

■ Teaching

Learning about the molecular basis of plant responses to UV-B: a laboratory class at the University of Glasgow

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Abstract

Plant responses to UV-B provide an excellent system for students to learn about the regulation of gene expression following stimulus perception. This article concerns a laboratory class for undergraduate students at the University of Glasgow that is based on molecular responses to UV-B in *Arabidopsis thaliana*. During the class students design and plan experiments, interpret and discuss their results with other students and present the findings. Hence they learn valuable research skills. Some examples of students' work are presented and students' perceptions of the class are summarized.

Introduction

Ultraviolet-B (UV-B) wavelengths (280–315 nm) have numerous regulatory effects on plant growth and development (Jenkins 2009; Jordan 1996; Robson et al. 2014; Vanhaelewyn et al. 2016). It is well established that these effects result from UV-B-stimulated differential expression of large

numbers of genes (Heijde and Ulm 2012b; Jenkins 2009). Responses to UV-B may involve several different perception and signal transduction processes, but many are mediated by the UV-B photoreceptor UV RESISTANCE LOCUS 8 (UVR8) (Jenkins 2014, 2017; Ulm and Jenkins 2015). Responses to UV-B are important because they modify biosynthesis, chemical composition and nutritional quality of plants, resistance to attack by pests and pathogens, and various aspects of development (Wargent and Jordan 2013). Moreover, UV-B responses affect both agriculturally important species (Wargent and Jordan 2013) and plants growing in natural ecosystems (Robson et al. 2014). Given their wide-ranging impact, it is important to raise awareness of plant responses to UV-B, and this is one of the aspirations of UV4Plants, the international association for plant UV research. Furthermore, it is vital to train and enthuse the next generation of researchers who will extend understanding of plant responses to UV-B and apply the knowledge gained in crop production, crop improvement and biotechnology. There is a partic-

ular onus on university teachers to do this.

This article concerns a laboratory class for undergraduate students at the University of Glasgow that is based on plant responses to UV-B. In Glasgow, life sciences students normally study for a BSc Honours degree over four years (<http://www.gla.ac.uk/schools/lifesciences/undergrad/>). The first two years provide a broad foundation in biological subjects and the final two years are dedicated to a particular degree subject. Most life sciences students encounter plant biology at some point in their courses and students can opt to take plant science as a specialism in the degree of Molecular and Cellular Biology (with Plant Science). In the University of Glasgow, most research in plant science concerns the molecular basis of responses to the environment, and several research groups are focused on plant photobiology. Responses to UV-B provide a good vehicle for students to learn about plant environmental perception and differential gene expression.

Students taking the degree courses in Genetics (<http://www.gla.ac.uk/undergraduate/degrees/genetics/>) and Molecular and Cellular Biology (<http://www.gla.ac.uk/undergraduate/degrees/molecularcellularbiology/>) take a number of extensive laboratory classes in their third year. The laboratory class described here occupies 2.5 days per week for four weeks and is usually taken by over 80 students. The class is intended to introduce students to methods used to study gene regulation and also to working with *Arabidopsis*, but a major aim is to develop skills in planning and designing experiments. The students are given scope to select genes for study, to choose questions to address in their experiments and to plan their work. They work in teams to design, execute and interpret their experiments, which encourages discussion and promotes learning through experience. This article provides information about what the laboratory class

involves, examples of outcomes, and the experiences and perceptions of students who take it.

Outline of the laboratory class

The focus of the class is to investigate the regulation of gene expression in response to UV-B exposure of *Arabidopsis*. Students examine the expression of selected genes and the role of the UVR8 photoreceptor in mediating these responses. UVR8 detects UV-B radiation and triggers responses to UV-B in plants (Jenkins 2014; Ulm and Jenkins 2015). The processes involved in UVR8 action are outlined in Figure 6.1. In the absence of UV-B, UVR8 protein forms homodimers that do not initiate UV-B signal transduction. The dimer subunits are held together by salt bridges between charged amino acid residues at the dimer interface, in particular between arginine, aspartate and glutamate amino acids (Christie et al. 2012; Wu et al. 2012). The UV-B stimulus converts UVR8 to the monomeric state (Rizzini et al. 2011). Differently to other photoreceptors, which detect radiation with chromophores, UVR8 perceives UV-B through specific tryptophans in the dimer interface (Christie et al. 2012; Wu et al. 2012). When stimulated, the tryptophans transfer excited electrons to specific charged amino acids, which become neutralized, resulting in destabilization of salt bridges and subsequent UVR8 monomerization (Christie et al. 2012; Mathes et al. 2015). In its monomeric form, UVR8 binds to CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) (Rizzini et al. 2011). In darkness, COP1 is part of an E3 ubiquitin ligase complex that targets proteins involved in the UV-B response for proteolysis, especially the ELONGATED HYPOCOTYL 5 (HY5) transcription factor. However, when bound to UVR8 monomer, COP1 is not involved in ubiquitin ligase activity allowing HY5 to accumulate (Huang et al. 2013). UVR8 and COP1 together regulate transcription of numerous UV-B response genes (Favory et al.

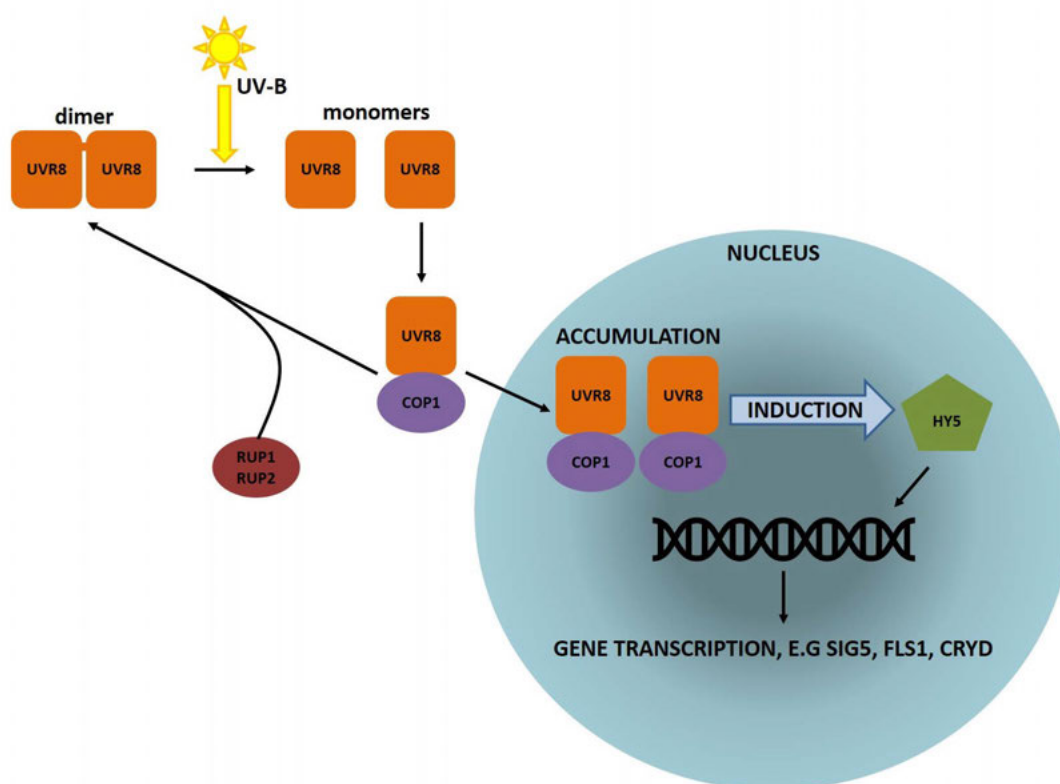


Figure 6.1: Schematic representation of UVR8 function in plant cells upon illumination with UV-B. Upon excitation with UV-B light, detected by tryptophan chromophores, the UVR8 dimer dissociates to produce monomers. In the cytoplasm, the UVR8 monomers can be bound by COP1 to initiate downstream signalling. UVR8 accumulates in the nucleus, where, together with COP1, it induces rapid accumulation of the HY5 transcription factor. This results in transcription of over 100 genes regulated by the UVR8 signalling pathway, such as *SIGMA FACTOR 5 (SIG5)*, *FLAVONOL SYNTHASE (FLS1)* and *CRYPTOCHROME DASH (CRYD)*. RUP1 and RUP2, also induced by UVR8 signalling, disrupt the UVR8-COP1 interaction and promote re-dimerisation of UVR8.

2009; Jenkins 2014), including those encoding the HY5 and HY5 HOMOLOG (HYH) transcription factors. When the UV-B stimulus ceases, UVR8 re-dimerizes, re-establishing the initial conditions. UVR8 re-dimerization is facilitated by binding of REPRESSOR OF PHOTOMORPHOGENESIS (RUP) 1 and RUP2 (Heijde and Ulm 2012a). RUP protein expression is stimulated by UV-B, detected by UVR8, resulting in a negative feedback mechanism (Gruber et al. 2010). Furthermore, RUP proteins compete with COP1 for binding to the C27 region of UVR8, which comprises residues 397 to 423 in the C terminus of UVR8 (Cloix et al. 2012). This way, RUP1 and RUP2 not only repress the UV-B response

by promoting re-dimerization, but also by diminishing the binding of UVR8 to COP1.

In the first, computer based session, students examine transcriptome analysis data from wild-type and *uvr8* mutant *Arabidopsis* exposed, or not, to UV-B (Brown et al. 2005; Brown and Jenkins 2007) to identify potential genes to study, and search for publications to find further information. Students then form teams based on which gene(s) they want to study. Members of the teams must work together to plan and execute experiments, initially using RT-PCR to examine gene expression. The students can use wild-type and mutant plants (such as *uvr8*, *hy5*, and *hy5 hyh*) and expose them to treatments such as

broadband or narrowband UV-B for different times and at different fluence rates; stresses such as salt treatment, or high/low temperature, to find out how these conditions influence the expression of their gene of interest. Based on the initial results, teams then plan and perform a second set of experiments. After they have the results of all their RT-PCR assays, they select a number of RNA samples to quantify the transcripts of specific genes through the use of real-time qPCR. To investigate UV-B signal transduction by UVR8, students carry out a yeast 2-hybrid assay to examine interaction with COP1. This allows them to test the importance of particular domains/amino acids of UVR8, especially the C-terminal region, in the interaction with COP1. In addition, the students use transgenic *Arabidopsis uvr8* mutant plants expressing wild-type or mutant forms of UVR8 fused to Green Fluorescent Protein (GFP). They visualize the protein by immunodetection using anti-GFP antibodies on western blots, enabling them to monitor the dimer/monomer status of UVR8 following UV-B exposure. Finally, students expose purified wild-type UVR8 protein to UV-B or other selected treatments, and examine dimer/monomer status by gel electrophoresis. The students keep records of their experiments and write a lab report, which is assessed.

Examples of student work

Gene expression

In the gene expression experiments undertaken by the 2017 class, over 10 different genes and a variety of treatments were studied. The data below are illustrative of the results obtained.

Expression of *RUP1* and *RUP2* genes was examined in response to different levels of UV-B exposure. Wild-type Landsberg erecta (*Ler*) and *uvr8-1* mutant *Arabidopsis thaliana* plants were grown in a growth cabinet at 20°C for 21 days in a low fluence rate (25

$\mu\text{mol m}^{-2} \text{s}^{-1}$) of fluorescent white light lacking UV-B, essentially as described by Brown and Jenkins (2007). Plants were then exposed to 0, 5 and 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of narrowband UV-B for 4 hours (total doses¹ of 0, 72 and 144 mmol m^{-2} respectively). The narrowband source has a peak emission at 312 nm and is effective in activating UVR8 (Favory et al. 2009). Plants were harvested, RNA isolated and *RUP1* and *RUP2* transcript levels quantified relative to control *ACTIN2* transcripts, which are unaffected by UV-B exposure, using RT-qPCR with gene-specific primers. Figure 6.2 shows increased expression of *RUP1* and *RUP2* in a UVR8-dependent manner at 72 mmol m^{-2} . At 144 mmol m^{-2} expression drops drastically in wild-type plants suggesting that fluence rate plays a key role in *RUP1* and *RUP2* expression. A 4-fold greater increase of *RUP2* transcript levels compared to *RUP1* was observed in the wild-type plants at 72 mmol m^{-2} . Such a difference in levels of expression was not observed by Gruber et al. (2010). The observations add to previous studies showing that *RUP1* and *RUP2* expression is transient and induced by different light qualities (Gruber et al. 2010), but the experiments need to be repeated and extended to learn more about the regulation of the *RUP* genes.

ZINC FINGER OF ARABIDOPSIS THALIANA12 (*ZAT12*) is a zinc finger protein that has been functionally characterized to play a role in response to abiotic and biotic stress factors (Davletova 2005). *ZAT12* gene expression increases rapidly in response to a range of stress treatments, including UV-B (Kilian et al. 2007), and regulation in response to oxidative stress may underpin these responses Hahn et al. 2013. The aim of the experiment was to determine whether *ZAT12* expression was induced by UV-B and oxidative stress independently and in combination. Wild-type

¹Editor's note: although frequently used when *photon exposure* is meant, according to the IUPAC Gold Book, this use is discouraged as dose describes photons or energy absorbed per unit volume or mass (see <https://goldbook.iupac.org/>)

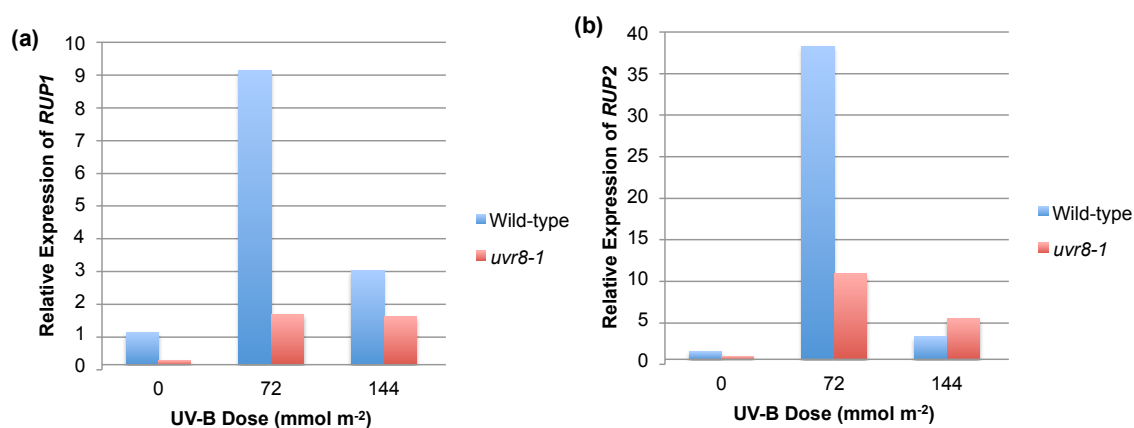


Figure 6.2: *RUP1* and *RUP2* gene activation in response to narrowband UV-B in wild-type and *uvr8-1* mutant *Arabidopsis thaliana* plants. Wild-type Ler and *uvr8-1* mutant plants grown in fluorescent white light lacking UV-B were illuminated with 0, 5 or 10 $\mu\text{mol m}^{-2} \text{s}^{-1}$ narrowband UV-B for 4 hours, corresponding to doses of 0, 72 and 144 mmol m^{-2} UV-B. Transcript levels of *RUP1* (a) and *RUP2* (b) were determined by quantitative RT-PCR analysis and normalized to the level of control *ACTIN2* transcripts. Transcript levels are presented relative to expression in wild-type without UV-B illumination.

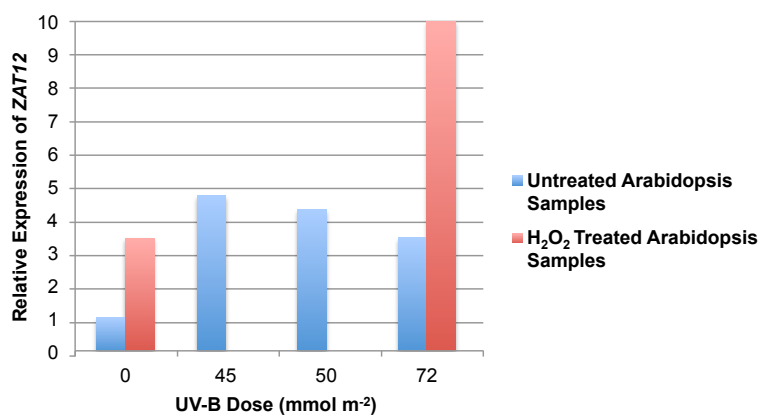


Figure 6.3: Relative *ZAT12* expression in UV-B treated and H_2O_2 treated *Arabidopsis thaliana* plants. Wild-type Ler *Arabidopsis* plants grown in fluorescent white light lacking UV-B were given different doses of broadband UV-B by varying fluence rate and duration of exposure. Where indicated, H_2O_2 treatment was provided by spraying the plants 3 times with a 1% (v/v) solution. *ZAT12* transcript levels in RNA samples were assayed by quantitative RT-qPCR and normalised to levels of control *ACTIN2* transcripts. *ZAT12* expression in the different samples is expressed relative to that in the non-treated sample.

A. thaliana plants grown as above were exposed to different doses of UV-B provided by a broadband source (spectrum described by Cloix et al. 2012) by altering the fluence rate or duration of exposure. *ZAT12* transcripts were assayed by using gene-specific primers, and normalized against transcripts of the control *ACTIN2* gene. Figure 6.3 shows that a significant increase of *ZAT12* expression was observed following exposure to all UV-B doses used. Additionally, under oxidative stress (caused by spraying plants with hydrogen peroxide), it was found that *ZAT12* expression was much higher, both in the presence and absence of UV-B. This is in line with previous studies which show that *ZAT12* expression is induced by UV-B stress and oxidative stress. It is likely that the moderate UV-B doses used here did not generate a sufficient level of reactive oxygen species to induce maximal *ZAT12* expression, which would explain why additional expression occurred when hydrogen peroxide was applied.

Interaction between UVR8 and COP1

A yeast 2-hybrid assay was used to examine protein-protein interactions between wild-type UVR8, a deletion mutant of UVR8 lacking amino acids 397-423 (termed the C27 region) in the C-terminus (UVR8^{ΔC27}), and COP1. The methods of Cloix et al. (2012) were used. UVR8 and the UVR8^{ΔC27} mutant were each cloned as a fusion with the DNA-binding domain of the yeast GAL4 transcription factor, whereas COP1 was fused to the activation domain of GAL4 in a separate plasmid vector. Expression of both fusion proteins in yeast and interaction between them resulted in the reconstruction of GAL4 from two separate polypeptides, which enabled growth on a selective medium; no interaction resulted in the absence of growth. Mammalian T antigen and p53 proteins that interact strongly were used as a positive control, whereas empty vectors were used as a negative control.

As shown in Table 6.1, all colonies grew in non-selective media, confirming viability of the yeast cells. Yeast cell growth was also observed in over 90% of selective plates containing the positive control, both in darkness and under illumination with UV-B. On the contrary, no growth was observed for the negative control. Under UV-B exposure, yeast cell colonies transfected with UVR8 and COP1 plasmids grew in approximately 81% of plates (Table 6.1) while in darkness, no growth was observed. In contrast, when illuminated, 92% of yeast co-transfected with UVR8^{ΔC27} and COP1 did not form colonies (Table 1). Similarly, in darkness, growth was observed in only 1 out of 26 plates.

Since yeast colony growth reflects interactions between the proteins of interest, the results indicated that UVR8 interacts with COP1 in the presence of UV-B light. However, the mutant UVR8 protein lacking the C27 region did not interact with COP1, indicating that C27 is required for UV-B dependent interactions of UVR8 and COP1 in yeast. This finding is in agreement with that of Cloix et al. (2012), who additionally found that C27 is required for interaction with COP1 in plants. They further reported that transgenic plants expressing UVR8^{ΔC27} had impaired responses to UV-B radiation, including *HY5* expression, which has a key role in mediating UVR8 responses. However, another study (Yin et al. 2015) reported that COP1 can interact with UVR8 lacking the C-terminal amino acids that include C27. These researchers discovered two distinct domains of UVR8 interacting with COP1, the first being the C27 region and the second the β -propeller domain, which interacts with the WD40 region of COP1 in a UV-B dependent manner. Nevertheless, it should be mentioned that this study employed less stringent selection to test interaction than used here.

Table 6.1: Yeast 2-hybrid assay of the interaction between UVR8 and COP1. Yeast 2-hybrid plasmids containing the GAL4 DNA binding domain (BD) or activation domain (AD) fused to the proteins indicated were co-transformed in yeast. Negative control: plasmid vectors with no inserts (-); positive control: plasmid vectors containing mammalian p53 and antigen T; test interactions: plasmid vectors containing either wild-type UVR8 or a mutant with a deletion of the C27 region (UVR8^{ΔC27}) and COP1. Yeast growth was tested on non-selective media (for viability) and selective media (for interaction) in darkness or under 0.1 μmol m⁻² s⁻¹ narrowband UV-B. The numbers in the table indicate growth (+) or no growth (-) and are the data of 26 groups of students.

		Darkness				UV-B			
Culture Medium		Non-sel.		Selective		Non-sel.		Selective	
Yeast growth		+	-	+	-	+	-	+	-
BD	AD								
-	-	26	0	3	23	26	0	2	24
P53	T-Ant	26	0	24	2	26	0	25	1
UVR8	COP1	26	0	1	25	26	0	21	5
UVR8 ^{ΔC27}	COP1	26	0	1	25	26	0	2	24

Effect of UV-B radiation on UVR8

An experiment was undertaken to examine the effect of UV-B exposure on wild-type and mutant UVR8 proteins expressed in *Arabidopsis uvr8-1* as GFP fusions. Extracts were prepared from plants and illuminated on ice with UV-B as described by Cloix et al. (2012). Following UV-B exposure, plant extract samples were run on a SDS-PAGE gel without boiling, followed by immunoblotting with a GFP-specific antibody. This method permits detection of the dimer and monomer forms of UVR8 (Rizzini et al. 2011). The western blot (Figure 6.4) showed an increase in the intensity of the monomer band following UV-B illumination of plant extracts with 1 μmol m⁻² s⁻¹ broadband UV-B for 15 to 60 minutes (approximate monomer size was identified by complete UVR8 denaturation in a boiled control). Quantification of band intensity with ImageJ showed that monomer proportion correlates with UV-B dose (Figure 6.5); while 10% of total UVR8 was present as monomer in the non-illuminated control, monomer proportion had increased to 65% following a 1-hour UV-B treatment. This observation is consistent with research demon-

strating that UV-B exposure induces UVR8 monomerization. Monomers then interact with proteins downstream in the signalling pathway (Rizzini et al. 2011).

Deletion of the C27 region did not alter UVR8 response to increasing dose of UV-B as compared to GFP-UVR8 (an unpaired *t*-test confirmed an insignificant difference). Despite the demonstrated importance of this region for interaction with COP1 and the induction of UVB-mediated photomorphogenic responses (Cloix et al. 2012; Yin et al. 2015), our results indicate it that it does not influence dimer formation or UV-B induced monomerization (Figures 6.4 and 6.5). Interestingly, C-terminal deletion has been observed to hinder re-dimerization (Heilmann and Jenkins 2012) as the C27 region is necessary for binding of RUP proteins (Cloix et al. 2012), which facilitate this process (Heijde and Ulm 2012a). It would therefore have been interesting to leave illuminated samples in the dark and inspect their rate of re-dimerization as compared to GFP-UVR8.

On the other hand, replacement of tryptophan 285 with phenylalanine prevented UVR8 monomerization upon UV-B exposure (Figure 6.4), demonstrating the critical im-

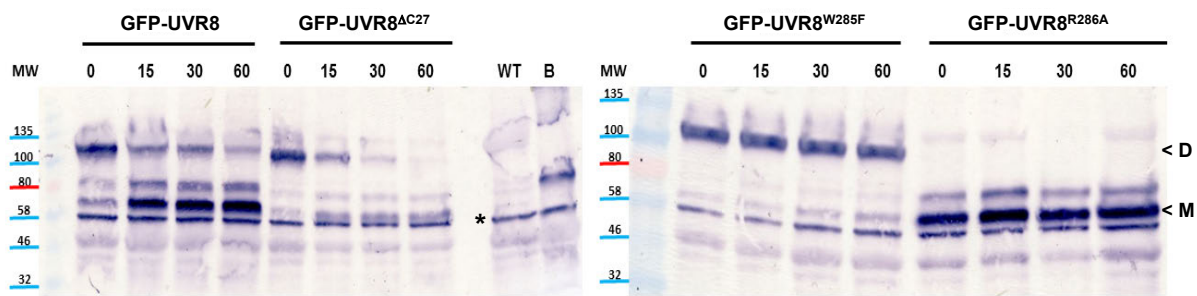


Figure 6.4: Effects of UV-B illumination on GFP-UVR8 mutants. Protein extracts of *Arabidopsis uvr8-1* expressing GFP-UVR8, GFP-UVR8^{ΔC27}, GFP-UVR8W285F or GFP-UVR8R286A, were left untreated or illuminated for 15, 30 or, 60 min with broad-band UV-B of 1 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Samples were run on SDS-PAGE without boiling and a western blot was performed for detection of GFP-UVR8 protein in dimer (D) and monomer (M) states using anti-GFP antibody. A non-illuminated wild-type (WT) sample was used to detect non-specific antibody binding (indicated by an asterisk). A boiled sample (B) was used as a control for monomerization which occurs after boiling UVR8 in SDS. MW: molecular weight marker proteins, in kDa.

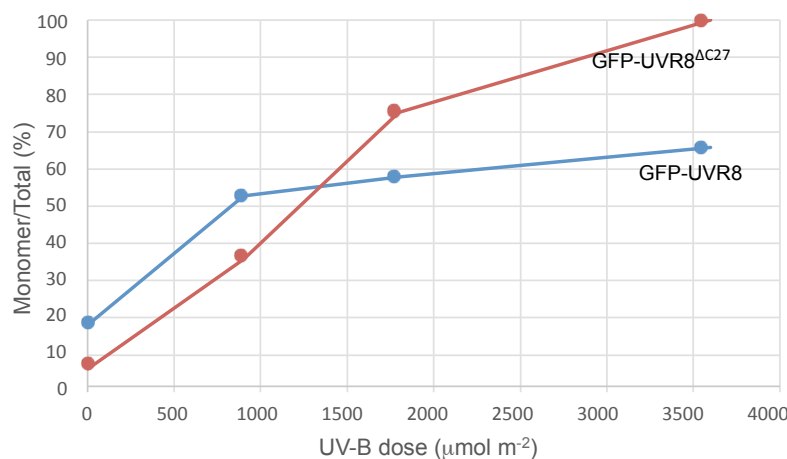


Figure 6.5: GFP-UVR8 and GFP-UVR8^{ΔC27} monomerization following UVB exposure. ImageJ was used to quantify UVR8 dimer and monomer band intensities.

portance of this residue for UV-B photoreceptor activity (Christie et al. 2012; Rizzini et al. 2011; Wu et al. 2012). UVR8 therefore differs from other photoreceptors, which absorb light using cofactors as chromophores (Jenkins 2014).

Replacement of arginine 286 with alanine resulted in UVR8 monomerization even in the absence of UV-B light (Figure 6.4), demonstrating a key role of this residue in the maintenance of the UVR8 homodimer. R286 is located at the interface of interaction between the two monomers and forms two

hydrogen bonds with D107 and a hydrogen bond with D96 on the opposing monomer. The monomer interaction interface contains many charged amino acids, which similarly form salt bridges with residues of complementary charge on the opposing monomer (Jenkins 2014). Due to the denaturing properties of the SDS buffer, we would expect disruption of all weak interactions, so weakened salt bridges could be disrupted in the gel but remain intact in vivo. However, size exclusion chromatography demonstrates that salt bridges are indeed disrupted following a

R286A mutation, causing constitutive monomerization (Christie et al. 2012; Heilmann et al. 2016; Wu et al. 2012). Therefore, R286 is critical for formation of salt bridges that maintain the dimer.

The monomerization of purified UVR8 was also monitored, to draw conclusions on the dose of UV-B required to initiate and complete the monomerization of photoreceptor dimers. The purified protein (Christie et al. 2012) was exposed on ice to a broadband UV-B source at different fluence rates (1 to 5 $\mu\text{mol m}^{-2} \text{s}^{-1}$) for different durations, for a maximum of 3 hours. The samples were then run, without boiling, on a SDS-PAGE gel to resolve the UVR8 dimer and monomer (Christie et al. 2012). After the gels were stained, ImageJ was used to quantify the band intensities and the $[\text{UVR8}^{\text{monomer}}] / [\text{UVR8}^{\text{total}}]$ percentages were calculated and plotted against increasing UV-B dose.

As can be observed from Figure 6.6a, UVR8 monomerization in response to increasing UV-B dose follows a logarithmic trend. UVR8 monomerization is initiated at relatively low exposures of UV-B, and the first monomers are detected after a UV-B dose of 30 $\mu\text{mol m}^{-2}$ (Figure 6.6b). The relative level of monomer reaches 50% at a dose of less than 200 $\mu\text{mol m}^{-2}$, while complete dimer dissociation requires a minimum dose of approximately 5200 $\mu\text{mol m}^{-2}$ of UV-B (Figure 6.6a). Moreover, a plot of the data on a semi-log graph reveals that the UV-B dose-response relationship is linear (Figure 6.6c). Our findings are consistent with those of Christie et al. (2012), who treated purified UVR8 with 450, 1350, 2700 and 5400 $\mu\text{mol m}^{-2}$ of narrowband UV-B and showed a rise in monomer formation with increasing UV-B doses, and those of Wu et al. (2012) and Zeng et al. (2015) who used undefined doses. The results of this study extend previously published findings on UVR8 in that a set of quantitative data points was used to determine a dose-response relationship.

Student experiences and perceptions

Most laboratory classes that students take in the early years of their undergraduate studies are designed to reinforce theoretical concepts and teach practical skills, so there is limited opportunity for investigation. The present laboratory class was intended to introduce students to some of the skills used in research, which potentially would help them in their final year project work and after graduation. Nevertheless, having to formulate questions and design experiments was a new and challenging experience for the students.

To discover students' perceptions, anonymous questionnaires were collected from over 120 students who took the class in years 2016 and 2017. Interestingly, 85% agreed, or strongly agreed, that the laboratory class helped them to understand how to plan and design experiments (0% disagreed; 15% neutral). Similarly 74% agreed, or strongly agreed, that it helped them think how to ask scientific questions (5% disagreed). Several students stated that the class encouraged active engagement by allowing them the freedom to choose a gene of interest and create an appropriate aim. Moreover, it fostered a lot of collaboration amongst students as it was vital to work together to discuss results of their experiments, and the results obtained sometimes forced changes in approaches and feasible objectives. Students commented that the approach promoted independence, confidence and an ability to communicate ideas to other students, and that receiving frequent feedback helped them further improve their experiments and realize their strengths and limitations. In addition, some felt they were encouraged to learn throughout the duration of the class.

In the questionnaire, 72% of students said that the class helped them to interpret data. Some stated that they helped each other and discussed how they were analysing the data

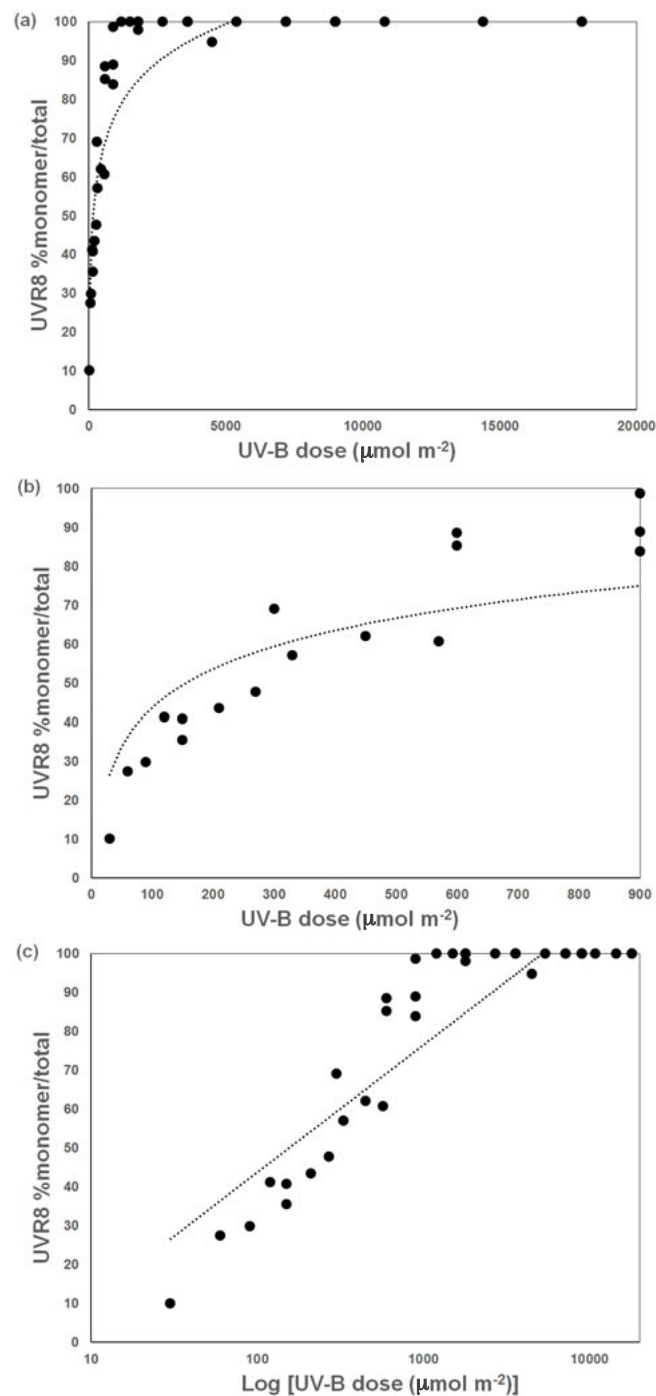


Figure 6.6: Monomerization of purified UVR8 with increasing dose of UV-B. Aliquots of purified UVR8 were exposed to increasing doses of broadband UV-B, produced by varying fluence rate and duration, and harvested. Non-boiled samples were run on SDS-PAGE gels and stained with Coomassie Blue to display dimer and monomer bands. For each sample, the relative abundance of the monomer and dimer bands was analyzed using ImageJ. The data are plotted and lines fitted to the data points using Excel. (a) Linear-scale plot of the increase in $[\text{UVR8}^{\text{monomer}}] / [\text{UVR8}^{\text{total}}]$ (shown as 'UVR8 % monomer/total') of the whole data set. (b) Detail on the increase in $[\text{UVR8}^{\text{monomer}}] / [\text{UVR8}^{\text{total}}]$ (shown as 'UVR8 % monomer/total') from 0 to 900 $\mu\text{mol m}^{-2}$, using a subset of the data. (c) Semi-log plot of the whole data set. Data compiled from experiments done by 4 teams of students.

and that they were able to compare and evaluate the methods they used. Due to the freedom to work on genes and aspects of regulation that were sometimes not fully characterized in the literature, students said that they felt their work had significance. This is likely why 76% stated that the laboratory class gave them a taste of what it would be like to do research, and 82% liked the idea that some of their experiments may not have been done previously. Overall, 89% agreed or strongly agreed that the class was a valuable learning experience, and 75% thought it would help them after they had completed their degree. Some felt the class allowed them to develop problem-solving and teamwork skills essential for future research work. UV4Plants members will be pleased to learn that the class generated interest in plant responses to UV-B. As expected, most (59%) students said they had little knowledge of how plants sense and respond to UV-B prior to doing the laboratory, but 72% that they learnt about it as a result. It was mentioned that the focus on analyzing their own data fostered a deeper interest in plant biology, specifically on the role of UVR8 and the chosen genes.

Concluding remarks

The laboratory class helps students develop practical skills in molecular biology but, more importantly, they start to develop key research skills that cannot easily be taught, including: formulating questions, having ideas, working collaboratively in a team, acquiring the confidence to express an opinion, developing independence. Moreover, they develop their ability to interpret data and evaluate observations in relation to the methods used to obtain them. Being given the scope to select genes and treatments for study promotes engagement and a taste for research. Importantly, gaining an appreciation of how new knowledge is generated encourages students to critically appraise published data. However, while many students

relish the freedom this type of class gives, others find it difficult. For instance, some students commented that they prefer classes focused on learning techniques and some felt the time for team discussion slowed down the work. Nevertheless, most found the class enjoyable and realized that they gained from the experience. From the class leader's perspective, there are organizational challenges that arise in undertaking an investigative approach with a large class. In addition, it can be difficult achieving the correct balance between giving students independence and intervening to ensure their experiments are well designed. But ultimately all the students produce data and learn from the experience, and it is satisfying that the approach generates an interest in the subject.

Plant responses to UV-B provide an excellent system for students to learn about the regulation of gene expression. The stimulus is easily applied and a wide range of genes can be studied. Moreover, *Arabidopsis* mutants are available lacking UVR8 and the downstream transcription factors HY5 and HYH. Activity of the photoreceptor itself can readily be monitored both with respect to monomerization and interaction with COP1, and mutants in the UVR8 protein are available. Hence there are numerous questions that students can define and address. Many of the experiments undertaken have not been reported in the literature and some of the findings are interesting and generate ideas for further research. Furthermore, when the whole class work on the same task valuable data can be generated, as illustrated with the dose-response experiment presented in Figure 6.6, where the findings extend published information. Evidently, the class is facilitated by the availability of resources and expertise generated in research projects in the University of Glasgow, consistent with the University's strategy that teaching should be research-led.



Figure 6.7: A number of authors of the paper enjoying the Glasgow sunshine.

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