Factors Affecting UV-B-Induced Changes in Arabidopsis thaliana L. Gene Expression: the Role of Development, Protective Pigments and the Chloroplast Signal

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Gene expression is known to change in response to UV-B radiation. In this paper, we have investigated three factors in Arabidopsis leaves that are likely to influence these changes: development, protective pigments and the ‘chloroplast signal’. During late leaf development the major change in pigment composition, after exposure to UV-B radiation, is an increase in UV-absorbing pigments. Chl and Chl a/b ratio do not change substantially. Similarly Chl fluorescence is not altered. In contrast, RNA transcripts for photosynthetic proteins are reduced more in older leaves than in young leaves. To determine the role of flavonoids in UV-B protection, plants of Arabidopsis mutant tt-5, which have reduced flavonoids and sinapic esters, were exposed to UV-B and RNA transcript levels determined. The tt-mutants were more sensitive to UV-B radiation than wild-type. To examine the role of the chloroplast signal in regulating UV-B-induced changes in gene expression, Arabidopsis gun mutants (genome uncoupled) have been used. The results show that UV-B-induced down-regulation still takes place in gun mutants and strongly suggests that the chloroplast signal is not required. Overall, this study clearly demonstrates that UV-B-induced changes in gene expression are influenced by both developmental and cellular factors but not chloroplastic factors.

Key words: Arabidopsis thaliana — Chloroplast signal — Flavonoids — Gene expression — Leaf development — Ultraviolet-B radiation.

It has been established that depletion of the stratospheric ozone layer is increasing the level of ultraviolet-B radiation (UV-B: 280–320 nm) reaching the earth’s surface (Blumthaler and Ambach 1990). A major effect of UV-B radiation on plants is an inhibition of photosynthesis (reviewed in Bornman 1989, Jordan 1993, 1996). Extensive studies have revealed a wide range of diverse functions that are affected, reflecting both the large numbers of molecules absorbing UV-B and the energetically unstable nature of the chloroplast (Bornman 1989, Strid et al. 1990). These changes in photosynthetic function, however, take place over different time periods ranging from minutes to days (Strid et al. 1994). One of the earliest effects of UV-B irradiation is an alteration in gene expression. For instance, mRNA levels for photosynthetic genes such as Lhcb and RbcS are substantially reduced within hours by supplementary UV-B radiation (Jordan et al. 1991, 1992, 1994, A.-H.-Mackerness et al. 1997, Strid et al. 1994). The rapid inhibition of chloroplast protein biosynthesis can account for the slower UV-B-induced reduction in photosynthetic function (Strid et al. 1994). The relative sensitivity of photosynthetic genes to UV-B radiation has been shown to be genespecific, with a number of ‘defense genes’ up-regulated while photosynthetic genes are down-regulated (Strid 1993, Jordan et al. 1994) and dependent on the developmental stage of the tissue studied (Jordan et al. 1994, A.-H. Mackerness et al. 1997). For example, in pea, supplementary UV-B reduced the relative levels of Lhcb transcripts in leaves and, to a lesser extent, in apical buds. In contrast, Lhcb levels were increased during greening of etiolated buds exposed to UV-B under the same conditions. The factors involved in this regulation are, therefore, fundamental to the reduction in photosynthetic efficiency.

Down-regulation of genes for photosynthetic proteins, especially those encoding the light-harvesting complex proteins (Lhcb), are frequently cited as evidence for a chloroplast signal transduction pathway (Taylor 1989, Susek and Chory 1992). This signal is associated with a lack of chloroplast development or oxidative damage to the chloroplast. At present, it is unclear whether the signal is a positive (Hess et al. 1994, Taylor 1989) or a negative regulatory signal (Susek et al. 1993). UV-B radiation is known to produce oxygen radicals and peroxides and lead to oxidative stress (Arnotts and Murphy 1991, Murphy and Huerta 1990, Beggs et al. 1986). Due to the high level of reactive molecules within the chloroplast it is likely that one of the primary sites of oxidative damage is within the chloroplast and consistent with this thylakoid membranes are perturbed very rapidly on exposure to UV-B radiation (Chow et al. 1992). Thus, it is possible that UV-B-induced oxidative...
damage to the chloroplasts results in the repression of gene expression for chloroplast proteins via the chloroplast signal transduction pathway. To investigate the role of this signal transduction pathway in UV-B regulation of photosynthetic transcripts, we have used an Arabidopsis mutant, gun (genome uncoupled), in which gene expression for chloroplast proteins continues under conditions of oxidative damage (Suszek et al. 1993).

Plants can intercept a large percentage of solar radiation and assimilate it into organic material. This requires that visible radiation penetrates through the epidermal layers of leaves to the underlying palisade and mesophyll tissues where the majority of photosynthesis takes place. A similar penetration of UV-B radiation into these cells would, however, be harmful to the plant. In most plants that have been studied reflectance from the leaf surface is relatively low (Day et al. 1993, Robinson et al. 1993) and attenuation by UV-B-absorbing pigments screens out this harmful radiation (Caldwell et al. 1983, Tevini et al. 1991, Robberecht and Caldwell 1986). The major protective pigments are flavonoid compounds, a group of secondary products which includes the flavones, flavonols, isoflavonoids and anthocyanins. Exposure to UV-B has been shown to induce a rapid and coordinated increase in the expression and activity of enzymes of the phenylpropanoid biosynthetic pathway (Feinbaum and Ausubel 1992, Lois et al. 1989, Kubasek et al. 1992, Schmelzer et al. 1988) and a subsequent increase in the level of these flavonoid pigments (A.-H.-Mackerness et al. 1997, Jordan et al. 1994, Schnitzler et al. 1996). Other related phenolic compounds such as sinapate esters also accumulate in epidermal cell vacuoles after exposure to UV-B radiation and have also been indicated as having a protective role against UV-B radiation (Sheahan 1996, Li et al. 1993). Direct evidence for the protective function of these phenolic pigments has come from the use of Arabidopsis transparent testa (tt) mutants, tt-4 which has reduced flavonoids (Li et al. 1993) and tt-5 which has both reduced flavonoids and sinapic esters (Ormrod et al. 1995, Li et al. 1993). Both of these tt-mutants were found to be more sensitive to UV-B irradiation than the wild-type plants. Thus both types of phenolic compound are involved in UV-B protection. In the present study, we have used the tt-5 mutants to investigate the protective role of these compounds on gene expression.

In this study we have investigated changes in gene expression in Arabidopsis leaves and related them to photosynthetic function and pigment composition. Three aspects were investigated for their role in regulating UV-B-induced changes in gene expression: 1, Development; 2, The role of protective pigments; 3, The chloroplast signal.

### Materials and Methods

**Plant material and growth conditions**—The wild type Arabidopsis thaliana Columbia and Landsberg ecotypes were used routinely in experiments as indicated in the text. In addition, we have used the Arabidopsis mutants gun (genome uncoupled) and poca (as transformed wild type controls containing the same Cab-3 promoter-driven transgene as gun mutants) in Columbia ecotype (Suszek et al. 1993) and it-5 mutant in Landsberg ecotype (Li et al. 1993).

The Arabidopsis seeds were germinated in a mixture of Levin F2 : sand : vermiculite (6 : 1.5 : 1) and grown for either three or six weeks in a controlled environment cabinet at 22°C. Irradiance of 40 W m~2 (200 μmol photons m~2 s~1; 400-700 nm) was provided by 9 Philips TL 40 W cool white fluorescent tubes during a 12 h photoperiod. The gun mutants were grown for 5 weeks under the same conditions and then transferred to a glasshouse where they were watered normally as control plants or with 5 mM Sandoz 9789, a herbicide which leads to oxidative photo-destruction of chlorophyll and bleaching of leaf tissue (Frosch et al. 1979). After 3 d the plants had bleached and were returned to the controlled environment cabinets prior to experimental procedures. After the growth period, half of the plants were then transferred into another cabinet that supplied an equal amount of photosynthetically active radiation but supplemented by a UV-B lamp (Phillips TL 12, 40 W). Plants were watered or treated with Sandoz and samples taken over a time period up to 7 d. The levels of UV-B radiation were 14 and 35 mW m~2 nm~1 at 297 nm and 313 nm respectively, as determined by using an IL1700 Research Radiometer with calibrated photodetector/filters (International Light, Newburyport, U.S.A.). To eliminate UV radiation below 290 nm the UV-B tube was wrapped in cellulose acetate which was changed daily to prevent increased transmission of the shorter wavelengths due to degradation of the cellulose acetate. This source emits very low levels of UV-A and blue light, which we have found are insufficient to affect the expression of the genes and levels of pigments studies. Comprehensive details of the UV-B treatments are given in Jordan et al. (1994).

The inner 3 whorls of leaves and the outer leaves from the rosettes were used in all experiments unless otherwise stated. For the purpose of this study, this division was considered to differentiate leaves at two different developmental stages. All experiments were independently duplicated. Samples were taken at random from different parts of the cabinets and from separate plants selected for their uniformity.

**Measurement of PSII Chl fluorescence**—Chl fluorescence was determined by using a Hansetech plant efficiency analyzer (Hanse-tech, Kings Lynn, U.K.). The leaves were dark adapted for 5 min prior to measurement for 30 s.

**Pigment analysis**—Leaf tissue (0.5 g) was ground to a fine powder in liquid nitrogen. The powder was then extracted with 50 μM Tris-HCl pH 7.5 (final volume 10 ml). The mixture was clarified by centrifugation at 5,000 xg for 10 min in a Sorvall RCS centrifuge. Chl was determined in the supernatants by the method of Porra et al. (1989).

For UV-absorbing pigment determination, the frozen powder was extracted in methanol (5 ml per 0.5 g of fresh weight), centrifuged and the absorbance scanned between 200 nm and 450 nm as described in Markham (1982). Anthocyanins were determined by extraction of the frozen powder in 1% HCl (v/v) in methanol (5 ml per 0.5 g), centrifuged and the absorbance scanned between 480 nm and 700 nm (maximum anthocyanin absorbance being at 530 nm) as described by Hirose et al. (1990).
The experiment was repeated twice with similar results and data from one set of experiments is presented.

**Purification of total RNA, Northern blotting and hybridisation**—RNA was extracted as described by Jordan et al. (1992) and stored at −70°C. The RNA was separated on a 1.5% agarose gel containing 6% (v/v) formaldehyde in 2×MOPS (Maniatis et al. 1982). Each gel was examined under UV light both to check RNA integrity and to ensure that equal amounts of RNA were loaded in each lane (data not shown). The RNA blotting, prehybridisation, hybridisation and washing were carried out as described in Jordan et al. (1992). Autoradiography of the filters was at −70°C using Fuji RX film with a single intensifying screen. Blots presented are representative of blots obtained from two independent experiments.

**Protein extraction and Western blotting**—Total protein was extracted and analysed by Western blotting essentially as described in A.-H.-Mackerness et al. (1997). The LHCB antibody used was a monoclonal antibody specific for the light harvesting complex II (LHCII) and is described in Darr et al. (1986).

**DNA sequences**—The Lhcb cDNA sequence (pAB 96) is a 1.8 kbp cDNA clone from pea and is described in Coruzzi et al. (1983). The RbcS sequence is a genomic fragment from Arabidopsis and is described in Krebbers et al. (1988). The psbA sequence is an 850 bp HindIII fragment containing the 3' 60% of the gene from spinach (Jordan et al. 1989). The rbcL sequence is a 657 bp PstI fragment from Maize (Jordan et al. 1989). The Cns sequence is a 3.8 kbp HindIII genomic clone from Arabidopsis (Feinbaum and Ausubel 1992).

**Results**

**Developmental variation in response to UV-B irradiation**

**Pigment composition and morphology**—The effect of UV-B on the morphology and pigment composition of 3 week old seedlings and the inner (younger) and outer (older) leaves of 6-week-old Arabidopsis var Columbia plants was determined.

Young seedlings were allowed to establish (3 weeks from germination) and then half were transferred into a supplementary UV-B cabinet. The seedlings were visually inspected daily and showed very little physical differences compared to control plants after a period of 3 weeks exposure to supplementary UV-B radiation. Significant changes in Chl content per fresh weight (Control 1.41 ± 0.181 mg g⁻¹; UV treated 1.39 ± 0.02 mg g⁻¹) or a/b ratio (3 : 1) were not detected. However, UV-B exposure did result in an increase in the levels of UV-absorbing pigments (Fig. 1a) and anthocyanins (Fig. 1b).

In contrast to seedlings, 6 week old rosettes of Arabidopsis inner and outer leaves showed visible damage to the leaf surface after only a single day of exposure to UV-B radiation. This took the form of a silvery 'glaze', predominantly on the older outer leaf whorls. Chl content was significantly increased in only the inner leaves in response to UV-B exposure, with no significant changes detectable in the outer leaves (Fig. 2a). In addition, no significant change was observed in the Chl a/b ratio at either developmental stage (Fig. 2b). Methanol extracts from inner and outer leaves treated with and without UV-B were scanned in an absorption spectrophotometer between 200 and 500 nm. There was a rapid increase in absorption within the UV region in the outer (Fig. 3b) and, to a greater extent, inner leaves (Fig. 3a) on exposure to UV-B. This increase was detectable after 8 h and continued over the experimental period. The absorption above 380 nm was slightly decreased in outer leaves and increased in inner leaves compared to con-

![Fig. 1](image-url) The (a) flavonoid and (b) anthocyanin profiles obtained from 3 week old Arabidopsis seedlings treated with (—) or without (---) supplementary UV-B for 3 weeks.
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Fig. 2 Pigment content in inner and outer leaves of 6 week old Arabidopsis plants exposed to supplementary UV-B for a period of 7 d. (a) Total chlorophyll. (b) Chlorophyll a:b ratio. Each bar represents the mean value obtained from three independent experiments and error bars indicate S.E.

trols and paralleled the duration of exposure to UV-B. This is mainly due to changes in Chl content which were determined more accurately using acetone extracts and described above. In contrast, no change in anthocyanin levels was detected in response to the UV-B treatment in the 6 week old plants (data not shown).

From these experiments, the effect of UV-B on older rosettes was shown to be more pronounced than in younger seedlings and, therefore, more extensive studies were carried out using 6 week old Arabidopsis plants. The effect of UV-B was determined in both the inner and outer leaves in order to further access variation in response between leaves at different developmental stages, to supplementary UV-B.

**Chi fluorescence**—The effect of UV-B on the Chi fluorescence of both the inner younger leaves and the older outer leaves of 6 week old Columbia rosettes were determined for 3 d (data not shown). The maximum quantum yield of PSII photochemistry, as monitored by Fv/Fm ratio, was not affected by UV-B treatment in the inner leaves while a small decrease was detected in the outer leaves. However, the maximum reduction in ratio was only ca. 10% after 3 d.

**Transcript levels**—The mRNA transcript levels for a number of genes (representing nuclear-encoded, chloroplast-encoded and defense genes) were determined in young and older leaves of 6 week old Arabidopsis plants (Fig. 4). The response to UV-B irradiation of Lhcb mRNA transcripts differed between the outer (old) and inner (young) leaves (Fig. 4a). In inner leaves, a reduction in Lhcb mRNA levels was detectable after 3 d of UV-B exposure at which time transcripts had declined by approximately 50%. This reduced level was maintained over the 7 d period. The reduction in Lhcb mRNA was considerably faster in the outer leaves than in the inner leaves (Fig 4a). Thus, after only 1 d the Lhcb mRNA transcripts had declined by over 50% and the level continued to fall until the last sampling day (7 d). To determine if the decline in Lhcb mRNA levels is reflected in LHCB protein content, protein...
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was isolated from inner and outer leaf tissue after various lengths of UV-B exposure. The proteins were separated by SDS-PAGE and Western blotting (Fig. 5). Using LHCB antibody, no change in LHCB protein levels could be detected in the inner or outer leaves in response to supplementary UV-B, although there was an overall decline in the levels with time, particularly in the outer leaves (Fig. 5). The effect of UV-B on transcript levels of another nuclear-encoded chloroplast protein, the small subunit of RUBISCO, encoded by \textit{RbcS}, was similar to that on \textit{Lhcb}, with levels in both inner but, to a greater extent, outer leaves falling after 3 d (Fig. 4b).

The effect of UV-B on two chloroplast-encoded genes, \textit{rbcL} and \textit{psbA}, encoding the large subunit of RUBISCO and the D1 polypeptide of PSII, respectively, was also studied (Fig. 4c, d). The effect of UV-B on \textit{rbcL} transcripts was similar to that of the \textit{Lhcb} transcripts but not as rapid (Fig. 4c). Thus, the mRNA level in the inner leaves was maintained for 3 d, before declining. Again in the older leaves, the \textit{rbcL} mRNA level decreased more rapidly, within 1 d, and remained at a lower level throughout the period of exposure. In contrast, \textit{psbA} mRNA transcript levels, after an initial decline (1 d) were maintained at a high level throughout the subsequent days of UV-B exposure in both inner and outer leaves (Fig. 4d).

To test the response of an important defense gene, the mRNA levels of chalcone synthase (\textit{Chs}) was studied (Fig. 4e). In control tissue the \textit{Chs} mRNA levels were undetectable or present only in trace amounts. UV-B exposure resulted in a dramatic increase in \textit{Chs} mRNA transcript levels in both the inner and outer leaves. After only 1 d of UV-B exposure the levels have increased in inner leaves by 10-fold and in the outer leaves by more than 5-fold. By 3 d, however, the levels in the leaves started to decline and continued to fall up to the last sampling day (7 d). The maximum \textit{Chs} mRNA transcript levels in the older outer leaves was 70\% of the level in the younger inner leaves.

The effect of UV-B radiation on Arabidopsis \textit{tt-5} mutants—Arabidopsis \textit{tt-5} mutants and wild type Landsberg were grown for 6 weeks and then exposed to UV-B radiation for 3 d. Inner and outer leaves were then analyzed for \textit{Lhcb}, \textit{psbA}, \textit{rbcL} and \textit{Chs} gene expression (Fig. 6). The responses of Landsberg and Columbia ecotypes were very similar (Compare Fig. 4, 6). The reduction in \textit{Lhcb}, \textit{rbcL} and \textit{psbA} mRNA was more extreme in response to UV-B in both the outer and inner leaves of the \textit{tt}-mutants than in the wild type plants (Fig. 6a, b, c). The effect of UV-B on the \textit{tt}-3 mutant, similar to the observation made for wild type plants, was greater on the nuclear-encoded than chloroplast-encoded genes and the effect on transcripts in outer leaves was more pronounced than that in the inner leaves. To extend these data, \textit{Chs} mRNA levels were also determined in \textit{tt-5} mutants as well as in wild type plants (Fig. 6d). The wild-type Arabidopsis Landsberg ecotype responded like Columbia with a rapid increase in \textit{Chs} mRNA. In contrast, the \textit{tt}-5 did not show a similar dramatic increase, although the levels were higher in UV-treated plants than in control plants. In addition, similar to the wild type, the increase was to a higher level in the inner leaves of \textit{tt-5} than the outer leaves (Fig. 6d). The level of flavonoids and anthocyanins were also measured in the inner and outer leaves of the \textit{tt}-mutants in response to UV-B.
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The level of flavonoids and anthocyanins were ca. 10 x and 1,000 x lower, respectively, in the tt-5 mutants than in the wild type at both developmental stages. They were similar at both developmental stages and no increase in either compound could be detected in tt-5 on UV-B exposure (data not shown).

The role of the chloroplast signal in UV-B responses—The herbicide Sandoz 9789 inhibits phytoene desaturase and blocks the synthesis of β-carotene. This leads to oxidative photo-destruction of Chl and bleaching of leaf tissue (Frosch et al. 1979). Under the conditions of the controlled environment cabinets application of herbicide had no effect on the plants due to the relatively low irradiance. Consequently, Sandoz was supplied to 5 week old plants which were then transferred to a glasshouse under natural irradiance. Within 3 d the inner younger leaves (within the inner 3 whorls) were completely photobleached and contained no Chl. The outer older leaves remained green and showed little evidence of photobleaching. The plants were then transferred back to growth cabinets for UV-B treatments. The herbicide-treated plants lasted up to 3 d in a suitable condition for experimentation. To determine the role of the chloroplast signal in UV-B-induced changes in gene expression, we determined the effect of UV-B on the level of photosynthetic transcripts in the inner leaves of the Arabidopsis gun mutant treated with Sandoz or water (Fig. 7) and Arabidopsis gun and poca (transgenic controls) plants treated with Sandoz (Fig. 8). Although gun mutants photobleached with Sandoz had reduced levels of the nuclear-encoded Lhcb and RbcS mRNAs compared to the water treated controls (Fig. 7a, b), the expression levels were still substantial. After 3 d of UV-B irradiation the control water-treated gun mutants showed a small reduction in Lhcb and RbcS mRNA levels. In contrast, Sandoz-treated gun mutants exposed to UV-B showed severe reduction in Lhcb and RbcS mRNA levels (Fig. 7a, b). The mRNA transcript levels for the two chloroplast-encoded genes, psbA and rbcL, were also determined in Sandoz and water treated plants exposed to supplementary UV-B (Fig. 7c, d). In water-treated controls, rbcL and psbA expression was decreased slightly after 3 d exposure to UV-B radiation. Again, in Sandoz treated tissue the levels of these transcripts were reduced in control plants and declined further on exposure to UV-B radiation (Fig. 7c, d). In addition, using poca, which expresses the same Cab-3 promoter-driven transgene as gun mutants, as the control, the effect of supplementary UV-B was determined in poca and gun mutants treated with Sandoz (Fig. 8). The level of all photosynthetic transcripts in poca and gun mutants prior to any treatment, was comparable (data not shown) as previously reported (Susek et al. 1993). However, relative to poca, Lhcb and RbcS transcript levels remained relatively high in gun

Fig. 6 Northern blots showing levels of (a) Lhcb (b) rbcL (c) psbA and (d) Chs transcripts in inner (I) and outer (O) leaves of 6 week old Arabidopsis wild-type (wt) and tt-5 mutant (tt5) treated with supplementary UV-B for a period of 3 d.
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(a) Lhcb
(b) RbcS
(c) rbcL
(d) psbA

Fig. 7 Northern blots showing levels of (a) Lhcb (b) RbcS (c) rbcL and (d) psbA transcripts in inner leaves of 6 week old Arabidopsis gun mutants sprayed with Sandoz (S) or water and treated with (U) or without (C) supplementary UV-B for a period of 3 d.

Fig. 8 Northern blots showing levels of (a) Lhcb (b) RbcS (c) rbcL and (d) psbA transcripts in inner leaves of 6 week old Arabidopsis gun and poca plants sprayed with Sandoz (S) and treated with (U) or without (C) supplementary UV-B for a period of 3 d.

Discussion

The sensitivity to UV-B was found to be heavily dependent on the developmental stage of the leaves studied. The effect of UV-B was monitored primarily in the inner (young) and outer (old) leaves of 6 week old rosettes of Arabidopsis var Columbia plants. The parameters used to determine sensitivity were changes in physical appearance, Chl fluorescence and transcript levels encoding three key photosynthetic proteins (Fig. 4). Considering all parameters, the outer leaves were found to be more sensitive to UV-B than the inner leaves. Physically the outer leaves showed symptoms earlier and Fv/Fm was affected by UV-B only in these leaves (data not shown). Lois (1994) also found a gradient of sensitivity to UV-B in Arabidopsis with the older leaves becoming damaged by UV-B faster and more extensively than the younger leaves. The effect of supplementary UV-B on transcripts encoding photosynthetic proteins has been previously reported at different developmental stages, to our knowledge, only in pea seedlings (A.-H.-Mackerness et al. 1997, Jordan et al. 1994). Similar to studies in pea, supplementary UV-B resulted in the down-regulation of photosynthetic genes, with nuclear-encoded transcripts (Lhcb and RbcS) (Fig. 4a, b) reduced at a greater rate than those encoded by the chloroplast (psbA) (Fig. 4d). However, compared to psbA transcripts, the decline in the chloroplast-encoded rbcL transcripts was more rapid in response to UV-B and possibly, as in pea, reflects the co-ordinated control of rbcL with RbcS transcripts (Fig. 4c). The difference in response of genes encoded by the nucleus and the chloroplast appears to be a reflection of the different ways in which UV-B affects transcript abundance: nuclear-encoded photosynthetic transcripts are regulated primarily at transcription while those encoded by the chloroplast are primarily regulated post-transcriptionally (A.-H.-Mackerness et al. 1997 for more detailed discussion). UV-B resulted in down-regulation of photosynthetic transcripts faster in the outer older leaves than the inner younger leaves (Fig. 4) as has been previously reported for pea (A.-H.-Mackerness et al. 1997, Jordan et al. 1994). Therefore, as in pea, although supplementary UV-B resulted in the down-regulation of photosynthetic transcripts at both stages of leaf development, the inhibition and hence sensitivity of the outer older leaves was greater than the younger inner leaves.

Exposure to supplementary UV-B resulted in an increase in the levels of UV-absorbing pigments in both inner (Fig. 3a) and outer (Fig. 3b) leaves of six week old Columbia plants. However, the increase was faster and to a higher level in the inner leaves which is consistent with previous
data (Lois 1994, Jordan et al. 1994). To our knowledge, spectral analysis of UV-absorbing pigments in inner and outer leaves of Arabidopsis, as defined in this paper, in response to UV-B, have not been determined. However, the spectra obtained in this study from outer leaves, under control and UV conditions, was similar to the profiles obtained from whole rosettes (Lois and Buchanan 1994). The inner leaves comprise a smaller proportion of whole rosettes and thus it is not surprising that whole plant spectra resemble those obtained from the outer leaves.

Increases in UV-absorbing pigments, particularly flavonoids, in response to UV-B have been shown to be due to dramatic changes in the rate of transcription for enzymes of the phenylpropanoid pathway (Feinbaum and Ausubel 1992, Chappell and Hahlbrock 1984). In Arabidopsis, UV and blue light appear to be primarily involved in regulating Chs expression in older seedlings (Fuglevand et al. 1996, Kubasek et al. 1992, Feinbaum et al. 1991, Kaiser et al. 1995). Our results are thus in agreement with previous results showing a UV-B inducible, rapid but transient increase in Chs mRNA (Fig. 4e) and subsequent comparable increases in flavonoid levels (Chappell and Hahlbrock 1984, Jordan et al. 1994, A.-H.-Mackerness et al. 1997).

A tight coupling between the flavonoid content and UV-resistance in developing Arabidopsis seedlings has been reported and suggests a role for flavonoids in UV-B protection (Lois 1994). As discussed above, a similar correlation was found between level of UV-absorbing compounds and sensitivity of inner and outer leaves to UV-B exposure. In order to determine the importance of these phenolic pigments in the UV-B mediated effects on photosynthetic transcript levels at different developmental stages we have used tt-5 mutants which have lower levels of flavonoid and sinapic esters (Li et al. 1993). Our results indicate that the level of these protective pigments does affect the accumulation of the photosynthetic transcripts in response to UV-B as the level of transcripts declined faster in the tt-5 mutants than in the wild-type plants (Fig. 6). However, the absence of these pigments did not affect the relative levels of the photosynthetic transcripts in the inner and outer leaves indicating that the differences in photosynthetic transcript accumulation, at different developmental stages, is unlikely to be correlated to the levels of phenolic pigments present in the tissue. One of the proposed roles of flavonoids in response to UV-B exposure has been shielding DNA from UV-induced damage (Harborne 1988, Stafford 1990, Stapleton and Walbot 1994). It is likely, therefore, that the greater reduction in photosynthetic transcripts and diminished increase in Chs expression in tt-5 mutants, compared to wild-type plants, in response to UV-B (Fig. 6), was due to an increase in UV-induced damage to DNA in the mutants. However, as the developmental variation in transcript accumulation was still apparent in tt-5 mutants, although there was no difference in the levels of flavonoids and anthocyanins between inner and outer leaves in these mutants (data not shown), it is unlikely that DNA damage could be the primary mechanism by which transcripts are up- or down-regulated in response to UV-B in wild-type plants.

A key component of plastid development is the coordination of gene expression between two different genomes with the nucleus playing a major role in determining the developmental fate of the chloroplast (Taylor 1989, Kirk and Tilney-Bassier 1978). However, the developmental stage of the chloroplast itself also appears to regulate the expression of nuclear genes coding for chloroplast proteins. Notably, under conditions of photooxidative damage or in plants were chloroplast development is arrested, the expression of nuclear-encoded chloroplast RNAs and cytosolic mRNAs encoding chloroplast proteins is blocked (Mayfield and Taylor 1987, Burgess and Taylor 1988). It has been hypothesised that lack of chloroplast development and photooxidative damage to the chloroplast leads either to the destruction (Hess et al. 1994, Taylor 1989) or generation (Susek et al. 1993) of a factor or signal of chloroplast origin that regulates transcription of nuclear genes encoding chloroplast proteins, for example, Lhcb and RbcS. The nature of this signal is at present unknown but direct evidence for its existence has come from the isolation of Arabidopsis gun mutants that allow the partial expression of nuclear-encoded chloroplast proteins in the absence of chloroplast development (Susek et al. 1993). In plants, UV-B exposure is known to lead to generation of active oxygen species and eventually result in oxidative stress (Arnotts and Murphy 1991, Murphy and Huerta 1990, Beggs et al. 1996). Chloroplast membranes, rich in polyunsaturated fatty acids, are particularly vulnerable to oxidative damage and one of the consequences of UV-B exposure is in fact perturbation of the chloroplast membrane (Chow et al. 1992). It is possible, therefore, that the chloroplast signal could be involved in the down-regulation of nuclear genes encoding photosynthetic proteins. The treatment of the gun and poca mutants with Sandoz 9789 illustrated the absence of this signal in gun mutants. The levels of nuclear-encoded transcripts for chloroplast proteins were maintained at higher levels in the gun mutants than in poca under the conditions of photobleaching and hence photooxidative stress (Fig. 8a, b) as reported previously by Susek et al. (1993). The experiments with gun mutants clearly show that UV-B-induced down-regulation of Lhcb and RbcS occurs even in the absence of the chloroplast signal (Fig. 7a, b, 8a, b). Reduction of chloroplast-encoded transcripts in response to oxidative stress is not thought to be mediated by the chloroplast signal but is a result of the loss of chloroplast function and hence transcription. As reported previously (Susek et al. 1993), low levels of chloroplast-encoded transcripts (rbcL and psbA) were detected in the photobleached gun mutants. The levels
of these transcripts were further reduced on exposure to UV-B indicating that the down-regulation of chloroplast-encoded photosynthetic proteins, as would be expected, is not affected in these mutants (Fig. 7c, d, 8c, d). Thus it is unlikely that the chloroplast signal is involved in the down-regulation of Lhcb and Rbcs in response to supplementary UV-B.

In conclusion, we have shown that there is a clear developmental sensitivity to UV-B with respect to effects on photosynthetic transcripts and that this is not a result of differences in protective pigments present at the different developmental stages. Protective pigments do, however, play an important role in reducing UV-B-induced down-regulation of gene expression, as illustrated by the experiments with tt-mutants. In addition, the results presented in this paper clearly indicate that the down-regulation of nuclear-encoded photosynthetic transcripts, in response to UV-B exposure, is not dependent on the chloroplast signal.

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