THESES OF PhD DISSERTATION

MOLECULAR GENETIC ANALYSIS OF UV-B SIGNAL TRANSDUCTION IN *ARABIDOPSIS THALIANA*: DESCRIBING THE ROLE OF ANAC13 TRANSCRIPTION FACTOR

Judit Sáfrány

Budapest

2011

PhD School

Name:	Doctoral School of Horticultural Sciences
Field:	Crop Sciences and Horticulture
Head of Ph.D. School:	Prof. Dr. Magdolna Tóth Doctor of the Hungarian Academy of Sciences Head of Department of Fruit Sciences Corvinus University of Budapest, Faculty of Horticultural Sciences
Supervisor:	Dr. György Dénes Bisztray Associate Professor, PhD Corvinus University of Budapest, Institute of Viticulture and Oenology Department of Viticulture
Outer consultant:	Dr. Géza Dallmann Candidate of biological sciences, Senior Scientist Wheat Genetics Group Agricultural Biotechnology Center, Gödöllő

The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.

....	....
Head of Ph.D. School	Supervisor

PUBLICATIONS IN THE TOPIC OF THE THESES

Journal articles with impact factor

Safrany, J., Haasz, V., Mate, Z., Ciolfi, A., Feher, B., Oravecz, A., Srec, A., Dallmann, G.,Morelli, G., Ulm, R., Nagy, F. (2008): Identification of a novel cis-regulatory element for UV-B-induced transcription in *Arabidopsis*, The Plant Journal 54, 402-414.

International conferences (abstract)

Safrany J., Haasz V., Dallmann G. Critical promoter elements in UV-B induced expression of ANAC13. Plant Abiotic Stress – from signalling to development, 2nd meeting of the INPAS, 14-17 May 2009 Tartu, Estonia p. 111.

Hungarian conferences (proceedings)

Sáfrány J, Haász V, Máté Z, Dallmann G, UV-B érzékelés és jelátvitel növényekben. Keszthely, Ifjúsági Tudományos Fórum, 2007. március 22. Konferencia-kiadvány: CD, 5 oldal

Sáfrány J, Haász V, Máté Z , Az UV-B jelátvitelben meghatározó szerepű promóter elem azonosítása pontmutánsok segítségével *Arabidopsis thaliana*-ban. Kecskemét, 2007. augusztus 27-28. Erdei Ferenc Konferencia, II.kötet, p. 951-954.

Haász V, Sáfrány J, Dallmann G, UV-B válaszban résztvevő promóter elemek azonosítása az *Arabidopsis* HY5 génjében. Budapest, Tavaszi Szél Konferencia, 2007. május 17-19. p. 213-218.

Hungarian conferences (abstract)

Sáfrány J, Haász V, Máté Z, Dallmann G, Nagy F, UV-B indukálta génválaszban szerepet játszó promóter elemek azonosítása *Arabidopsis thaliana*-ban. Balatonfüred, VII. Magyar Genetikai Kongresszus, XIV. Sejt- és Fejlődésbiológiai Napok. 2007. április15-17. p.160-161.

Sáfrány J, Máté Z, Dallmann G, A NAM transzkripciós faktor UV-B válaszban betöltött szerepének jellemzése *Arabidopsis thaliana*-ban. "Lippai János-Ormos Imre-Vas Károly" Tudományos Ülésszak, 2007. November 7-8. Összefoglalók p.132-133.

Sáfrány J, Haász V, Máté Z, Dallmann G, Az ANAC13 gén ultraviola-B válaszban szerepet játszó promoterelemei, Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak, 2009. október 28–30. Budapest, Összefoglaló, p. 58-59.

through independent cis-elements. ANAC13 promoter is induced for abscisic acid and for most abiotic stimuli including red light, salt and heat studied in our experiments. Our findings clearly indicate that the intact $UVBox^{ANAC13}$, at least in its dimerised form, is sufficient for conferring UV-B inducibility to the Pro_{35Smin}::Luc promoter construction. We demonstrated that the regulatory function of $UVBox^{ANAC13}$ is specific for UV-B as, unlike Pro_{ANAC13}::Luc and Pro_{DREB2A}::Luc constructions, expression of the $Pro_{2xUVBoxANAC13\text{-}35Smin}$::Luc transgene is not affected by any other biotic and abiotic stimuli tested in our experiments. These together suggest that $UVBox^{ANAC13}$ acts as a specific cis-regulatory element in mediating UV-B induced transcription.

INTRODUCTION AND THE AIMS OF THE RESEARCH

Sunlight is the ultimate energy source on our Earth. Solar radiation provides indispensable energy for all living organisms, although only plants and some bacterium species are capable of its direct absorption and its transformation into biologically utilizable chemical energy. However, for plants, sunlight is not a mere energy source that directs photosynthesis but it is also the most important medium of perception as it provides basic information about the environment. Furthermore, it can also serve as an environmental stimulus to direct growth and development. The capacity of plants to perceive and to react to continuously changing environmental factors such as light quality and quantity, differences in the duration of light exposure is indispensable for their optimal adaption. For this purpose, plants have developed a subtle light sensoring system which continuously monitors light conditions of the environment. Phytochromes are specialised for perceiving red and far-red regions of the light spectrum while cryptochromes and phototropins absorb blue and UV-A light. Radiation perceived by these photoreceptors is transmitted to a complex signal transduction cascade and through different regulatory pathways it is involved in controlling numerous morphological and physiological responses during the entire life cycle of a plant.

UV-B (Ultraviolet-B) radiation reaching the surface of the Earth is an integral part of the sun's electromagnetic radiation. As a consequence of their light-dependent and sessile lifestyle, plants are inevitably exposed to UV-B radiation. Higher intensity UV-B radiation induces different stress responses. However, UV-B is not only a stress factor. Similarly to other ranges of the light spectrum, lower intensity UV-B acts as an environmental signal carrying basic information. Several evidences suggest the existence of a specific UV-B detecting system which would be independent from both the damage/defence responses and the known photoreceptors perceiving the visible part of the light spectrum.

In sharp contrast with the perception and signal transduction of visible and UV-A light, elements of non-defensive UV-B specific responses are mainly unknown and the molecular structure of their photoreceptors is yet to be identified.

The purpose of our study is to define the molecular mechanisms underlying UV-B perception and signal transduction. As a first step, UV-B specific changes at transcription levels are to be revealed. These results may provide further basis

for the identification of new potential elements of UV-B response mechanisms and for the comparison of UV-B and visible light induced signal transduction.

MATERIALS AND METHODS

Plant material and microorganisms

In this study we used three wild-type *Arabidopsis thaliana* ecotypes (*Columbia* [Col], *Landsberg erecta* [Ler] and *Wassilewskija* [Ws]) and their different publicly accessible mutants. Cloning was performed by the DH5α strain of *Echerichia coli* and *Agrobacterium* conjugation was carried out by the S17-1 strain. Plants were transformed by the GV3101 strain of *Agrobacterium tumefaciens*.

Growth conditions

Until sowing, seeds of *A. thaliana* were stored in dark at 4ºC. Seeds were sown on the surface of COMPO SANA soil. Plants were grown in greenhouses without using artificial light from spring to autumn whereas in growth chamber (12/12h light/dark photoperiods, 80% relative humidity and 23±2ºC) during the winter period. Transformation was effectuated on 8-10-week-old plants bearing flowers. After harvesting, seeds from transformed plants were sterilised and spread on MS medium. After stratification (48 hours, 4ºC), plates were transferred into growth chambers and seedlings were grown under 12h light/12h dark photoperiods for six days. UV-B irradiation was effectuated on the seventh day. For red-light treatment, seeds were kept at 4ºC for two days after sterilisation. Next, for germination, they were transferred into 96-well format microtiter plates and kept in darkness at 23ºC during 4 days. They were subjected to red-light irradiation on the fifth day.

Arabidopsis thaliana transformation and generation of transgenic lines

Arabidopsis plants were transformed using the floral-dip method. The GV3101 strain of *Agrobacterium tumefaciens* containing binary vectors responsible for hygromycine resistance was used for the selection of transgenic plants. Primary transformants were selected upon their hygromycine resistance.

lower intensity (~5x). Thus, we speculate that regulatory elements in this region also contribute to red-light induction.

We started a luciferase-based genetic screening in *Arabidopsis*. Among individuals showing decreased luciferase activity after EMS mutagenesis, we focused on two mutants, recovered from independent pools, in which only UV-B mediated activation of the Pro_{ANAC13}:Luc transgene was compromised, but in which the UV-B mediated activation of the endogen ANAC13 remained unaltered. Our data showed that both mutants carried the same point mutation (C→G transition) at the -110 bp position of the promoter. In order to clarify the potential role of nucleotides flanking the cytosine located at the seemingly critical position for UV-B response, we generated a further point mutation at the -107 bp position (G→A). Analysis transgenic plants containing these mutant chimeric genes provided convincing evidence that each of these two point mutations is able to significantly reduce UV-B induction of Pro_{ANAC13}:Luc. This newly identified regulator element, which we designated $UVBox^{ANAC13}$, is a major cis-regulatory element controlling the UV-B response of the ANAC13 promoter. We favour the hypothesis that for maximal efficiency, MRE^{ANAC13} and $UVBox^{ANAC13}$ in the proximal promoter region and probably other ACE^{ANAC13} and MRE^{ANAC13} elements act cooperatively by binding different, still unidentified, transcription factors. We showed that $UVBox^{ANAC13}$ has no role in the red-light induction of ANAC13. This was proved by the fact that while the distal (from -1457 to -198) region of the promoter abolished red-light induction, point mutations affecting the $UVBox^{ANAC13}$ in the full-length promoter do not alter red-light activation. Nevertheless, our findings do not exclude the existence of a possible cooperation between yet-to-be-identified cis-elements in the proximal promoter region, ACE^{ANAC13} and MRE^{ANAC13} in order to mediate red-light response of ANAC13.

Light and other environmental signals often jointly mediate specific developmental responses, which suggests the existence of links between different signalling pathways. The UV-B induced signal transduction network has proved to be overlapping with wounding response. The majority of phenylpropanoids act as light-filters or/and protectors against herbivore attacks. Microarray analyses have identified a great number of UV-B induced genes that are also activated for other stress factors. Several members of the NAC protein family also participate in different biotic and abiotic stress-induced signal transduction. Taken together, we assume that a myriad of regulating factors is probably needed for controlling certain genes. In order to gain further understanding about the induction of the genes, it would be important to know whether different stimuli induce transcriptional responses through common or

corroborate and extend former results showing that complex and probably distinct signalling pathways regulate the expression of genes in the UVR8-dependent pathway (UVR8, COP1, HY5) and of ANAC13.

Most elements of the UV-B induced signal transduction are also known as actors in other light signalling pathways. HY5 has been the first and most intensively investigated positive regulator of photomorphogenic development. It probably acts downstream in signalling pathways of several light-induced and other development processes. The ubiquitin ligase COP1 also has a complex role in light-signalling which is shown by the fact that PhyA, PhyB, Cry1 and Cry2 photoreceptors are in direct interaction with COP1. One of the key enzymes of the phenilpropanoid pathway is the KALKON-SYNTASE the expression of which is both regulated by red/far-red and blue/UV-A light. Our study demonstrates that the expression of ANAC13 is induced both by UV-B and red light. Compared to the UV-B response, red-light induction reaches its maximum slightly more rapidly but its intensity remains significantly lower. Taken together, we propose that ANAC13 expression is regulated by complex and probably distinct signalling pathways.

UV-B and UV-A/blue light-induced changes in the expression of CHS and other phenylpropanoid biosynthesis genes (CFI, F3H and FLS) are mediated by complex light regulatory elements such as LRUs/LREs. In *Arabidopsis thaliana*, these light regulatory elements contain two different cis-regulatory elements: the bZIP-binding ACE and the MRE elements. Comparing the promoter regions of the above mentioned phenylpropanoid biosynthesis genes and of ANAC13, we found that the 1.5 kb-long promoter region of ANAC13 contains two MRE^{CHS} and three ACE^{CHS} elements, four of which are localised pairwise, similarly to the other investigated genes. In order to establish the role of the putative cis-elements and to define whether UV-B and red-light induction of ANAC13 is regulated by independent or common cis-regulatory elements, we analysed the expression patterns of a series of ANAC13 promoter mutants in *Arabidopsis* seedlings. As a result of these experiments, we can conclude that two promoter regions are needed for maximal UV-B induction of ANAC13: a short sequence in the proximal promoter region (from -110 to +24) and a second short sequence which contains the putative MRE^{ANAC13} regulatory element (from -146 to -110). Furthermore, red-light induction of the above mentioned promoter also requires two promoter regions. Contribution of a yet unknown element in the distal (from -1457 to -198) promoter region is essential for its functioning, as the chimeric gene containing the truncated ANAC13 promoter (from -110 to +24) displays no red-light induction. When the -110 - +24 promoter region was replaced by the CaMV35S minimal promoter, red-light response was again detectable albeit at

Abiotic and biotic stress treatments and measurement of luciferase activity

For UV-B and quartz treatments the six-day-old seedling were placed into 96-well microtiter plates which contained 200 µl MS medium per well. 20 µl 2mM D-luciferin solution was added to each plant. Light treatments were carried out on 7-day-old seedlings. Light-induced luminescence changes in seedlings carrying the luciferase gene were measured by TopCount™ automated luminometer. A lamp containing eight Philips TL 40W/12 UV tubes was used for UV-B (25 min) and quartz (15 min) treatments. The required range of the light spectrum was obtained was by the use of a 3-mm transmission cut-off filter (WG305 quartz) placed between the lamp and the microtiter plates. For red-light experiments, the 4-day-old etiolated seedlings were pre-incubated for one day in luciferin solution before being subjected to red-light irradiation on the fifth day. For these red-light treatments (60 min), a light source containing 936 red-light emitting diodes was used. For hormone treatments, 100 µM ABA solution, for salinity stress 250 mM NaCl solution and for osmotic stress 100mM mannitol was added to the seedlings. For heat and cold stress, seedlings were incubated for 6 hours in a growth chamber at 37ºC and in a cold room at 4ºC, respectively.

Molecular biological methods

Isolation and manipulation of nucleic acids (purification of plasmid DNA, isolation of plant DNA, RNA extraction, electroforetic separation, DNS fragment isolation, polymerase chain reaction, Southern-analysis, RNA analysis and sequencing), transformation of *E. coli,* conjugation of *A. tumefaciens*, introduction of site directed point mutations were performed as described in scientific literature or according to the manufacturers' instructions.

EMS mutagenesis

Following vernalisation, a stock of approximately 1 g (~50 000 pieces) seeds were pre-soaked in water for a few hours than gently shaken in 0.2% etilmethan-sulfonate solution for 12 hours. After this treatment, seeds were washed in abundant water and finally sown into 800 pots. Plants harvested from each pot were considered as distinct pools and were subjected to screening. From each pool, approximately 150-200 seeds were spread onto MS medium. Germination rate was about 50%, thus luciferase activity measurements could be performed on approximately 100 seedlings per pool. Measurements were carried out by TopCount™ automated luminometer. Seedlings exhibiting reduced luciferase activity were grown to maturity and their seeds harvested. RNA isolated from

seedlings grown from this M3 seed-population was subjected to semi-quantitative PCR examinations.

RESULTS

Results of full genome microarray analysis from 7 day-old seedlings of light-grown *Arabidopsis thaliana Wassilewskija (Ws)* ecotype subjected to UV-B irradiation had been reported before our study. 15 minute-long UV-B treatment at different wavelengths caused changes in the expression of a large number of genes. Specifically, 100 genes showed at least double induction for low-intensity UV-B and 7 genes were repressed.

Analysis of UV-B induced transcriptional changes

On the basis of microarray analysis, we selected 10 genes which responded to low-intensity UV-B with the highest levels of induction. In order to determinate whether UV-B regulates expression of these genes at the level of transcription and/or mRNA stability, we used a luciferase reporter system as a marker of transcriptional activity. In this *in vivo* experimental system, promoter regions of the 10 most highly inducible genes, represented by approximately 1.5 kb fragments were fused to the luciferase reporter gene and these chimeric genes were then stably transformed into *Arabidopsis* plants. Following the exposure of 7 day-old transgenic plants to polychromatic UV-B radiation, luciferase activity was measured by an automated luminometer. These measurements show that longer wavelength UV-B irradiation induces rapid changes in the expression of Pro$_{HY5}$, Pro$_{ELIP2}$, Pro$_{At5g52250}$ and Pro$_{ANAC13}$ promoters while unfiltered UV-B enhances the transcriptional activity of Pro$_{ZAT12}$, Pro$_{UGT72C1}$ and Pro$_{ANAC13}$ promoters. It is to be noted that Pro$_{ANAC13}$ displays similar expression patterns to longer wavelength and unfiltered UV-B.

Spectral characteristics of the early UV-B response of ANAC13 gene

From the 10 genes analysed, ANAC13 was chosen for further experiments due to its special expression pattern. Notably, not only it showed higher induction than average but its induction was similar in the two analysed spectra. Being a yet uninvestigated element, it seemed to be the ideal candidate for further studies. Four different UV-B spectra were created for UV-B treatments: WG327 (negative UV-B control, mainly UV-A), WG305 (low-level UV-B),

chimeric genes upon longer-wavelength and unfiltered UV-B treatments supported and extended our data obtained from microarray analysis. Furthermore, they indicate that the UV-B response of all ten selected genes is mainly regulated at transcriptional level. Among the four different spectrum ranges obtained by the use of cut-off filters, WG295 was able to trigger the most abundant transcript accumulation as far as the UV-B treatment of ANAC13 is concerned. This finding also corroborates the result of microarray analysis. In fact, transcriptional activity of ANAC13 is further induced when UV-B treatment is extended towards shorter wavelengths.

In the majority of development processes several photoreceptors are involved in light perception resulting in a complex network of interactions between different light-induced signalling pathways. As evidence is growing that plants operate several distinct UV-B signalling pathways, it becomes more likely that existing photoreceptors participate in at least some of these pathways. Several findings indicate that UV-B induced transcription is mediated by a specific UV-B receptor or UV-B receptors. Further studies suggest a link between UV-B and cryptochrome signalling pathways. On the basis of this hypothesis/these observations, we further investigated ANAC13 and demonstrated that its transcriptional-level UV-B response is independent from known cryptochromes ant phototropins although we can not exclude potential multiple overlapping between the function of different photoreceptors. In cryptochrome 1 and 2 double mutants (*cry1cry2*) the expression rate for shorter and longer-wavelength UV-B treatments was shifted towards the longer-wavelength. Thus, we presume that cryptochromes play a role in the UV-B response of ANAC13.

Some key regulators of UV-B induced photomorphonegetic development such as the bZIP transcriptional factor HY5, the E3 ubiquitin ligase COP1 and the UVR8 protein have already been identified. The direct interaction of UVR8 and COP1 proteins in the nucleus of plant cells has been established. Downstream the COP1 and UVR8 proteins, HYH and especially HY5 play a crucial role in UV-B induced signal transduction. In UV-B specific photomorphogenetic responses, expression of most genes depends upon COP1 and UVR8. In the semiquantitative RT-PCR analysis of our study we compared the expression patterns of the endogene ANAC13 gene in UV-B-induced wild-type plants and in *cop1-4, uvr8-1* single mutants and *cop1-4/uvr8-1* double mutants. We examined the UV-B inducibility of the ProANAC13::Luc reporter construction in wild-type individuals and in *cop1-4* mutants. As no significant differences have been found, we can conclude that ANAC13 expression is partially independent from COP1, UVR8 and HY5 genes. These findings

4. We demonstrated that ANAC13 expression is also induced by red light and we proved that the red-light and the UV-B response of ANAC13 are partly regulated through distinct cis-elements.
5. Using EMS mutagenesis, we identified a point mutation in the promoter region of the ANAC13 gene; a point-mutation which significantly reduces the UV-B induction of the Pro$_{ANAC13}$ ::Luc transgene.
6. In order to clarify the role of the above point mutation, we generated a further point mutation and identified a new cis-regulatory element that we named UVBox^{ANAC13}. This newly identified regulatory element plays a crucial role in controlling the Uv-B response of ANAC13. Our study suggests a cooperation of the UVBox^{ANAC13} with the MRE^{ANAC13} in the proximal promoter region and possibly with other ACE^{ANAC13} and MRE^{ANAC13} elements in order to achieve maximal UV-B response. This cooperation would be mediated by binding different transcriptional factors which are yet to be identified.
7. By further investigating the newly identified UVBox^{ANAC13} we showed that it is not only necessary but sufficient for UV-B response. Furthermore we demonstrated that its regulatory function is UV-B specific.

DISCUSSION

Rapid and transient transcriptional changes induced by UV-B and the strong presence of transcriptional factors among UV-B activated genes suggest that, similarly to signalling pathways triggered by visible light, complex regulatory networks are activated by the UV-B signal. Our microarray analysis shows that after irradiating *Arabidopsis thaliana Wassilewskija* ecotype plants by broad-band UV-B light for 15 minutes, among the 100 induced genes, 62 were only activated by longer-wavelength UV-B while the remaining 38 genes, including ANAC13, were also induced by shorter-wavelength WG295 and quartz treatments. It is of note, because WG305, WG295 and quartz treatments cover the same ranges of the spectrum with the only difference that WG295 and quartz are also extended to shorter wavelengths. This indicates that shorter-wavelength UV-B down-regulates the transcription of genes induced by low-level UV-B, suggesting the presence and interaction of at least two UV-B signalling pathway.

Results of the microarray analysis prompted us to investigate those genes which showed the highest induction levels. Changes in the luciferase activity of

WG295 (higher level UV-B) and quartz (unfiltered, strong UV-B). Full RNA was isolated from the samples for Northern analysis. Transcript accumulation was stronger under WG305, WG295 and quartz treatments than in the control group. Stronger signal was detected in the case of WG295 than for WG305 and quartz treatments.

Rapid and transient UV-B induction of ANAC13

Seven-day old seedlings were exposed to UV-B irradiation provided by WG327 and WG305 cut-off filters. Samples for full RNA extraction were collected 0.5, 1, 2, 4 and 8 hours after the onset of irradiation. Results of Northern analysis show that the UV-B response of ANAC13 is quick and transient. Maximum level of transcript accumulation was detected at approximately 1 h followed by a gradual decline in transcript levels.

Transcript-level UV-B response of ANAC13 is partially independent from known photoreceptors

We investigated whether known photoreceptors play a role in the transcript level UV-B response of ANAC13. We compared UV-B inducibility of ANAC13 genes from phytochrome A and B (*phyAphyB*), cryptochrome 1 and 2 (*cry1cry2*) and phototropin 1and 2 (*phot1phot2*) double mutants with the same gene from wild-type plants. Phytochrome and phototropin photoreceptor double mutants and wild type plants displayed similar UV-B responses in all investigated spectra**.** However, in the case of cryptochrome double mutants, ANAC13 expression rate under longer and shorter wavelength irradiation was shifted towards longer wavelengths.

Position of ANAC13 in UV-B signal transduction

In order to determine whether the ANAC13 gene is part of the COP1, UVR8 and HY5 determined signal transduction pathway or it is regulated by an other signalling cascade, semi-quantitative RT-PCR was performed to compare the expression patterns of endogenic ANAC13 in UV-B irradiated wild-type plants and in different mutants. We found that UV-B induction of ANAC13 was not significantly affected in *cop1-4* mutants in contrast with the induction of HY5. Furthermore, UV-B response of ANAC13 was also only slightly affected in *uvr8-1* and *cop1-4/1* double mutants compared to wild-type plants (Col).

Activation of ANAC13 upon red-light treatment

Apart from UV-B, red light also induces ANAC13 expression. Pro_{ANAC13} expression showed six-fold induction after red-light treatment of etiolated seedlings.

We compared the sequence of ANAC13 promoter to that of CHS and of some other genes involved in phenilpropanoid biosynthesis (CFI, F3H, FLS). We found the ANAC13 promoter contains two MRE^{CHS} and three ACE^{CHS} elements, four of which localised pairwise. Elimination of the -1457 / -198 region of the promoter did not significantly alter UV-B induction by longer wavelengths or upon unfiltered irradiation, but it abolished red-light inducibility. Removal of the -1457 / -146 region did not cause any significant changes in the UV-B response. However, elimination of the putative ACE^{ANAC13} and MRE^{ANAC13} elements of the proximal promoter region had drastic consequences on UV-B inducibility. Similarly to the CaMV35S minimal promoter, the expression of this short (-110 / +24) construction was not altered by UV-B. Substitution of the short (-110 / +24) promoter region by the CaMV35S minimal promoter significantly diminished UV-B induction, notably to one tenth of its original level (~2.5x). In contrast, response to red light was only slightly weakened (~4-5x). Targeted mutation of the putative MRE^{ANAC13} element completely abolishes the UV-B responsiveness of the construction while it only reduces the intensity of red-light responsiveness to its third. Simultaneous destruction of putative ACE^{ANAC13} and MRE^{ANAC13} elements caused the complete absence of both UV-B and red light responses.

Identification of point mutants by luciferase-based genetic screening

In order to identify the elements of the UV-B dependent signalling cascade, we performed luciferase-based genetic screening in *Arabidopsis*. Expression of the Pro_{ANAC13}::Luc transgene remained unchanged in individuals carrying two independent mutations, whereas it was induced in wild-type plants. Sequence information shows that both mutants carry a point mutation in the same position (-110) of the proximal region of their promoters. With directed point-mutation method we generated a further point mutation at the -107 position. Each of these two point mutations, independently from each other, is able to reduce the UV-B inducibility of the promoter. No significant changes were observed in the red-light inducibility of the five-day-old seedlings carrying either the chimeric genes containing the point mutations or the wild-type construction.

The newly identified $UVBox^{ANAC13}$ regulatory element confers UV-B specific responsiveness to the CaMV35S minimal promoter

A 12 bp-long fragment of the proximal promoter region containing the putative core sequence of $UVBox^{ANAC13}$ regulatory element was used in a dimerized form. Using the CaMV35S promoter we created two constructions: one contains the dimerized form of the wild-type $UVBox^{ANAC13}$ followed by the CaMV35S minimal promoter; while in the second one, the mutant $UVBox^{ANAC13}$ dimer is placed before the 35Smin promoter. Both were fused to a luciferase reporter. ($Pro_{2xUVBoxANAC13-35Smin}$::Luc, mut$Pro_{2xUVBoxANAC13-35Smin}$::Luc). Similarly to the Pro_{ANAC13} ::Luc transgene, $Pro_{2xUVBoxANAC13-35Smin}$::Luc is also induced both upon longer-wavelength and unfiltered UV-B treatment. Considering the basal level expression, relative UV-B induction is stronger in $Pro_{2xUVBoxANAC13-35Smin}$::Luc than in Pro_{ANAC13} ::Luc. No UV-B response was observed for the chimeric gene containing the point mutation (mut$Pro_{2xUVBoxANAC13-35Smin}$::Luc). Apart from UV-B, salinity, heat and - to a minor extent – ABA and red light also induce the Pro_{ANAC13} ::Luc construction. In contrast, $Pro_{2xUVBoxANAC13-35Smin}$::Luc was only induced by UV-B irradiation, and remained unaffected by all other treatments performed in our experiments.

New scientific achievements

1. We provided evidence that the UV-B response of the ANAC13 gene is mainly regulated at transcriptional level. Among the four different spectra utilized, transcript accumulation was the strongest upon WG295 irradiation, suggesting that transcriptional activation of ANAC13 is further induced when UV-B treatment is extended towards shorter wavelengths.
2. We showed that the transcriptional level UV-B response of ANAC13 is independent from the known phytochromes and phototropins although we can not exclude the existence of multiple overlapping in the role of different photoreceptors. Cryptochromes presumably play a role in the UV-B response of ANAC13.
3. We proved that ANAC13 gene is partially independent from COP1, UVR8 and HY5 genes. Thus elements of the UVR8 signalling pathway (UVR8, COP1, HY5) and ANAC13 are likely to be regulated by distinct signal transduction pathways.