, the direction and the rhythmicity of the surrounding light. In the widely used model plant, *Arabidopsis thaliana*, the following photoreceptors were identified: the blue/UV-A light absorbing phototropins, cryptochromes and Zeitlupe-type receptors, the red/far-red absorbing phytochromes (phyA-phyE) and the UV RESISTANCE LOCUS 8 (UVR8) UV-B photoreceptor.

UV-B radiation (280–315 nm) is a part of sunlight which reaches the Earth's surface. This part of the sunlight has the largest energy content, so UV-B can easily damage macromolecules (DNA, proteins, lipids, *etc*.), causing stress, or even irreversible damages. For this reason, it is crucial for the plants to perceive UV-B and start signalling cascades in order to adapt to UV-B irradiation and even initiate repair mechanisms to reduce the damage caused by UV-B irradiation.

It has long been assumed that UV-B radiation can result in photomorphogenic changes irrespective of considerable DNA damage. It took many years to identify a UV-B-specific receptor in *Arabidopsis*, the UVR8. This discovery boosted the research of UV-B signalling cascades driven by photomorphogenic (weak) UV-B, leading the expanding knowledge of these pathways. UVR8 is a seven-bladed β-propeller protein that normally forms homodimers, which monomerize upon UV-B irradiance, leading to the enrichment of physiologically active UVR8 monomers. Nuclear UVR8 accumulation is necessary, however not sufficient step of UVR8 signalling cascades. An early step of the UVR8 signalling cascade after the receptor monomerization is the interaction between UVR8 and an E3 ubiquitin ligase, named CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), which is needed for the nuclear import of UVR8. Later, due to the interaction, COP1 is not able to ubiquitinate its target proteins. COP1 enables protein ubiquitination and therefore degradation of the target proteins via the 26S proteasome pathway, such as ELONGATED HYPOCOTYL 5 (HY5) and HY5 HOMOLOG (HYH) positive regulators of photomorphogenesis. When UVR8 is active, it inhibits HY5 and HYH ubiquitination by COP1, and in addition, UVR8 induces the expression of the *HY5* and *HYH* genes. Thus, UVR8 promotes both photomorphogenesis and photoprotection by inducing high levels of HY5 and HYH proteins, leading to different responses such as inhibition of hypocotyl elongation in one hand, and enhanced synthesis of photoprotective pigment molecules on the other hand by upregulating, for example, the *CHALCONE SYNTHASE* (*CHS*) flavonoid biosynthesis gene

leading to better survival under UV-B radiation. This is the most accepted model of the basic UVR8 signalling mechanisms, but there must be many other, yet unknown pathways, which are independent of HY5, but still dependent of UVR8. For example, UV-B can adjust the circadian clock via UVR8 in a HY5 independent way by upregulating *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *PSEUDO-RESPONSE REGULATOR 9* (*PRR9*) genes, which encode components of the central circadian oscillator.

UVR8 cascade is inhibited by REPRESSOR OF UV-B PHOTOMORPHOGENESIS 1 (RUP1) and RUP2 proteins, which are responsible for UVR8 redimerization and sequestering COP1 from UVR8, thus inactivating the cascade.

UVR8 signalling is tightly linked to several pathways, regulated by other photoreceptors and hormones. The intimate relationship between UVR8 and auxin signalling has been examined extensively. The most important molecular link which connects these pathways is the HY5, since HY5 can inhibit auxin responses (under UV-B irradiation also).

It is known, that there are signals which do not spread between different tissues in the plant, these are termed tissue-autonomous or tissue-specific. Such processes were discovered and examined in the signalling pathways of different photoreceptors. The hypocotyl bending response of phyA can be triggered exclusively by phyA in the mesophyll but not in the epidermis or vasculature. It was also found that some responses are controlled by inter-tissue signal spreading, like regulation of certain genes involved in cell growth. Despite the gained data from different photoreceptors, the tissue autonomous or inter-tissue aspects of UVR8 signalling has not yet been studied. To achieve this, we developed a transgenic plant-based experimental system using promoters which are active in certain tissues and we monitored the chosen proteins of interest at tissue level using confocal laser scanning microscopy. We supplemented these observations with diverse phenotyping studies obtained from different development stages of the universal dicot model plant, *Arabidopsis thaliana*.

Research objectives

It has long been known that UV-B irradiance has deleterious effects on plants, but especially at lower dose, it also triggers responses (photomorphogenesis, phototropism, defense responses, *etc*.) regardless of any damages. In the last few years, it turned out that the majority of these latter processes are controlled by the UVR8 UV-B receptor, but our knowledge of its signalling is rather rudimentary. Tissue-dependent or independent types of responses were discovered in other photoreceptor-controlled signalling pathways, but UVR8 signalling has not been investigated in this respect yet. Thus, we generated transgenic *uvr8* mutant plants, expressing YFP-UVR8 (YFP=YELLOW FLUORESCENT PROTEIN, fused to UVR8) under the control of endogenous *ProUVR8* promoter and other promoters, which are active in different tissues. We also created additional reporter constructs to monitor the tissue-dependent aspects of UVR8 signalling. We set the following aims:

- Investigating the spatial pattern of UVR8 expression *in planta*;
- Examining the effects of YFP-UVR8 expressed in different tissues on UV-B induced photomorphogenesis, phototropism and UV-B acclimation responses;
- Exploring differences between the UVR8 signalling mechanisms in seedlings and in adult plants;
- Investigating the spatial expression and accumulation pattern of the most important players involved in UVR8 signalling, and revealing these physiological relevance.

Materials and methods

Plant growth conditions and light treatments

For our experiments (and for making new plant lines) we used *Arabidopsis thaliana* L (Heynh.) *uvr8-6*, *uvr8-7*, *hy5-ks50*, *phot1phot2amiUVR8* mutants, Wassilewskija (Ws) and Columbia (Col) wild-type plants.

Seeds were surface sterilized and subsequently stratified. The seedlings were grown in 12 h white light (WL, 80 μmol m^{-2} s^{-1})/12 h dark at 22 °C for 6 days. Non-damaging photomorphogenic (low-fluence) supplemental UV-B intensity was around 1,4 μmol m^{-2} s^{-1} for seedlings, 7-15 μmol m^{-2} s^{-1} for adult plants. For microscopic analysis, the seedlings were grown in 12 h white light (WL, 80 μmol m^{-2} s^{-1})/12 h dark at 22 °C for 6 days and then placed under continuous white light supplemented with UV-B for 16 h at 22 °C. For hypocotyl and cotyledon measurements, seedlings were grown for 3 days in light/dark chambers before being exposed to continuous WL supplemented with UV-B for 4 days or 5 days. For morphogenic, survival and chlorophyll-content experiments, adult plants were grown in 8 h white light/white light plus UV-B (WL: 120 μmol m^{-2} s^{-1}, UV-B: 2/12 μmol m^{-2} s^{-1})/16 h dark at 22 °C for 7 weeks. For flavonoid accumulation, bending, and reporter genes microscopy experiments plants were grown in 16 h white light (65 μmol m^{-2} s^{-1})/8 h dark for 5-6 weeks on 22 °C. Before microscopy experiments, plants were exposed to 1,5 μmol m^{-2} s^{-1} UV-B plus 2,5 μmol $m^{-2}s^{-1}$ intensity white light (for 12 h), except for bending assay, where only 1,5 μmol m^{-2} s^{-1} unilateral UV-B irradiance was used (over night).

Applied experimental methods

- Measurement of hypocotyl length and cotyledon area.
- Measurement of stem bending kinetics using a custom built automated web-camera-based time-lapse recording system.
- Chlorophyll level determination.

- Sectioning of plant tissues for microscopy experiments using vibratome.
- YFP/GFP signal detection by Confocal Laser Scanning Microscopy (CLSM).
- Determination of flavonoid level and distribution in the hypocotyl and stem using CLSM.
- Molecular cloning for making reporter constructions.
- Protein isolation from plant tissues and Western blot analysis.
- Determination of transcript levels by qRT-PCR.

Results and discussion

Investigating the expression pattern of UVR8

In order to monitor the expression pattern of UVR8 in different tissues we generated transgenic lines expressing the YFP-UVR8 fusion protein under the control of the endogenous *ProUVR8* promoter in *uvr8* mutant background (chosen line named ProUVR8) and determined the expression pattern using CLSM. *ProUVR8* drove the expression of the YFP-UVR8 protein in the epidermis and, to a lesser extent, in the mesophyll/subepidermal cells of cotyledon and the hypocotyl of seedlings, and in epidermis and cortex of adult plants. We could not detect YFP-UVR8 signal in the vascular bundles, but it should be noted that our plant line expressed much less total YFP-UVR8 (~10%) compared to endogenous UVR8 level of wild type plants, thus it cannot be excluded, that UVR8 is present in the vascular bundles in wild type plants. However, if so, UVR8 in the vasculature must have only a limited role (if any) in sensing UV-B irradiation, since UV-B penetrates rather poorly into deeper tissues.

Characterization of transgenic plant lines expressing YFP-UVR8 in different tissues

In order to investigate the role of UVR8 in different tissues, we generated transgenic lines which expressed YFP-UVR8 fusion protein under the control of *ProML1* or *ProCAB3* or *ProSUC2* promoters (*ML1=MERISTEM LAYER 1, CAB3=CHLOROPHYLL A/B BINDING PROTEIN 3, SUC2=SUCROSE-PROTON SYMPORTER 2* genes). These promoters have already been widely used to express the gene of interest in epidermal (*ProML1*), mesophyll/cortex (*ProCAB3*), and companion cells (*ProSUC2*), respectively. We named the transgenic lines as ProML1, ProCAB3 and ProSUC2 for simplicity. We found that the ProML1 line expressed YFP-UVR8 in the epidermis and the ProCAB3 contained YFP-UVR8 mainly in the mesophyll/cortex. However, in the ProSUC2 line we could detect YFP-UVR8 signal not only in the vasculature, but also in the subepidermal cells of hypocotyls and cotyledons, and in the cortex of adult plants.

Western blot analysis showed that the total YFP-UVR8 protein levels in seedlings grown under supplemental UV-B, are ~5% in ProML1 line, ~75% in ProCAB3 line and ~25% in ProSUC2 line, compared with the endogenous UVR8 level of the wild type. We also compared the amount of YFP-UVR8 in particular tissues by CLSM to facilitate direct comparison. The amount of YFP-UVR8 in the epidermis of ProUVR8 and ProML1 lines was similar, whereas it was about four- to fivefold higher in the mesophyll of ProCAB3 line, and almost the same in the mesophyll of the ProSUC2 compared with the same tissue of the ProUVR8 line. We could not test the YFP-UVR8 accumulation in the vasculature by this method.

Investigating the UV-B related responses of the seedlings expressing YFP-UVR8 in different tissues

We wanted to assess the function of UVR8 in different tissues, thus we examined typical photomorphogenic responses of our transgenic seedlings exposed to UV-B irradiation. The applied UV-B treatment resulted in hypocotyl growth inhibition in the wild type plants, whereas the *uvr8* mutant seedlings showed almost no response. All transgenic lines, except for ProSUC2, showed hypocotyl growth inhibition in UV-B, however, neither of them could fully complement the *uvr8* mutant phenotype. Cotyledon area is also affected by UV-B irradiation. The cotyledon size expansion of the *uvr8* mutant seedlings decreased, whereas it slightly increased in wild type plants. Neither ProCAB3 nor ProSUC2 line showed complementation of the *uvr8* background, whereas ProUVR8 and ProML1 line, which have YFP-UVR8 in their epidermis, could show wild-type-like response.

We can conclude that the YFP-UVR8 fusion protein indeed works as a functional photoreceptor in our transgenic lines. Our results also revealed that in seedlings the UVR8 signalling contributes to the inhibition of the hypocotyl elongation both in the epidermal and mesophyll tissues, but only epidermal UVR8 has influence on the cotyledon size, which therefore appears to be a tissue-autonomous response. We also note that the vascular UVR8 has limited, if any role in regulating the above responses.

UV-B-induced phototropism in adult plants

It has been revealed previously, that *Arabidopsis* seedlings bend towards unilateral UV-B irradiation and this response is controlled mainly by phototropins, but also by UVR8. Despite the advances in studying seedling development, no results were published in the examination of UV-B-induced phototropic responses in adult plants so far. Thus we decided to investigate the bending of the inflorescence stems towards UV-B. We treated adult plants having about 5-10 cm tall inflorescence stems with unilateral UV-B irradiation, than monitored their bending towards the irradiation source using an automated web-camera-based time-laps recording system. Interestingly,

whereas *uvr8* mutant seedlings showed wild-type-like hypocotyl bending due to the action of phototropins, the stems of adult *uvr8* plants showed only negligible bending towards unilateral UV-B. This means that phototropins have major role in bending only during seedling stage and we could use our phototropin-containing adult plants for tissue-specific analysis of UVR8-mediated bending. Our results showed that the ProUVR8 and the ProCAB3 line, which have high levels of YFP-UVR8 in their cortex, bent like wild type plants. In contrast, the ProSUC2 plants having lower levels of YFP-UVR8 in their cortex, could only partly complement the *uvr8* mutant phenotype. This result suggests the influence of UVR8 levels in this process. The epidermal expressor ProML1 plants showed bending similarly to ProSUC2 despite the much lower total YFP-UVR8 content, which suggests high efficiency of UVR8 action in the epidermis.

Taken together, it seems that in adult plants UVR8 can initiate bending towards unilateral UV-B irradiation in the epidermis and cortex/mesophyll tissues also, with a major role in the epidermis. Most likely in case of ProSUC2 line the complementation was due to the YFP-UVR8 expression in cortical tissues, not due to vascular YFP-UVR8, however we cannot exclude the possibility that vascular UVR8 contributes to initiate bending towards UV-B irradiation.

UVR8-dependent flavonoid accumulation

High level of UV-B-induced flavonoid accumulation can be observed in different tissues of several examined plant species. We wanted to investigate whether the flavonoid accumulation occurs in a tissue-autonomous manner using the DPBA molecule forming complexes with flavonoids, that can be visualized by CLSM. The applied UV-B treatment induced flavonoid accumulation in wild type, but not in *uvr8* mutant seedlings indicating that under our experimental conditions the flavonoid accumulation is controlled by UVR8. All of the examined transgenic lines expressing YFP-UVR8 in different tissues accumulated flavonoids with a pattern similar to wild type, but to lower levels. All examined line accumulated flavonoids at levels that were similar to each other, despite the much lower YFP-UVR8 level in the ProML1 line compared to that in the ProCAB3 plants. It indicates that the epidermal YFP-UVR8 is more effective in this response as YFP-UVR8 expressed in the mesophyll and cortex.

We also examined the flavonoid accumulation in the stems of adult plants using DPBA staining on cross sections made from the upper part of the stems irradiated with unilateral UV-B. CLSM analysis revealed that accumulation of flavonoids depends on UVR8 and flavonoids play major role in the epidermis. It is not a surprise, since epidermis is the first defence line of scavenging the UV-B radiation, so flavonoids must be there in case of strong UV-B irradiation. However, compared to seedling stage, in adult plants UVR8 also plays a very important role in the cortex, since the ProCAB3 (YFP-UVR8 expressed only in the cortex/mesophyll) line accumulated huge amount of flavonoids after UV-B irradiation. Similarly, ProUVR8 also accumulated flavonoids in the cortex.

The lower cortical level of YFP-UVR8 in the ProUVR8 line (compared with the ProCAB3) can explain the different flavonoid accumulation level and this observation indicates that not only the presence, but also the amount of the photoreceptor can affect this response.

The pattern of flavonoid accumulation is very similar to HY5 and HYH accumulation in the stem, most likely because HY5 (and HYH) can upregulate the *CHS* gene, which is a key gene in flavonoid production. In wild type and transgenic plants we could observe flavonoid gradient between the irradiated and the shaded side of the stem, having much more flavonoids on the irradiated side. This finding shows that the UVR8 signal does not spread from the UV-B irradiated side towards the shaded side of the stem.

Another interesting result is that flavonoids seem to be transported from the cortex to the epidermis, but not from the epidermis towards the deeper tissues: in ProCAB3 and ProSUC2 lines we could detect huge amounts of flavonoids in the epidermis, despite the fact that these lines do not express YFP-UVR8 in epidermis. On the other hand, in ProML1 line (YFP-UVR8 in the epidermis), only epidermal flavonoid accumulation was observed. The flavonoid transport from the cortex to the epidermis makes sense, since flavonoids are most effective in scavenging UV-B in the epidermis. In strong UV-B irradiance, UV-B can reach the deeper tissues, activating the UVR8, which can trigger flavonoid production in the cortex. Despite UV-B penetrates poorly into deeper tissues, the accumulated flavonoids can protect the photosynthetic apparatus here and we can speculate that the produced flavonoids can migrate into the epidermis to enhance the protection against UV-B. We cannot exclude, however, that UVR8 signalling in the cortex triggers flavonoid production in the epidermis.

Acclimation and survival of adult plants in UV-B irradiation

UVR8 also plays an important role in the acclimation of adult plants to UV-B. Weak supplemental UV-B irradiation resulted in rosette growth inhibition and shorter petioles compared to the white light-grown wild type plants, whereas *uvr8* mutant plants showed a limited rosette growth reduction and had light green leaves. These responses show that this UV-B treatment elicits mostly UVR8-mediated photomorphogenic responses. All lines developed equally in white light, and all of our transgenic lines displayed wild-type-like acclimation after weak supplemental UV-B irradiation treatment: the lines had similar rosette development and chlorophyll accumulation, except for the ProCAB3 line, which developed smaller rosettes and accumulated chlorophylls to higher levels.

In order to examine the role of UVR8 in UV-B acclimation and survival we applied stronger supplemental UV-B irradiation, which was lethal to *uvr8* mutant plants, but not to wild type plants. All transgenic plants survived, and additionally, ProCAB3 and ProSUC2 plants developed small

rosettes similar to the UVR8-overexpressor plants described in previous reports. We found that the latter two transgenic adult plants indeed overexpressed YFP-UVR8.

These results show that in adult plants, both epidermal and cortical UVR8 can facilitate photomorphogenesis, acclimation and survival under UV-B irradiation, with the major role of UVR8 in the epidermis, but also in the cortex. We assume that UVR8 in the mesophyll is needed for the efficient protection of the photosynthetic apparatus under UV-B irradiation, maybe by maintaining the necessary levels of the D1 and D2 proteins. We also note that the epidermal UVR8 of the ProML1 and ProUVR8 lines contributes for proper rosette development.

UVR8 regulated *HY5* expression and HY5 accumulation

One of the early steps of UV-B induced signalling cascades is the induction of the *HY5* gene and the subsequent accumulation of the HY5 transcription factor. In order to examine the tissue specificity of these responses, we introduced the *ProHY5:HY5-GFP* or *ProHY5:GUS-GFP-NLS* transgenes into our lines which express YFP-UVR8 in different tissues. The first reporter was designed to monitor the HY5-GFP accumulation in different tissues, whereas the latter is suitable for determining the tissue-specific induction of *HY5* transcription. Our results revealed that significant HY5-GFP accumulation can be observed in only those cells, which contain detectable amount of YFP-UVR8, thus it is a tissue-autonomous process. Similarly, the UVR8-dependent induction of *HY5* transcription also found to be restricted to YFP-UVR8 expressing tissues.

We also examined the accumulation pattern of HY5 (and its closest homologue, HYH) in the stem of adult plants. For this purpose, we introduced *ProHY5:HY5-YFP* transgene to *hy5* and *hy5uvr8* mutant backgrounds and monitored the YFP signal accumulation using CLSM after unilateral UV-B treatment. We found that the UV-B-induced accumulation of HY5 is indeed UVR8-dependent since HY5-YFP was only detectable in the *hy5* background line, but not in the *hy5uvr8* plants. Additionally, we found, that the accumulation pattern of this fusion protein in unilaterally UV-B irradiated plants was detectable only in the illuminated side of the stem. This observation indicates, that HY5 is a regulator of UVR8-induced stem bending towards UV-B, since HY5 is known to be a repressor of auxin signalling. HY5 can inhibit auxin induced growth responses locally, which leads to bending towards UV-B. Thus, the HY5 action in stem bending is similar as it was observed in seedlings bending towards UV-B.

We also monitored the HYH-YFP accumulation pattern in lines, which express the *ProHYH:HYH-YFP* transgene in *hyh*, or *hy5hyh* mutant background. These transcription factors accumulate only in the illuminated side of the stem, just like HY5-YFP. We could detect much more HYH-YFP in *hyh* mutant plants compared to *hy5hyh* (even after only 4,5 h UV-B irradiation), which indicates that HY5 not only triggers its own expression but the expression of HYH too.

We also investigated the *HY5* and *HYH* transcript accumulation pattern between the illuminated and the shaded sides of the inflorescence stems of adult plants. We cut the stems of wild type plants vertically in half after the unilateral UV-B irradiation treatment, and measured the transcript levels in each sides using qRT-PCR analysis. The data showed that the *HY5* and also the *HYH* gene transcript levels were much higher in the illuminated part of the stem, compared to the shaded side.

Based on the above data we can speculate that the UVR8 induced *HY5* (and *HYH*) induction and HY5 accumulation only in the irradiated side of the stem is one of the main reasons of the bending towards unilateral UV-B and it happens, because there is no UVR8 or HY5 (and HYH) signal spreading from the irradiated side towards the dark side of the stem.

UVR8 induced transcription of HY5-dependent and HY5-independent genes

In order to get insight into the tissue-related organization of later steps of UVR8-signalling, we introduced the *ProELIP2:GUS-GFP-NLS* or the *ProPRR9:GUS-GFP-NLS* transgenes into the transgenic lines which express YFP-UVR8 in certain tissues. EARLY LIGHT INDUCED PROTEIN 2 (ELIP2) is involved in the photoprotection of thylakoid membranes. *ELIP2* is induced by UV-B and this requires UVR8 and HY5. Our microscopic images show that *ELIP2* gene activity is strongly enhanced by UV-B irradiation, compared to its activity in white light, and the UV-B induction was observed only in those tissues, which also contained detectable amount of YFP-UVR8: in the epidermis of ProML1 lines and in the cortex of ProCAB3 lines. These data indicate that the UVR8 regulates the expression of *ELIP2* gene in a tissue-autonomous manner.

The PSEUDO RESPONSE REGULATOR 9 (PRR9) protein is a component of the circadian clock. UV-B irradiation induces *PRR9* in UVR8-dependent manner, but it is independent of HY5. In contrast to the *ProELIP2* promoter, *ProPRR9* was active only in the sub-epidermal cells of the cotyledons of our transgenic lines. UV-B irradiation strongly enhanced the activity of *PRR9*, but only in those sub-epidermal cells that contained YFP-UVR8. Elevated expression of *GUS-GFP-NLS* was not observed in the epidermis neither in the ProUVR8 nor the ProML1 lines, although these plants express YFP-UVR8 in their epidermal cells. Taken together, the elevation of *PRR9* activity under UV-B irradiation occurs in a tissue-autonomous manner. Additionally, it seems that *ProPRR9* has an own cell-specific type of regulation, which inhibits its expression in epidermal cells.

Conclusions

There are tissue-autonomous mechanisms working together with non-autonomous regulation processes in the light-signalling system. UVR8 is taking part in this by triggering tissue-autonomous and non-autonomous sub-processes as well, which are needed together for proper UV-B responses just like photomorphogenic growth inhibition, phototrophic stem bending, or flavonoid accumulation. To expand our knowledge about the UVR8 signaling we developed transgenic plants expressing the YFP-UVR8 protein in particular tissues of the *uvr8* mutant and investigated their UVR8-related UV-B responses. Taken together, our results revealed, that:

- YFP-UVR8 was detectable in the epidermis and in the mesophyll/cortex, where the fusion protein was expressed under the control of its own endogenous promoter. However, we cannot exclude that UVR8 is present in the vasculature.
- The mutant complementation tests revealed that YFP-UVR8 could trigger photomorphogenic hypocotyl elongation inhibition if it is present in the epidermis and in the mesophyll/subepidermal cells.
- YFP-UVR8 could restore cotyledon expansion phenotype of the *uvr8* mutant background, when it was expressed in the epidermis. The UVR8 in the mesophyll/subepidermal cells does not have a role in this response, thus this process is strictly tissue-autonomous.
- YFP-UVR8 expressed in the vascular bundles has a very limited, if any role in regulating hypocotyl elongation and cotyledon expansion.
- The weak supplemental UV-B treatment we applied was suitable for investigating the photomorphogenic UV-B responses of adult plants. The stronger supplemental UV-B treatment, however, triggered the responses needed for acclimation and survival. Our results suggest that in the regulation of chlorophyll accumulation, photomorphogenic and acclimation responses, UVR8 plays a major role in the mesophyll/cortex, rather than in the epidermis. It seems that these responses are influenced by the overall UVR8 level of the plants.
- UVR8-dependent flavonoid accumulation can be detected in all of our transgenic seedlings, however the flavonoid levels were lower compared to the wild type. It seems that UVR8 plays a major role in the epidermis, since much less epidermal than cortical/subepidermal UVR8 is enough for inducing comparable flavonoid accumulation during seedling stage.
- In case of adult plants, epidermal UVR8 plays a major role in triggering flavonoid accumulation, however cortical UVR8 become more important compared to seedling stage. We could detect flavonoids not only in those tissues, which expressed YFP-UVR8: in ProCAB3 and ProSUC2 plants we could detect high amount of flavonoids in the epidermis, despite the fact that these plants do not express YFP-UVR8 in this tissue. In the ProML1 line which expresses YFP-UVR8 exclusively in the epidermis, we could detect flavonoids only in

the epidermis. Taken together, flavonoid transport can happen between neighbouring tissues, but it is directed only from cortex to epidermis, not *vice versa*.

- Under unilateral UV-B irradiation, the UVR8-dependent induction of *CHS* (*CHALCONE SYNTHASE*, coding for a key enzyme in flavonoid biosynthesis) was much stronger in the UV-B irradiated side of the stem, compared to its shaded side. We found that flavonoids accumulate in a very similar manner, and the overall flavonoid level was influenced by the YFP-UVR8 level. UVR8 can lead to *CHS* upregulation, only in those parts of the plant where it is irradiated.
- Unilateral UV-B treatment triggers bending of the stem towards UV-B irradiation. This response depends mainly on UVR8 and has negligible phototropin influence compared to young seedlings. Both in the epidermis and in the mesophyll/cortex, UVR8 can trigger stem bending towards UV-B, and it seems that UVR8 signalling is more effective in the epidermis.
- The UVR8-dependent *HY5* gene induction and HY5 accumulation are tissue-autonomous, since these responses only happen in those particular tissues, where detectable YFP-UVR8 is present in our lines. This finding shows that there is no signal spreading between tissues regarding these regulatory processes.
- Both *HY5* and *HYH* gene induction as well as HY5 and HYH protein accumulation take place in the UV-B irradiated side of the stem, which contributes to site-specific inhibition of auxin triggered growth responses. This phenomenon enhances the stem bending towards UV-B, because of the unequal growing between the irradiated and the shaded side of the stem. This finding also proves that there is no UVR8 signal spreading from the irradiated side towards the shaded side of the stem (just like in the case of *CHS* gene activation, and flavonoid accumulation). Additionally HY5 can enhance *HYH* induction during UV-B irradiation.
- The HY5-dependent *ELIP2* gene induction, and the HY5-independent *PRR9* gene induction are also regulated by UV-B in a UVR8-dependent manner. Both of these responses are strictly tissue-autonomous.

Taken together, there are tissue-autonomous and non-tissue-autonomous processes working together in UVR8 signalling system in order to trigger proper UVR8 responses. Despite the increasing knowledge of UVR8 signalling cascades, there are still many unknown parts of it, so in the future there will be many possibilities to plan new experiments. Revealing more and more about the UVR8 signalling system will give us the chance to manipulate particular UV-B related sub-processes in order to enhance development and survival, or defence against herbivores. Moreover, favourable illumination of crop plants with supplemental weak UV-B, combined with gene manipulation could increase crop yield of plants in horticultures.

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