Arabidopsis *UVH6*, a Homolog of Human *XPD* and Yeast *RAD3* DNA Repair Genes, Functions in DNA Repair and Is Essential for Plant Growth[1]

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To evaluate the genetic control of stress responses in Arabidopsis, we have analyzed a mutant (*uvh6-1*) that exhibits increased sensitivity to UV light, a yellow-green leaf coloration, and mild growth defects. We have mapped the *uvh6-1* locus to chromosome I and have identified a candidate gene, *AtXPD*, within the corresponding region. This gene shows sequence similarity to the human (*Homo sapiens*) *XPD* and yeast (*Saccharomyces cerevisiae*) *RAD3* genes required for nucleotide excision repair. We propose that *UVH6* is equivalent to *AtXPD* because *uvh6-1* mutants carry a mutation in a conserved residue of *AtXPD* and because transformation of *uvh6-1* mutants with wild-type *AtXPD* DNA suppresses both UV sensitivity and other defective phenotypes. Furthermore, the UVH6/AtXPD protein appears to play a role in repair of UV photoproducts because the *uvh6-1* mutant exhibits a moderate defect in the excision of UV photoproducts. This defect is also suppressed by transformation with *UVH6/AtXPD* DNA. We have further identified a T-DNA insertion in the *UVH6/AtXPD* gene (*uvh6-2*). Plants carrying homozygous insertions were not detected in analyses of progeny from plants heterozygous for the insertion. Thus, homozygous insertions appear to be lethal. We conclude that the *UVH6/AtXPD* gene is required for UV resistance and is an essential gene in Arabidopsis.

DNA damage is a challenge for all organisms exposed to UV irradiation. UV photoproducts consist primarily of cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidinone dimers (Mitchell and Nairn, 1989; Pfeifer, 1997). These lesions inhibit DNA replication and transcription and also promote mutagenesis (McGregor, 1999). The effects of UV irradiation are especially detrimental in plants, where sunlight is both a source of damage and a requirement for photosynthesis.

Increasing evidence suggests that plants repair UV-damaged chromosomes using mechanisms similar to those found in humans (*Homo sapiens*) and yeast (*Saccharomyces cerevisiae*). These mechanisms include the nucleotide excision repair (NER) pathway, a process which involves recognition of UV lesions, incision of the damaged strand on both sides of the lesion, removal of the damaged fragment, and repair by gap filling and ligation (Batty and Wood, 2000; de Boer and Hoeijmakers, 2000; Prakash and Prakash, 2000). Several potential plant homologs of human and yeast NER genes have been identified. Genetic analyses of these plant genes, including studies of the phenotypes of plants carrying mutations within these genes, provide support for the idea that the NER pathway is conserved in plants.

Lesion recognition during NER involves the homologous heterodimers XPC:HR23B (human) and RAD4:RAD23 (yeast; Balajee and Bohr, 2000; Batty and Wood, 2000; de Boer and Hoeijmakers, 2000; Prakash and Prakash, 2000). The Arabidopsis genome contains potential homologs of both *XPC/RAD4* and *HR23B/RAD23* (Arabidopsis Genome Initiative, 2000). *HR23B* expression occurs in Arabidopsis, rice (*Oryza sativa*), and carrot (*Daucus carota*), and the carrot gene complements the repair defect in a yeast *rad23* mutant (Schultz and Quatrano, 1997; Sturm and Lienhard, 1998).

Damage-induced incision in NER involves two nucleases. The first nuclease makes cuts 5′ to the lesion and consists of homologous heterodimers XPF:ERCC1 (human) and RAD1:RAD10 (yeast). A homologous complex has been implicated in plant DNA repair based on the UV sensitivity of Arabidopsis derivatives carrying either a mutation in the plant

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XPF homolog (Fidantsef et al., 2000; Liu et al., 2000) or an antisense construct to this gene (Gallego et al., 2000). Arabidopsis XPF mutants are also defective in repair of UV photoproducts (Liu et al., 2000; Li et al., 2002). Homologs of the ERCC1 component of this first nuclease occur in Arabidopsis (Arabidopsis Genome Initiative, 2000) and *Lilium longiflorum* (Xu et al., 1998), and the *L. longiflorum* gene complements a repair defect in ERCC1-deficient Chinese hamster (*Cricetulus griseus*) ovary cells. The nuclease that incises 3′ to DNA lesions is called XPG in humans and RAD2 in yeast. A mutation in the Arabidopsis XPG homolog results in UV sensitivity (Liu et al., 2001b). In addition, a second mutation (*uvr1*), which fails to complement the *AtXPG* mutation (Z. Liu and D. Mount, unpublished data) results in defective repair of 6-4 photoproducts (Britt et al., 1993).

Finally, NER requires DNA unwinding by two DNA helicases, called XPB and XPD in humans and RAD25 and RAD3 in yeast. Two potential Arabidopsis homologs of *XPB/RAD25* have been identified (Ribeiro et al., 1998; Arabidopsis Genome Initiative, 2000). *AtXPB1* complements a repair defect in yeast *rad25* mutants, and plants carrying T-DNA insertions in this gene are sensitive to alkylating agents (Costa et al., 2001b). Arabidopsis also encodes a presumed homolog of the *XPD/RAD3* genes (Arabidopsis Genome Initiative, 2000).

The *AtXPD* gene is the subject of the present study. We have examined an Arabidopsis mutation (called *uvh6-1*) that dramatically increases sensitivity to UV light. We present evidence that this mutation results in a missense change within the Arabidopsis *XPD/RAD3* gene. Our findings further support the possibility that the AtXPD/UVH6 gene product functions within the plant NER pathway. In addition, we observe that a T-DNA insertion in *AtXPD* (*uvh6-2*) is lethal, suggesting that this gene serves an essential function during plant development.

RESULTS

Identification of the *UVH6* Gene and the *uvh6-1* Mutation

We previously identified a UV-sensitive mutant of Arabidopsis (originally called *uvh6* but renamed *uvh6-1* in this study) and used it to map the *UVH6* locus to the top 6 centimorgans on chromosome I (Jenkins et al., 1995). As shown in Figure 1A, we have further refined the location this gene to the region between physical markers nga59 and AtEAT1. Within this region, we have also identified a candidate gene at locus At1g03190 (Bac locus F15K9). We have designated this gene as *AtXPD* because it exhibits strong similarity to *ERCC2* (*XPD*) from Chinese hamster and *RAD3* (yeast; The Arabidopsis Information Resource, http://www.arabidopsis.org).

Because mammalian *XPD* and yeast *RAD3* genes are required for NER and because the *uvh6-1* mutant is UV sensitive, indicative of a repair defect, we hypothesized that the *uvh6-1* mutation maps within the *AtXPD* gene. To test this possibility, we amplified *AtXPD* cDNA from wild-type (C10) and *uvh6-1* mutant plants (as described in "Materials and Methods") and determined their corresponding sequences. Compared with the wild-type sequence, we found a single-nucleotide change at codon 521 in the *uvh6-1* mutant cDNA sequence. This mutation was also confirmed by sequencing mutant genomic DNA (data not shown). As depicted in Figure 1B, this mutation should result in a Gly to Glu missense substitution. Hence, it seems likely that the *UVH6* gene is equivalent to *AtXPD* and that the sequence change detected in the *AtXPD* gene is the *uvh6-1* mutation.

The *UVH6/AtXPD* sequence that we obtained from wild-type plants is nearly identical to two unpublished *AtXPD* mRNA sequences in GenBank (accession nos. AF188623 and AY062471) but contains a few, possibly polymorphic, differences within the coding region. In comparison with AF188623, we observed three differences, one at codon 541 (AGC/AGT), which did not alter the encoded Ser amino acid, and two at codon 554 (GAA/AAG), which changed the encoded Glu to a Lys in our sequence. In comparison with AY062471, one silent change is observed at codon 282 (CGA/CGG). The residues that we observed at all these positions were identical to those found in the Arabidopsis consensus genome sequence (Arabidopsis Genome Initiative, 2000), which, like our sequence, was derived from plants of the Columbia ecotype. Our cDNA sequence has been submitted to GenBank (accession no. AY090788) and is depicted in Figure 1B in relation to the corresponding genomic sequence.

Complementation of UV Sensitivity in the *uvh6-1* Mutant by *AtXPD* DNA

To confirm the identification of the *UVH6* gene, we characterized the phenotypes of *uvh6-1* mutant plants that had been transformed with wild-type (C10) *AtXPD* genomic DNA. Primary T_1 transformants were examined for UV sensitivity, and 22 of 26 transformants displayed a UV-resistant phenotype. This phenotype is depicted in Figure 2 for T_2 generation plants homozygous for the introduced *AtXPD* transgene. As shown, *uvh6-1* mutant plants are abnormally sensitive to UV-C irradiation, compared with the wild-type parent, and exhibit severe browning and death of rosette leaves within 3 d after treatment (compare Fig. 2A, middle with left). This finding confirms our previous report that *uvh6-1* plants are hypersensitive to both UV-C and UV-B irradiation (Harlow et al., 1994). In contrast, the transformed plants exhibit the same resistance to irradiation shown by the wild-type parents (right). Thus, the mutant phenotype is complemented by wild-type *AtXPD* DNA, indicating that the *UVH6/AtXPD* gene is required for resistance to UV irradiation.

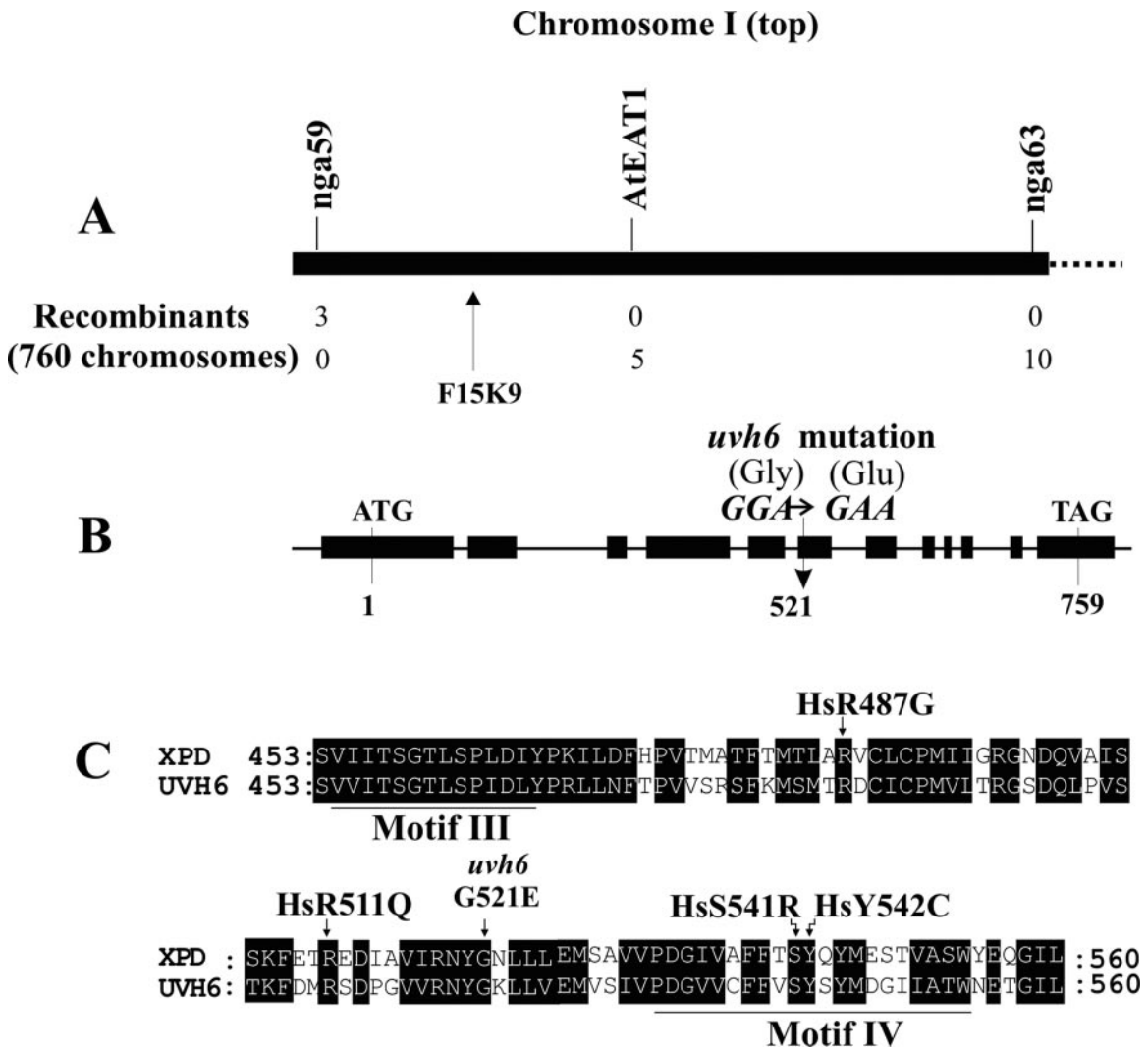

Figure 1. Mapping of the *UVH6* locus and organization of the corresponding *UVH6(AtXPD)* gene. A, Top 3.3 megabases of chromosome I are shown with corresponding physical markers nga59, AtEAT1, and nga63. The *UVH6* locus was localized between markers nga59 and AtEAT1, as described in "Materials and Methods," based on an analysis of DNA from 380 UV-sensitive F_2 progeny (760 chromosomes) obtained from a cross between *uvh6-1* (Columbia ecotype) and wild-type (Landsberg ecotype) plants. Three UV-sensitive recombinants carrying the Landsberg nga59 polymorphism were identified, and all three lacked both the Landsberg nga63 and AtEAT1 markers. Ten recombinants carrying Landsberg nga63 were also obtained. Five of these carried the Landsberg AtEAT1 marker, whereas none carried the Landsberg nga59 marker. The position of Bac clone F15K9, which carries the candidate repair gene *AtXPD* (gene F15K9.20, locus At1g03190), is indicated. B, The *UVH6/AtXPD* gene is diagrammed, based on a comparison between the known genomic and cDNA sequences. The diagram depicts exons (thick bars), introns (thin lines), initiation and termination codons, and the *uvh6-1* mutation at codon 521. C, Alignment of the central portion of the human XPD (accession no. CAA36463) and UVH6/AtXPD (accession no. AY090788) protein sequences is shown, encompassing conserved helicase motifs III and IV. The *uvh6-1* mutation at amino acid 521 is indicated, and the sites of known human mutations (designated Hs), which produce the diseases xeroderma pigmentosum or trichothiodystrophy, are indicated.

Growth Defects in the *uvh6-1* Mutant

In addition to radiation sensitivity, *uvh6-1* mutant plants exhibit growth defects that are also suppressed in plants transformed with wild-type *UVH6/AtXPD* genomic DNA. As shown in Figure 2B (middle), the leaves of *uvh6-1* mutant plants are yellow-green, as previously reported (Jenkins et al., 1997). This phenotype is observed when mutant plants are grown under normal (non-stress) conditions and may result from the low chlorophyll content in these plants described previously (Jenkins et al., 1997). In addition, mutant *uvh6-1* plants are also substantially smaller when compared with wild-type plants of the same age, as seen in Figure 2C (left). In contrast, *uvh6-1* plants that have been complemented with *AtXPD* genomic DNA are dark-green and exhibit

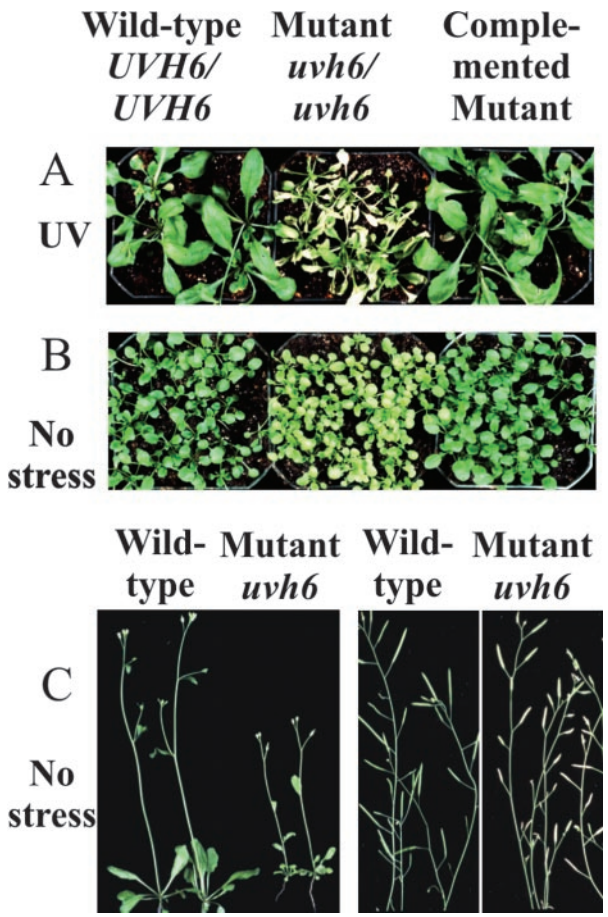

Figure 2. Recovery of UV resistance and normal growth properties after complementation of a *uvh6-1/uvh6-1* homozygous mutant with *UVH6/AtXPD* DNA. Mutant *uvh6-1* plants were transformed with *UVH6/AtXPD* genomic DNA (from C10 wild-type plants) as described in "Materials and Methods." C10 wild-type (*UVH6/UVH6*), *uvh6-1* mutant (*uvh6/uvh6*), and T_2 generation-complemented mutant plants were grown for 2 to 3 weeks and subsequently exposed to UV-C irradiation (300 J m^2) as described in "Materials and Methods" (A) or maintained under normal growth conditions for 3 weeks (B), 4.5 weeks (c, left), or 6.5 weeks (c, right).

normal growth rates (Fig. 2B, right). Thus, these additional mutant phenotypes appear to result from the same mutation that causes UV sensitivity.

Relationship of AtXPD to Presumed Human and Yeast Homologs

We originally identified the *AtXPD* gene based on the similarity of its encoded protein sequence to human XPD and yeast RAD3 proteins, which have well-established roles in NER (Batty and Wood, 2000; de Boer and Hoeijmakers, 2000; Prakash and Prakash, 2000). We have performed further protein sequence comparisons to assess this similarity and to gain insight into the function of the Arabidopsis protein. Pair-wise alignments (data not shown) indicate that the Arabidopsis sequence (accession no. AAF14582) is 56% and 50% identical to the human and yeast sequences (accession nos. CAA36463 and AAB64698, respectively). The Arabidopsis sequence exhibits strong similarity to both homologs along its entire length, and the residue mutated in the *uvh6-1* mutant is conserved in both comparisons. Figure 1C depicts part of the alignment to the human sequence, including the *uvh6-1* mutation site. Additional alignments (not shown) with *XPD* homologs from *Schizosaccharomyces pombe*, *Caenorhabditis elegans*, and *Drosophila melanogaster* (accession nos. CAA93221, AAK95892, and AAD33587, respectively) also reveal strong sequence similarity and conservation of the G residue at the position of the *uvh6-1* mutation. This strong conservation of the *uvh6-1* residue in several homologs further suggests that this mutation disrupts the function of the UVH6/AtXPD protein.

Human *XPD* and yeast *RAD3* genes encode DNA helicases essential for repair (Winkler et al., 2000). Seven highly conserved motifs in these sequences are required for helicase activity (Gorbalenya et al., 1989). Our comparison of the *AtXPD* sequence with the human and yeast homologs revealed especially strong similarity in the regions of all seven motifs (data not shown). Sequence conservation involving motifs III and IV is illustrated in Figure 1C. Thus, it appears likely that the *AtXPD* plant gene acts as a helicase and performs the same role in DNA repair previously demonstrated in humans and yeast.

Repair of 6-4 Photoproducts in the *uvh6-1* Mutant

The UV sensitivity of our *uvh6-1* mutant suggests that this mutant might be defective in repair of UV photoproducts. To test this possibility, we examined the repair of 6-4 photoproducts in DNA from irradiated plants, comparing wild-type, *uvh6-1* mutant, and *uvh3* mutant plants. The *uvh3* mutant is also UV sensitive and carries a nonsense mutation in the *UVH3/AtXPG* gene, encoding the homolog of the human XPG 3′-incision endonuclease (Jenkins et al., 1995; Liu et al., 2001b). As shown in Figure 3A, wild-type plants exhibited significant removal (62%) of 6-4 products from their DNA by 24 h after irradiation. In comparison, the *uvh6-1* mutant (Fig. 3C) exhibited reduced levels of repair after 24 h (47% photoproduct removal), suggesting that it has a moderate repair defect. No photoproduct removal was detected in the *uvh3* mutant (Fig. 3B). This latter result confirms a previous report (Britt et al., 1993) of defective repair in a mutant (*uvr1*) that fails to complement our *uvh3* mutant in genetic tests. Finally, for both the *uvh6-1* and *uvh3* mutants, complementation by the appropriate wild-type genes resulted in restoration of the wild-type level of repair. The complemented *uvh3* mutant showed 66% photoproduct removal (Fig. 3B) and the complemented *uvh6-1* mutant showed 65% removal (Fig. 3C).

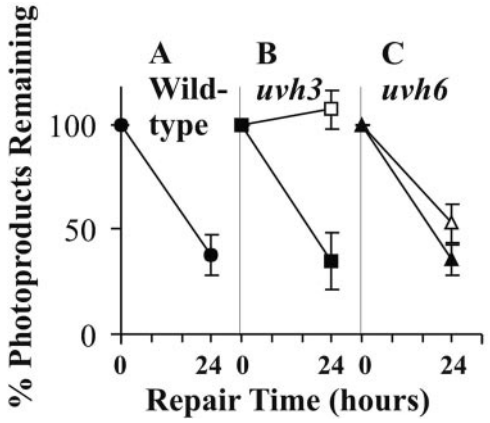

Figure 3. Repair of 6-4 photoproducts in DNA from UV-irradiated plants. DNA extracted from irradiated plants was assayed for photoproducts as described in "Materials and Methods." The plants studied were C10 wild-type (●), mutant *uvh3* (□), complemented mutant *uvh3* (■), mutant *uvh6-1* (△), and complemented mutant *uvh6-1* (▲). Each point represents the mean of four separate assays, and SDs of the means are shown. The difference between the 24-h data points for *uvh6-1* and the complemented *uvh6-1* plants is statistically significant ($P < 0.025$).

Characterization of a T-DNA Insertion in *UVH6/AtXPD* and Its Effect on Plant Viability

We have identified a line of Arabidopsis plants (called 825 BO5) carrying a T-DNA insertion (which we designate as *uvh6-2*) within the *UVH6/AtXPD* gene. The site of insertion was confirmed by DNA sequencing, as depicted in Figure 4. Insertion occurred within the sixth of 11 introns in the *UVH6/AtXPD* gene and presumably results in a truncated UVH6/AtXPD protein because of altered splicing of *UVH6/AtXPD* mRNA. Based on the site of insertion, 26% of the coding sequence and four of the highly conserved helicase motifs should be deleted.

In a preliminary analysis, we asked whether the 825 BO5 line was homozygous or heterozygous for the T-DNA insert. For this analysis, 10 plants were grown from the initial seed stock, and genomic DNA from each plant was subjected to a PCR analysis that distinguishes between wild-type and T-DNA-inserted alleles of *UVH6/AtXPD*. The locations of primers used in this analysis are shown in Figure 4. Gene-specific primers that anneal to sites flanking the insertion site were used to identify wild-type *UVH6/AtXPD* alleles. This test detects only wild-type alleles because the presence of the insertion results in an inter-primer distance (greater than 10 kb) that is too long for PCR amplification. To detect alleles carrying the insertion, a second PCR reaction was performed using one of the *UVH6*-specific primers and a primer that anneals within the T-DNA left border. Thus, DNA from plants heterozygous for the insert should be amplified with both sets of primers, whereas DNA from plants homozygous for either the wild-type or inserted allele should be amplified by only one set of primers. Results of this analysis revealed that five plants were heterozygous and five were homozygous for the wild-type allele. No homozygous T-DNA insertions were detected, suggesting that homozygous insertions are lethal.

To investigate this possibility, we analyzed two of the above heterozygous plants (called Ga-2 and Ga-3) to determine the frequency with which their progeny inherit the T-DNA insert. The heterozygote parents were allowed to produce seeds by self-fertilization, and DNA from individual F_1 seedlings was subjected to the same PCR analysis described above. In total, 97 progeny plants were examined. As seen in Table I, 56.7% of these plants were heterozygous and 43.3% carried only wild-type alleles, whereas no plants homozygous for the insert were detected. This result clearly deviates from the theoretical expectation that 25% of the progeny should carry homozygous T-DNA insertions under conditions where the insertion is not lethal ($P < 0.01$). This result also rules out the possibility that lethality is caused by a second mutation unlinked to the *UVH6/AtXPD* locus, although the presence of a lethal mutation very closely linked to *UVH6* is still possible. Most likely, the results indicate that homozygous *uvh6-2* insertions within *UVH6/AtXPD* are lethal and that the *UVH6/AtXPD* gene serves an essential function during plant growth.

Expression of the *UVH6/AtXPD* Gene

The mRNA expression pattern of the *UVH6/AtXPD* gene was analyzed as shown in Figure 5. Total RNA was extracted from several plant tissues, specific mRNA sequences were amplified by RT-PCR, and PCR products were visualized after electrophoresis. We previously found that the use of 50 ng of input plant RNA gave a PCR product that was proportional to the input RNA concentration, using 25 to 30

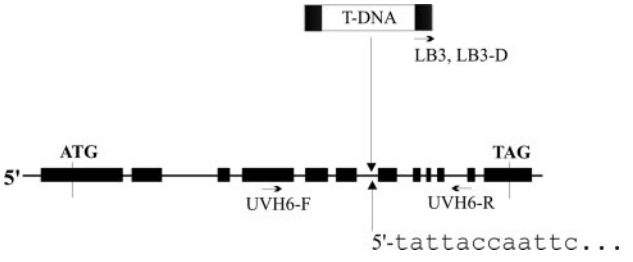

Figure 4. Site of T-DNA insertion (uvh6-2) within the *UVH6/AtXPD* gene. The position of the insert was confirmed by DNA sequencing, as described in "Materials and Methods." This site is shown with respect to the *UVH6/AtXPD* genomic DNA, and the *UVH6/AtXPD* DNA sequence adjacent to the left border (LB) is shown. The *UVH6/AtXPD* gene is depicted with exons (thick lines) and introns (thin lines). Positions of the primers (UVH6-F, UVH6-R, LB3, and LB3D) used for PCR reactions and DNA sequencing are indicated. For results shown in Table I, primers UVH6-F and UVH6-R (gene specific) were used to detect wild-type alleles; primers UVH6-R and LB3 (Ga-3 experiment) or LB3D (Ga-2 experiment) were used to detect T-DNA-inserted alleles.

Table I. *PCR analysis of DNA from progeny of Ga-2 and Ga-3 plants carrying a heterozygous T-DNA insertion (uvh6-2)* in the *UVH6/AtXPD* gene[a]

Genotype of Progeny Plants[b]	No. of Progeny Plants with the Specified Genotype		
	Progeny from Ga-3 parent	Progeny from Ga-2 parent	Total progeny[c]
Homozygous wild type	18	24	42(43.3%)
Heterozygous T-DNA insertion	29	26	55(56.7%)
Homozygous T-DNA insertion	0	0	0(0.0%)

[a] Genomic DNA was extracted from leaves of F_1 seedlings from two plants (Ga-3 and Ga-2) carrying heterozygous T-DNA insertions in *UVH6/AtXPD*, and the DNA was subjected to PCR analysis, as described in "Materials and Methods." [b] Genotypes were scored as homozygous wild type (amplification observed only with gene-specific primers), T-DNA insertion heterozygous (amplification observed with both sets of primers), or T-DNA insertion homozygous (amplification observed only with the T-DNA/gene-specific primer pair). Figure 4 shows the primer annealing sites. [c] A χ^2 analysis of these results generated a value of $P < 0.01$, indicating that the results are not consistent with the expected Mendelian genetic distribution of 1:2:1.

PCR cycles (Liu et al., 2001b). As seen in Figure 5, these conditions gave clearly detectable levels of product with our *UBQ10* control, a gene that expresses at a relatively high level. This control also serves to verify that equivalent quantities of RNA were present in each PCR reaction. However, to detect a PCR product for *UVH6/AtXPD*, which expresses at a relatively low level, we required additional input RNA (200 ng) and 40 PCR cycles. Under these conditions, we observed that *UVH6/AtXPD* mRNA is expressed to various degrees in leaf, root, stem, flower bud, and meristem tissues. Hence, *UVH6/AtXPD* appears to be expressed at low levels in all tissues, as expected for a gene required for general transcription.

DISCUSSION

We have identified a mutation (*uvh6-1*) in the Arabidopsis *AtXPD* gene and have characterized the phenotypes of *uvh6-1* mutant plants. *AtXPD* is predicted to be a homolog of the human *XPD* and yeast *RAD3* genes, which are required for NER of DNA. The *uvh6-1* mutation is a missense change from Gly to Glu at position 521 in the UVH6/AtXPD protein. This mutation appears to cause the extreme UV sensitivity exhibited by *uvh6-1* plants because mutant plants transformed with wild-type *UVH6/AtXPD* DNA regain wild-type radiation resistance. Mutant sensitivity to UV light is likely to result from a deficiency in NER, based on our finding that mutant plants exhibit a moderate defect in removal of 6-4 photoproducts from their DNA.

In addition, we have examined a mutant carrying a T-DNA insertion (*uvh6-2*) within the *UVH6/AtXPD* gene. This insertion is predicted to produce a truncated *UVH6* gene product. Our findings indicate that homozygous insertions within *UVH6/AtXPD* are lethal, suggesting that this gene encodes an essential function required during plant development.

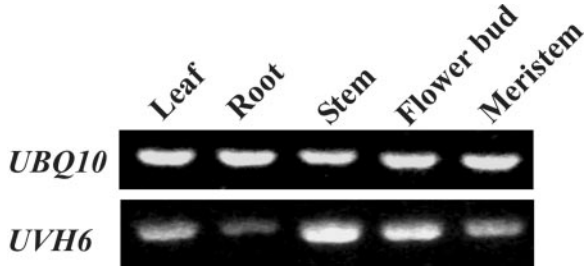

Figure 5. Expression of *UBQ10* and *UVH6* mRNA in Arabidopsis tissues, as measured by reverse transcriptase (RT)-PCR. Total RNA was isolated from the C10 wild-type plant tissues indicated and assayed, as described in "Materials and Methods." RT-PCR products were detected after agarose gel electrophoresis, and the product amplified from the fully spliced message is shown in each case. Controls without RT were run in each experiment (not shown) and failed to produce a product, thus ruling out any contribution of genomic DNA to amplification. RNA was prepared from unbolted flower buds, roots, stems, or leaves, using 4-week-old plants or from 2-week-old meristem tissue.

Role of AtXPD in DNA Repair

Genes involved in NER have been highly conserved from yeast to humans (Prakash and Prakash, 2000), suggesting a common mechanism. This mechanism also appears to be conserved in plants, based on the characterization of several presumed homologs of NER genes in Arabidopsis (Britt et al., 1993; Fidantsef et al., 2000; Gallego et al., 2000; Liu et al., 2000, 2001b; Li et al., 2002). Our findings that the *uvh6-1* mutant carries a mutation in the presumed Arabidopsis homolog of *XPD* (human) and *RAD3* (yeast) and that this mutant is both sensitive to UV light and has a moderate repair defect provide additional evidence that NER is conserved in plants.

Human XPD and yeast RAD3 proteins function as helicases during DNA repair, presumably unwinding DNA surrounding target lesions to facilitate incision (Sung et al., 1988; Winkler et al., 2000). We think it highly likely that the Arabidopsis homolog UVH6/AtXPD also acts as a repair helicase. The UVH6/AtXPD protein sequence contains seven highly conserved motifs that are required for helicase function (Gorbalenya et al., 1989). The *uvh6-1* mutation lies between motifs III and IV, suggesting that it might impair helicase activity. In addition, the *uvh6-1* mutation occurs in a G residue that is highly con-

served in humans, yeast, *S. pombe*, *C. elegans*, *and D. melanogaster*, further suggesting that this residue is important for the normal enzymatic function of UVH6/AtXPD.

Potential Role of AtXPD in Regulating Gene Expression and Plant Development

Mutant *uvh6-1* plants exhibit phenotypic defects when grown under normal lighting conditions, suggesting that the UVH6/AtXPD protein has a role during plant development in addition to DNA repair. Under normal, non-stress growth conditions, mutant plants are small compared with wild-type plants of the same age. They have a yellow-green appearance, contain subnormal levels of chlorophyll. (Jenkins et al., 1997), and exhibit poorly organized grana stacks within thylakoid membranes (M. Jenkins, unpublished data). Furthermore, the mutants are abnormally sensitive to prolonged exposure to high temperature (37°C; Jenkins et al., 1997). In addition, we have observed that T-DNA insertions within the *UVH6* gene are lethal, based on our failure to isolate plants that are homozygous for a T-DNA-insertion (*uvh6-2*) in this gene. Thus, UVH6/AtXPD appears to serve an essential function during plant growth.

To understand the role of UVH6/AtXPD during plant growth, it is informative to consider the known functions of the human and yeast homologs. Human XPD and the homologous yeast RAD3 proteins have dual roles in repair and transcription initiation (de Boer and Hoeijmakers, 2000; Prakash and Prakash, 2000; Lehmann, 2001). In this latter role, they facilitate initiation of transcription by RNA polymerase II as part of initiation factor TFIIH. Considerable evidence suggests that human *XPD* mutations can cause developmental abnormalities due to defects in transcription. These mutations produce three different genetic diseases (xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy; de Boer and Hoeijmakers, 2000; Lehmann, 2001) that all result in sensitivity to UV light and repair deficiencies but are distinguished by additional, distinct phenotypes. For example, trichothiodystrophy is associated with brittle hair and Cockayne syndrome with skeletal deformations. Substantial evidence supports the idea that these additional phenotypes result from transcription defects in differentiated tissues, rather than just loss of repair capacity (Bootsma and Hoeijmakers, 1993; de Boer et al., 1998; Lehmann, 2001; Liu et al., 2001a; Viprakasit et al., 2001; Keriel et al., 2002).

Reminiscent of patients with these human diseases, *uvh6-1* mutants exhibit developmental and stress-response defects. These phenotypes are consistent with the possibility that the *UVH6/AtXPD* gene is required for transcription in plants. Hence, we hypothesize that transcription is abnormal in the *uvh6-1* mutant, causing defects in expression of genes required for chlorophyll synthesis, normal growth rate, and heat resistance. However, we cannot rule out the possibility that *uvh6-1* mutants are not impaired in transcription per se but, due to their repair defect, accumulate DNA damage that blocks transcription.

Interaction of AtXPD within a Predicted TFIIH Complex

In humans and yeast, the TFIIH transcription initiation complex contains six core components and three additional proteins in an associated kinase CAK complex. Of these nine components, only three core proteins and one CAK protein appear to be conserved in Arabidopsis. Strong matches to Arabidopsis sequences are observed for human core components XPD, XPB (two matched loci), and p44. These matches occur at loci At1g03190, At5g41370, At5g41360, and At1g05050, respectively. The existence of two expressed *AtXPB* genes (Costa et al., 2001b) suggests that multiple forms of TFIIH may exist in plants. In addition, the CAK catalytic subunit appears to be conserved, based on clustering of an Arabidopsis kinase (locus At1g73690) with human (cdk7) and yeast (KIN28) CAK sequences in a phylogenetic tree of cyclin-dependent protein kinase sequences (http://kinase.ucsf.edu/ksd/). In contrast, sequences that are similar to the other three core and two CAK components are not found in Arabidopsis. Although a recent report (Costa et al., 2001a) claimed to have found two of these components (p52 and cyclinH) in Arabidopsis and sugarcane (*Saccharum officinarum* and *Saccharum spontaneum*), our sequence analyses suggest that this report may represent matches to isolated protein domains. Thus, only some of the potential TFIIH core and CAK components can be identified in Arabidopsis, suggesting either that these complexes do not exist or are composed of novel or highly diverged subunits in plants.

Analysis of T-DNA Insertions Suggests That *UVH6/AtXPD* Is an Essential Gene

In contrast to the subtle growth defects of *uvh6-1* mutant plants, we have failed to detect plants carrying homozygous T-DNA insertions in the *UVH6/AtXPD* gene. The specific insertion that we characterized (*uvh6-2*) is predicted to cause truncation of the UVH6/AtXPD protein, deleting 26% of its length and four of the seven conserved helicase domains. Thus, the overall structure and presumed helicase activity of this protein should be disrupted. Our failure to isolate plants carrying homozygous insertions in *UVH6/AtXPD* suggests that this gene is essential for plant growth. Our observation that *UVH6/AtXPD* is expressed in all plant tissues supports the possibility that this gene might be important for general transcription.

We have observed further an apparent reduction in transmission of the T-DNA insertion within the prog-

eny of plants carrying a heterozygous insertion. These progeny were either heterozygous for the insertion or had lost the insertion and were wild type. However, the observed ratio of heterozygotes:wild type was close to 1:1, rather than the expected 2:1. This finding suggests that transmission of the inserted allele might be impaired in either the female or male gametes (or both). We are currently testing these possibilities by backcrossing heterozygous plants to wild type.

A model to explain the differences observed between the *uvh6-1* point mutant and the *uvh6-2* T-DNA insertion mutant has been suggested by studies of yeast *RAD3* mutations. These mutations exist in two classes. Mutations in the first class are lethal and result in severe defects in RNA polymerase II-dependent transcription under restrictive conditions (Guzder et al., 1994; Prakash and Prakash, 2000), suggesting that the transcription defect causes the lethality. In contrast, the second class of mutations does not impair viability, but most of the mutants are sensitive to UV irradiation and exhibit defects in NER (Prakash and Prakash, 2000). Thus, this second class appears to affect DNA repair, while leaving transcription largely intact. Presumably, *XPD* mutations that produce diseases in humans are of this second type and cause repair defects with only subtle impacts on transcription. In accord with this model, the *uvh6-1* point mutation may also resemble this second class, causing a repair defect with minor effects on transcription, whereas the *uvh6-2* T-DNA insertions may resemble the first class, producing a lethal phenotype due to a severe transcription defect.

MATERIALS AND METHODS

Strains and Growth Conditions

The wild-type Columbia (C10) used was derived from a single plant isolate of Arabidopsis and served as the parent of the *uvh6-1* mutant, which was isolated as described (Harlow et al., 1994; Jenkins et al., 1995). Plants were normally grown at room temperature (22°C–24°C) under continuous lighting consisting of a combination of 40WT12 Excella and F40 agro bulbs (Philips Lighting Company, Somerset, NJ) positioned approximately 35 cm from the plants (30 μmol photons m^{-2} s^{-1}).

Seeds carrying a T-DNA insertion (*uvh6-2*) in the *UVH6* gene (garlic line 825 BO5) were obtained from the Torrey Mesa Research Institute (San Diego). Seeds were either surface sterilized and germinated on agar plates (Haughn and Somerville, 1986) or were germinated in soil and grown in a growth chamber at 22°C with 16 h of light (240 μmol photons m^{-2} s^{-1}) at 22°C and 8 h of dark at 21°C and 75% to 90% humidity.

UV Light Treatment

Two- to 3-week-old plants were irradiated with UV-C light (254 nm) as described by Liu et al. (2001b). They were then incubated for 3 d under F40 GO gold fluorescent lights (Philips Lighting Company, Somerset, NJ) to avoid reversal of UV photoproducts by photoreactivation, a mechanism activated by visible light, and then transferred to standard growth conditions for 7 d. Sensitivity was assessed by the extent of leaf yellowing and tissue death.

Genetic and Physical Mapping of the UVH6 Gene

Recombinant plants were generated by crossing a *uvh6-1/uvh6-1* homozygous mutant plant (Columbia ecotype) and a wild-type Landsberg *erecta* plant. F_2 progeny were screened to identify those which exhibited the UV sensitivity of the mutant (Columbia) parent, using protecting foam to shield the meristem during treatment, as described (Harlow et al., 1994). UV-sensitive progeny were then examined for the presence of Landsberg-associated simple sequence length polymorphism physical markers nga59, AtEAT1, and nga63. Polymorphisms were detected by agarose gel analysis of PCR assays (Bell and Ecker, 1994).

Mutant Complementation

A 5.6-kb fragment of *AtXPD* genomic DNA, which carries the *UVH6/AtXPD* coding region, 3′-untranslated sequences, and a 5′-flanking region (1.2 kb) containing the *AtXPD* promoter, was amplified by PCR from wild-type (C10) DNA, using the primer pair H6U186 (5′-CAACATTCCGATTTTCCGTCACCT) and XDL2532 (5′-CCTACAGTGAAAATTTGAGCTCCAACAATT). The amplified fragment was first cloned into the pCRII vector, using a TA Cloning Kit (Invitrogen, Carlsbad CA), and then transferred into plasmid pBIN19. The resultant recombinant (pAtXPD) was introduced into *Agrobacterium* strain GV3101 by electroporation, as described by Mozo and Hooykaas (1991). Transformed bacteria were selected on Luria-Bertani medium (Sambrook et al., 1989) containing 30 mg L^{-1} gentamycin and 60 mg L^{-1} kanamycin. DNA from the selected bacteria was transferred into *uvh6-1* mutant plants using the floral dip method (Clough and Bent, 1998), and transformed kanamycin-resistant plants were selected from the next generation seed on media containing one-half-strength Murashige and Skoog basal salt mix (Gibco/Life Technologies, Grand Island, NY), 1× B5 vitamins (Bamborg et al., 1968), 1% (w/v) Suc, 0.8% (w/v) Bacto-agar, and 60 mg L^{-1} kanamycin. Primary transformants were tested, as described by Liu et al. (2001b) to identify UV-resistant, complemented transgenic lines. The UV-resistant phenotypes were further confirmed in T_2 generation plants.

DNA Sequencing

To sequence the *AtXPD/UVH6* cDNA, partial cDNA fragments were amplified from C10 wild-type and *uvh6-1* mutant plants by RT-PCR, as described below, and each fragment was sequenced. A full-length cDNA sequence was assembled, and the *uvh6-1* mutation was identified by comparison of wild-type and mutant sequences. The primers used for amplification were: XDU169, 5′-GCCAATTTCGAGATCTAGGTAGGAGGAA; XDU740, 5′-TAAGGGCGTTTGGTAAGAATC; H6U1375, 5′-TACGCCATCTCGGCACCCAGGCT; H6L2699, 5′-AAGTACTGAAGTAATCGGCGT; XDL1740, 5′-ACTTCTCATGTCAAATTTGGTGCT; XDL2382, 5′-CCCATTGTACCCGCCTTATCAT; H6U1534, 5′-AACCGGTGTTTGATCGTTTCCAGT; H6U4835, 5′-CTGGTTGGATACTTTCGCATCTGC; and H6L5357, 5′-AGGAGCCGACGAAGTATTTCTTGA. To sequence the T-DNA insertion within *UVH6*, DNA was isolated from a plant carrying the insertion, subjected to PCR amplification using primers UVH6-R and LB3 to amplify the region surrounding the insertion site (see Fig. 4) and then sequenced using the LB3 primer (5′-TAGCATCTGAATTTCATAACCAATCTCGATACAC).

Repair Assays

Assays were performed as described by Liu et al. (2000). In brief, this procedure involved irradiating seedlings that had been sprouted on agar plates, isolating seedling DNA at 0 and 24 h after irradiation, and quantifying the 6-4 photoproducts present in this DNA by a lesion-specific radioimmunoassay (Mitchell, 1996).

PCR Analysis of *UVH6* Alleles

Genomic DNA was isolated from the leaves of seedlings grown in agar or from leaves of older plants grown in soil. To prepare seedlings, seeds were surface sterilized, plated on agar medium (containing 2.5 mM potassium phosphate [pH 5.5], 5 mM KNO_3, 2 mM $MgSO_4$, 2 mM $Ca[NO_3]_2$, 49 μM ethylenediamine-tetraacetic acid micronutrients, and 5 g L^{-1} Suc), incu-

bated at 4°C, and then germinated in a growth chamber. DNA was isolated from seedlings as described by Klimyuk et al. (1993) and from older plants as described by Bell and Ecker (1994). PCR reactions were conducted using 42 cycles (30 s of melting at 94°C, 1 min of annealing at 65°C, and 2-min extension at 72°C) for experiments with Ga-3 progeny and 35 cycles (1 min of melting at 95°C, 1 min of annealing at 57°C, and 2.5-min extension at 72°C) for experiments with Ga-2 progeny. Primers used were: UVH6-F, 5′-ATCGTCACTGAATTCTCAGGC; UVH-6-R, 5′-CATGACGGCTGTATC-TGCAAG; LB3, see above; and LB3-D, 5′-ATTTCATAACCAATCTCGA-TACAC. Reaction products were detected in 1.5% (w/v) agarose gels.

RT-PCR Analysis of Gene Expression

Total RNA was isolated from C10 wild-type plant tissues as described by Liu et al. (2001b). RT-PCR was conducted using the OneStep RT-PCR Kit (Qiagen Inc., Valencia, CA) with either 50 (*UBQ10*) or 200 (*UVH6*) ng of RNA. Either 26 (*UBQ10*) or 40 (*UVH6*) PCR cycles were used for amplification. Eight microliters of each 25-μL reaction was subjected to electrophoresis in 1% to 3% (w/v) agarose, and the identity of each RT-PCR product was confirmed by sequencing. Primers UBQ10U60 (5′-GA-CTCTCACCGGAAAGAAAT) and UBQ10L573 (5′-TTGTCTTGGATCTT-GGCTTTCA) were used to amplify the *UBQ10* gene. Primers XDU740 (5′-TAAGGGCGTTTGGTAAGAATC) and XDL2382 (5′-CCCATTGTACC-CGCCTTATCAT) were used to amplify the *UVH6* gene.

Bioinformatics

Multiple sequence alignments were obtained using the ClustalW program with default parameters (Higgins et al., 1996). The BLAST server (http://www.ncbi.nlm.nih.gov/) was used for database similarity searches (Altschul et al., 1997). Pair-wise alignments and alignment of the *AtXPD* cDNA and genomic sequences were obtained using LALIGN (Pearson and Miller, 1992), with minor adjustments to make the exon/intron junctions consistent with the consensus sequences for Arabidopsis splice junctions.

Distribution of Materials

Upon request, all novel materials described in this publication will be made available in a timely manner for noncommercial research purposes, subject to the requisite permission from any third party owners of all or parts of the material. Obtaining any permissions will be the responsibility of the requestor.

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