The EMBO Journal Vol.18 No.2 pp.490–499, 1999

Involvement of *Arabidopsis thaliana* ribosomal protein S27 in mRNA degradation triggered by genotoxic stress

Ekaterina Revenkova[1], Jean Masson, Csaba Koncz[2], Karin Afsar, Ludmila Jakovleva and Jerzy Paszkowski

Friedrich Miescher Institute, PO Box 2543, CH-4002 Basel, Switzerland and [2]Max Planck Institute for Breeding Research, D-5000, Cologne, Germany

[1]Corresponding author
e-mail: revenek@fmi.ch

A recessive *Arabidopsis* mutant with elevated sensitivity to DNA damaging treatments was identified in one out of 800 families generated by T-DNA insertion mutagenesis. The T-DNA generated a chromosomal deletion of 1287 bp in the promoter of one of three S27 ribosomal protein genes (*ARS27A*) preventing its expression. Seedlings of *ars27A* developed normally under standard growth conditions, suggesting wild-type proficiency of translation. However, growth was strongly inhibited in media supplemented with methyl methane sulfate (MMS) at a concentration not affecting the wild type. This inhibition was accompanied by the formation of tumor–like structures instead of auxiliary roots. Wild-type seedlings treated with increasing concentrations of MMS up to a lethal dose never displayed such a trait, neither was this phenotype observed in *ars27A* plants in the absence of MMS or under other stress conditions. Thus, the hypersensitivity and tumorous growth are mutant-specific responses to the genotoxic MMS treatment. Another important feature of the mutant is its inability to perform rapid degradation of transcripts after UV treatment, as seen in wild-type plants. Therefore, we propose that the ARS27A protein is dispensable for protein synthesis under standard conditions but is required for the elimination of possibly damaged mRNA after UV irradiation.

Keywords: *Arabidopsis thaliana*/genotoxic stress/mutants/ribosomal protein S27

Introduction

Cellular responses to genotoxic treatments include activation of DNA repair, temporal cell-cycle arrest and induction of stress signaling that modulates gene expression. Hypersensitivity to genotoxic challenges usually reflects impairment of one of these responses, as illustrated by numerous mutations of genes required for DNA repair, cell-cycle control or stress signal transduction. There are, however, several examples of defects in genes thought to be involved in cellular processes unlinked to genotoxic stress but causing genotoxic hypersensitivity. Furthermore, there are cases of DNA repair activities assigned to proteins that have been already allocated to another function. The discovery of such dual roles can reveal unexpected links and increase our insight into co-regulation of cellular functions. An example is the *Saccharomyces cerevisiae* transcription factor TFIIH; this was characterized initially as a component of the RNA polymerase II pre-initiation complex (Feaver *et al.*, 1991) and it was later shown that its five subunits are products of the *RAD3*, *SSL1*, *SSL2*, *TFB1* and *TFB2* genes, which are required for nucleotide excision repair (Feaver *et al*., 1993, 1997; Wang *et al*., 1994, 1995). This finding provided a direct connection between transcription and DNA repair. Similarly, surprising findings resulted from a search for human UV endonuclease III, an enzyme lacking in xeroderma pigmentosum group-D (XP-D) individuals. It was found that DNA endonuclease is a ribosomal protein S3 (Kim *et al*., 1995). Although S3 is present in ribosomes of XP-D cells, and protein synthesis is not affected, the endonuclease activity is absent, suggesting that separate S3 activities or different S3 isoforms are involved in translation and DNA repair. The biological importance of shared functions between protein synthesis and repair of DNA damage is still not clear; however, the significance of this intriguing connection is supported further by the involvement of yeast proteins SSL1 and SSL2 also in the initiation of translation (Gulyas and Donahue, 1992; Yoon *et al*., 1992).

Other prominent examples of 'multipurpose' ribosomal proteins are *Escherichia coli* S9, which interacts with *UmuC*, SOS repair protein (Woodgate *et al*., 1989) and S16, which is a DNA-binding protein with endonuclease activity (Oberto *et al*., 1996). The human protein P0 associated with ribosomes was also rediscovered in a search for apurinic/apyrimidinic endonucleases (Grabowski *et al*., 1991). Therefore, ribosomes appear to incorporate several proteins with possible functions beyond protein synthesis, including responses to DNA damage (for reviews, see Wool, 1996; Wool *et al*., 1996). Furthermore, it has been postulated that some unexpected structural features of certain ribosomal proteins, such as zinc finger motifs similar to those found in DNA-binding proteins, are vestiges of evolution and suggest possible recruitment of DNA-binding proteins to ribosomes (Wool, 1993). However, it is intriguing that such evolutionary relics are highly conserved in distant organisms such as yeast, animals and plants (Wool, 1993; this work), thus indicating constant selective pressure.

Generally it is difficult to provide genetic evidence for auxiliary roles of ribosomal proteins. The phenotypes of ribosomal protein mutants are rather severe and pleiotropic even in a heterozygous state (e.g. *Drosophila Minute* phenotypes) (Saeboe-Larssen *et al*., 1997, and references therein), suggesting unspecific effects of an altered translation apparatus. Loss-of-function mutations are usually lethal. The only known exceptions are two yeast ribosomal

proteins, L30 and S27a. Deletion of either gene results in slow-growing but viable strains (Warner *et al*., 1990). An additional difficulty in the genetic dissection of ribosomal protein functions is that they are usually encoded by more than one active gene, making the study of mutants rather complex or impossible, unless mutation modifies a specific subsidiary function, leaving translation unaffected.

Here we describe an *Arabidopsis* mutant identified in a search for individuals hypersensitive to DNA-damaging treatments. Among families of plants mutagenized by the T-DNA insertion (Koncz *et al*., 1989), one family was identified with a recessive mutation causing hypersensitivity to UV irradiation and the DNA-damaging agent, methyl methane sulfonate (MMS). The T-DNA insert was found to generate a null allele of one of three active genes coding for ribosomal protein S27. S27 was described previously as a ribosomal protein which displays DNA-binding properties, probably through a zinc finger domain of the C_2–C_2 type similar to those present in transcription factors and proteins involved in response to DNA injury (Chan *et al*., 1993; Fernandez-Pol *et al*., 1993). In an independent search for genes induced by transforming growth factor-βs, the same gene was also recovered and named metallopanstimulin-1 (MPS-1) (Fernandez-Pol *et al*., 1993). Elevated levels of MPS-1 (S27) were shown to be a characteristic feature of tissues from human tumors of several types. Moreover, MPS-1 (S27) protein was shown to be released into extracellular fluids and could be used as a serum tumor marker (Fernandez-Pol *et al*., 1993; Fernandez-Pol, 1996). The data presented here suggest that one isoform of ribosomal protein S27 is dispensable for translation but acts as a regulator of transcript stability in response to genotoxic treatments. We propose that this isoform of S27 is involved in the degradation of damaged RNAs.

Results

Isolation of the Arabidopsis ars27A mutant

A collection of 800 families containing random insertions of *Agrobacterium tumefaciens* T-DNA (Koncz *et al*., 1989) was screened for individuals with elevated sensitivity to two kinds of DNA-damaging agents: UV-C and MMS. UV-C was applied to root tips in a way similar to that previously used for the isolation of X-ray-hypersensitive mutants affected in recombinational DNA repair (Masson *et al*., 1997). The screen for MMS hypersensitivity was performed with 5-day-old seedlings transferred to MMS-containing liquid medium. Treatments with doses permissive for the wild type were used to search for families containing hypersensitive individuals by testing 20 randomly chosen plants from each family. One family was found to contain individuals with increased sensitivity to both UV-C and MMS. The evaluation of MMS sensitivity was faster and easier to perform than that of UV-C, and this trait was used for further genetic analysis (Figure 1). Nineteen random plants from this segregating family were grown to maturity and self-pollinated. Genetic segregation for hygromycin resistance encoded by the T-DNA and for MMS hypersensitivity was examined in the progeny of the individual plants. Southern blots revealed two unlinked T-DNA inserts (data not shown). A line homozygous for both hygromycin resistance and MMS sensitivity and containing only one T-DNA insert was identified and used for outcrosses with the wild type. Further genetic tests indicated co-segregation of this T-DNA insert with the MMS-hypersensitive phenotype, which was a recessive, monogenic trait (data not shown).

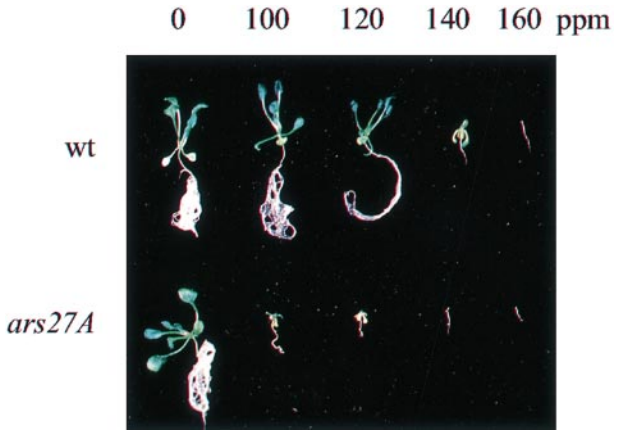

Fig. 1. The response of wild type and the *ars27A* mutant to MMS. Seedlings were pre-germinated in the absence of MMS and transferred to liquid medium containing MMS at the concentrations indicated. The photograph was taken after 3 weeks.

Molecular characterization of the mutant locus

DNA of plants homozygous for the T-DNA and uniformly sensitive to DNA-damaging treatments was used for cloning of the mutant locus. The T-DNA contained bacterial sequences which allowed rescue of a plasmid containing flanking plant DNA. The flank was then used as a probe to screen an *Arabidopsis* cDNA library, and several cDNA clones hybridizing to this probe were isolated. The longest clone contained a 455 bp insert terminated by poly(A). The open reading frame encoded a protein of 86 amino acids with a calculated mol. wt of 9.531 kDa. A database search revealed that the deduced amino acid sequence shares 77% identity and 83% similarity to the rat ribosomal protein S27 (Chan *et al*., 1993). The *Arabidopsis* protein and the rat protein share the characteristic features of primary structure (Figure 2A), such as a cluster of basic residues proximal to their N-terminus (amino acids 16–23 for rat S27), which may function as a nuclear localization signal, and a well conserved zinc finger domain of the C_2–C_2 type. A computer database search with the GeneQuiz system (http://columbia.ebi.ac.uk:8765/ext-genequiz) revealed structural similarities of S27 to a number of proteins known as transcriptional regulators (Figure 2B). The use of MEME motif discovery tool (Bailey and Elkan, 1994; http://www.sdsc.edu/meme) for the search of common motifs, among a set of sequences found by GeneQuiz, led to the detection of a motif VCHNCQNILCHP which occurred with a match score above a threshold in the following sequences: LSD1, *A.thaliana* negative regulator of cell death (Dietrich *et al*., 1997); VFBZIPZF, *Vicia faba* transcription factor containing a leucine zipper and a zinc finger (DDBJ/EMBL/GenBank accession No. X97904); *Schizosaccharomyces pombe* basic transcription factor subunit, a protein similar to the p34 subunit of human transcription factor TFIIH (GenBank/EMBL accession No.

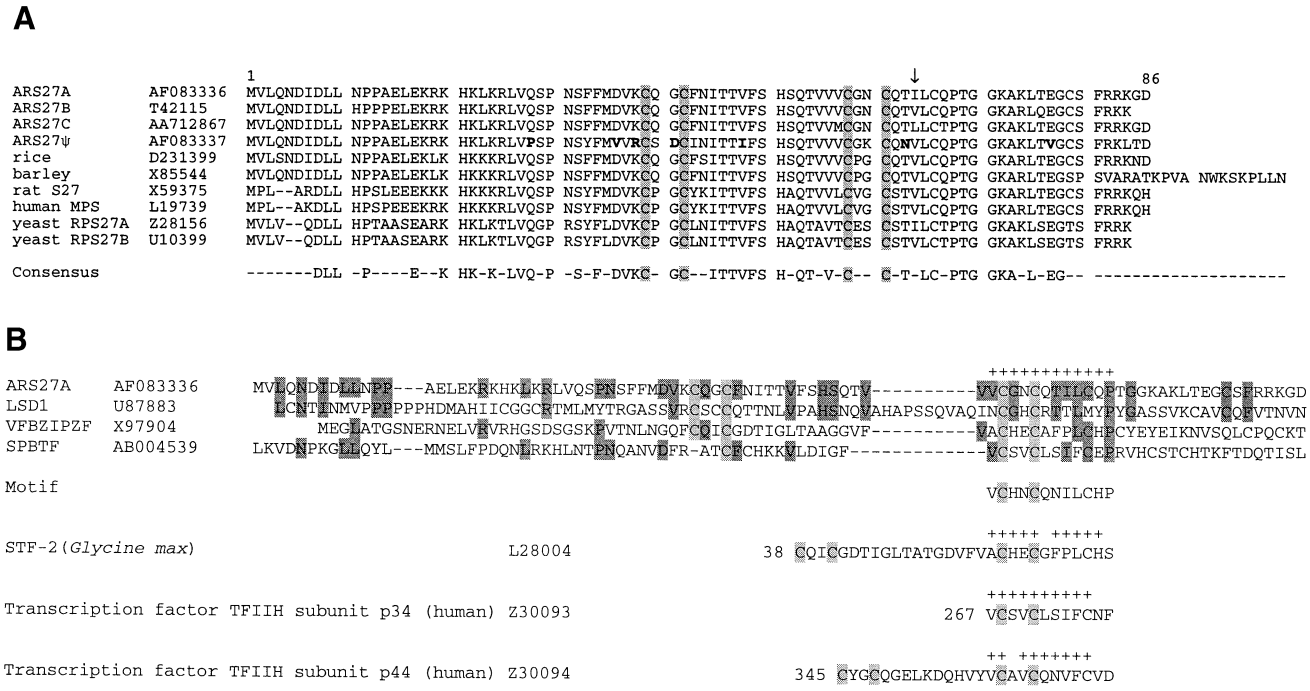

Fig. 2. (**A**) Sequence alignment of ARS27A and selected homologs of the ribosomal protein S27. Amino acid sequences were deduced from cDNA sequences [ARS27A, ARS27B, ARS27C, *A.thaliana* mRNAs; rice, *Oryza sativa* mRNA from callus; barley, *Hordeum vulgare* root mRNA; rat, *Rattus rattus* mRNA for ribosomal protein S27 (Chan *et al*., 1993); human MPS, human metallopanstimulin (MPS-1) mammary gland carcinoma mRNA (Fernandez-Pol *et al*., 1993)] or genomic sequences [yeast, *S.cerevisiae* RPS27A and RPS27B (Baudin-Baillieu *et al*., 1997) and ARS27Ψ, deduced from the *A.thaliana* genomic sequence]. DDBJ/EMBL/GenBank accession Nos are indicated. Conserved cysteines forming a zinc finger are highlighted. The amino acids in ARS27Ψ differing from the consensus are shown in bold. The positions of valine or isoleucine differentiating isoforms of *S.cerevisiae* and of *Arabidopsis* are indicated with a vertical arrow. (**B**) Sequence alignments (GeneQuiz system, EMBL, EBI, UK) of ARS27A and transcription factor-related proteins (only partial sequences are shown). LSD1, *A.thaliana* negative regulator of cell death (Dietrich *et al*., 1997); VFBZIPZF, *V.faba* transcription factor containing a leucine zipper and a zinc finger (direct submission to the database); SPBTF, *S.pombe* protein similar to the p34 subunit of human transcription factor TFIIH (direct submission to the database). Identical residues (dark gray) and cysteines forming zinc finger motifs (light gray) are highlighted. The motif VCHNCQNILCHP was calculated by the MEME system (San Diego Supercomputer Center, CA) using the sequences of the proteins presented in the alignment and other sequences showing similarities to ARS27A found with GeneQuiz. The motif VCHNCQNILCHP was found using the MAST program for database search (Bayley and Gribskov, 1998) in sequences shown at the bottom. STF-2, *Glycine max* TGACG-motif-binding factor (direct submission to the data base). + indicates a positive match score. DDBJ/EMBL/GenBank accession Nos are shown.

AB004539); *Dictyostelium discoideum* LIM domain protein LimA (DDBJ/EMBL/GenBank accession No. U83086), *Caenorhabditis elegans* putative serine/threonine protein kinase YLK3 (DDBJ/EMBL/GenBank accession No. P41951); and *Strongylocentrotus purpuratus* metallothionein B (DDBJ/EMBL/GenBank accession No. Q27287). Motif Alignment and Search Tool (MAST) software (Bailey and Gribskov, 1998) was used to search databases for the sequences containing this motif. Among high-scoring sequences containing this motif, the program identified two core subunits (p34 and p44) of transcription / DNA repair complex TFIIH (Figure 2B).

Alignment of S27 ribosomal protein sequences of different species showed high conservation (Figure 2A). In the DDBJ/EMBL/GenBank database, nine related *Arabidopsis* cDNAs were found, representing transcripts of three apparently different genes (Figure 2A), referred to as *ARS27A*, *ARS27B* and *ARS27C* throughout this manuscript. Comparison of mutant and wild-type DNA sequences in the area of the T-DNA insert revealed that T-DNA integration was accompanied by a chromosomal deletion of 1287 bp (Figure 3). The first 50 nucleotides of the *ARS27A* cDNA overlapped with the fragment replaced by the T-DNA in the mutant. Thus, the insertion of the T-DNA replaced the promoter and a sequence coding for the untranslated leader of the *ARS27A* gene. There is no functional T-DNA promoter close to the left border, suggesting that the mutation may cause lack of *ARS27A* expression. Indeed, no transcript of *ARS27A* was detected on Northern blots of RNA extracted from young seedlings and mature leaves of *ars27A* mutant plants (Figure 4A). In contrast, this message was abundant in the wild type. The depletion of the *ARS27A* transcript was not compensated by increased expression of the *ARS27B* gene (Figure 4B).

Further analysis of the genomic region around the T-DNA insertion revealed a second gene related to *ARS27A* in inverted orientation and separated from *ARS27A* by 0.4 kb of an intergenic sequence (Figure 3). Homology between this gene and the *ARS27A* probe should have allowed detection of a transcript from the neighboring gene under the hybridization conditions applied. The absence of a signal on Northern blots, the lack of corresponding cDNA in the database and the sequence analysis revealing amino acid changes in regions of high conservation (Figure 2A) suggest that the neighboring gene is a non-expressed pseudogene.

The T-DNA-tagged locus was mapped by hybridization to CIC yeast artificial chromososme (YAC) clones (provided by Arabidopsis Biological Resource Center,

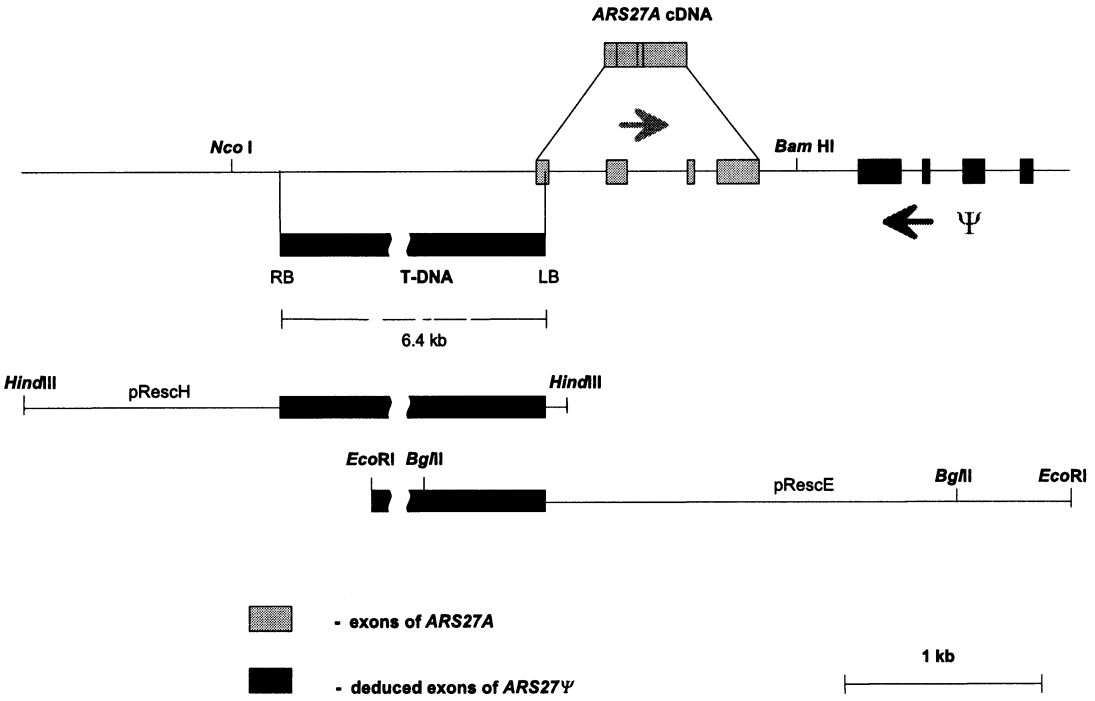

Fig. 3. Top: structure of the *ARS27A* locus in the wild type and *ars27A*. In *ars27A*, the sequence upstream of the *ARS27A* coding region is replaced by T-DNA. The direction of transcription is shown by an arrow. Bottom: genomic fragments rescued as plasmids after digestion of *ars27A* DNA with *Hin*dIII (plasmid pRescH) or *Eco*RI (plasmid pRescE). The *Bgl*II fragment of pRescE was used as a probe for screening the cDNA library. The *Nco*I–*Bam*HI fragment of the wild-type genomic clone was used for *ars27A* complementation.

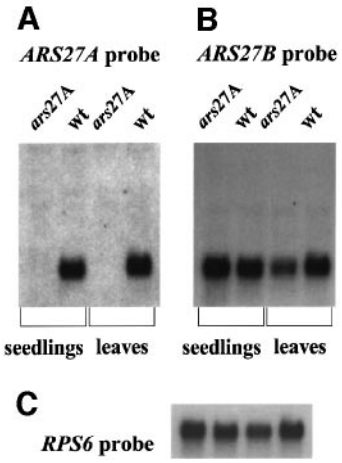

Fig. 4. Northern blot analysis of RNA isolated from 7-day-old seedlings and leaves of 6-week-old plants of *ars27A* and the wild type. The same blot was probed with (**A**) an *ARS27A*-specific probe, (**B**) an *ARS27B*-specific probe and (**C**) an S6-specific probe.

Ohio, USA) to chromosome 3 in the spl1–m424 region (YACs: CIC 3B6, 9B7, 7D1 and 1E8).

The 2.8 kb *Nco*I–*Bam*HI genomic fragment containing the *ARS27A* gene (Figure 3) was introduced into mutant *ars27A* by an *Agrobacterium*-mediated transformation procedure (Bechtold *et al*., 1993). Three independent transgenic lines were studied in detail. In all three lines, the level of *ARS27A* mRNA had reverted to that of the wild type. Homozygous derivatives of these lines displayed wild-type resistance to genotoxic treatments and the reversion of all mutant phenotypes described below.

Phenotype of the ars27A mutant

In contrast to the wild type, the growth of *ars27A* was strongly inhibited on media supplemented with MMS (100 p.p.m.) (Figure 1). This inhibition was accompanied by characteristic developmental abnormalities. Approximately 10 days after transfer to medium containing MMS, *ars27A* seedlings formed tumor-like structures on the main root at the sites of auxiliary root primordia (Figure 5A and C). Wild-type seedlings treated with the same concentration of MMS (Figure 5B and D), or with higher concentrations up to a lethal dose, never displayed such a trait, nor was this phenotype observed in *ars27A* plants in the absence of MMS or under other stress conditions such as osmotic stress, increased salinity, elevated temperature or oxidative stress (for details, see Materials and methods). Also, the short (3 min) exposure to intensive UV-C causes a rapid block in root development and thus no tumors. Therefore, hypersensitivity and tumorous growth are specific responses to the cumulative genotoxic action of MMS.

Considering the importance of ribosomal proteins for translation, manifested by usually drastic or lethal consequences of mutations in *Arabidopsis* ribosomal protein genes (Van Lijsebettens *et al*., 1994; Tsugeki *et al*., 1996), it was surprising that the development of *ars27A* plants under standard growth conditions as well as under a variety of abiotic stresses (listed above) was indistinguishable from the wild type, despite depletion of *ARS27A* transcript (Figure 5E).

Ribosomal protein S27A influences levels of transcripts after genotoxic treatments

In order to examine the transcription of the *ARS27* gene, in response to genotoxic treatments, 1-week-old seedlings

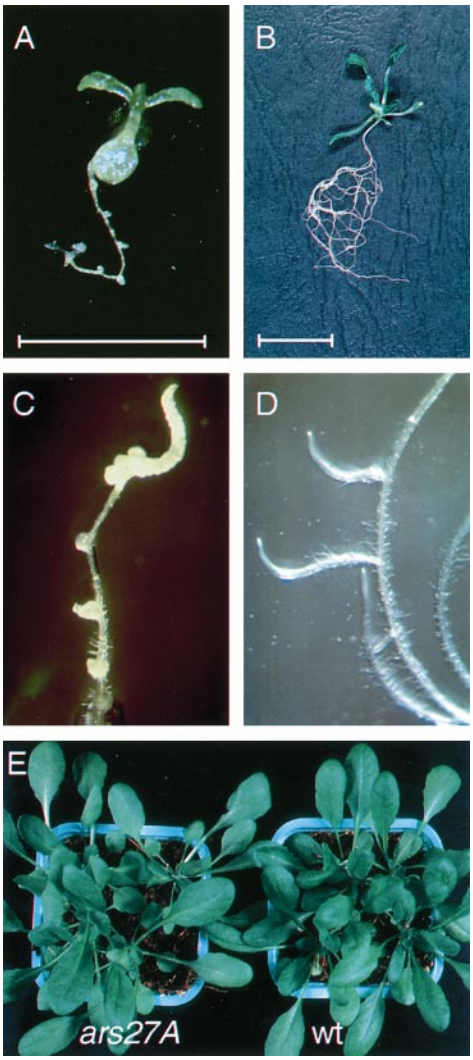

Fig. 5. Phenotype of the *ars27A* mutant. (A–D) Three-week-old seedlings grown for weeks 2 and 3 in the presence of MMS. (**A**) *ars27A* seedling; (**B**) wild-type seedling; (**C**) root of an *ars27A* seedling; (**D**) root of a wild-type seedling; bars, 1 cm. (**E**) One-month-old plants grown in soil under standard growth conditions.

of mutant and wild type were exposed to two doses of UV-C and the levels of S27 mRNA were examined by Northern blot hybridization. A low UV dose (1 kJ/m^2) apparently had no effect on survival of wild-type and *ars27A* seedlings, although root growth was inhibited in the mutant. A high dose (5 kJ/m^2) had no immediate visible effect, but resulted in growth arrest and chlorosis of both the mutant and the wild type, which was evident after 1 week of further culture. Control and irradiated material was harvested immediately (2 min) following UV treatment, or after 10, 30 or 60 min of dark incubation. The *ARS27A* transcript level decreased rapidly (already after 2 min) in the wild type compared with the non-irradiated control (Figure 6A, upper panel). Northern blot hybridization of different membranes with the probe for the *ARS27B* gene showed that the level of its transcript also decreased after the irradiation. In order to determine whether this reaction is specific to S27 mRNA, the same membranes were hybridized with probes for ribosomal protein S6, actin mRNA and 25S rRNA (Figure 6A and B). S6 and actin mRNA levels also decreased rapidly. In contrast to mRNAs, 25S rRNA appeared to be stable after the irradiation with 1 kJ/m^2, which allowed us to use 25S rRNA as a reference for quantification of relative mRNAs levels (Figure 6C and D). Surprisingly, the decrease of mRNA levels in *ars27A* was not as drastic as in the wild-type, and did not continue after an initial drop (Figure 6C and D). Therefore, the UV-responsive reduction of mRNA levels was clearly affected in the *ars27A* mutant (Figure 6).

We addressed the question of whether the phenomenon of UV-induced decrease is specific to mRNA. The same blots were hybridized with the probes specific to U2 small nuclear RNA (snRNA) and 7-2/MRP-like RNA. U2 snRNA in higher plants is transcribed by RNA polymerase II and its main pool is located in the nucleoplasm (Goodall *et al.*, 1991). *Arabidopsis* 7-2/MRP-like RNA is a small RNA transcribed by RNA polymerase III and was shown to be located in the nucleoli (Kiss *et al.*, 1992). In contrast to cytoplasmic transcripts, no significant change was observed in the amount of U2 snRNA and 7-2/MRP-like small nucleolar RNA (snoRNA) either in the mutant or in the wild type (Figure 6B).

After the irradiation with a high UV dose (5 kJ/m^2), the decrease of S6 mRNA was even more drastic compared with at 1 kJ/m^2. U2 snRNA and 7-2/MRP snoRNA appeared to be stable also under these conditions (Figure 7). Quanfication of the signals, using U2 as a loading standard, demonstrates that a decrease of S6 mRNA level in the *ars27A* was inhibited compared with the wild type (Figure 7).

In order to determine whether the reduced levels of RNA were the result of transcriptional suppression or the outcome of post-transcriptional RNA turnover, we determined the mRNA levels in a similar experimental set up, but UV irradiation was preceded by a treatment with cordycepin. Cordycepin was applied at a high concentration (600 μM) shown to be effective in preventing mRNA synthesis in plants (Seeley *et al*., 1992; Peters and Silverthorne, 1995; Phillips *et al*., 1997). The half-life of S6 mRNA in non-irradiated control plants was similar (~4 h) in wild type and mutant. In contrast, after UV irradiation, the characteristic slower transcript drop in *ars27A* was again evident (Figure 8), suggesting that post-transcriptional turnover of RNA after UV irradiation is impaired. Since, this turnover is also activated by MMS (data not shown), it could be envisaged that the ARS27A protein is required for elimination of transcripts affected by these genotoxic treatments.

Incorporating the data on the relative levels of transcripts, we concluded that UV irradiation triggers rapid degradation of cytoplamic mRNA but does not significantly affect the level of small non-coding RNAs located in the nucleus. The process of UV-induced mRNA degradation is impaired in the *ars27A* mutant. After reintroduction of *ARS27A* to *ars27A*, the three independently complemented lines regained UV-C and MMS resistance and lost MMS-induced tumorous growth. They also regained the wild-type ability for rapid mRNA degradation after exposure to UV irradiation (Figure 9). Thus, deficiency of the *ARS27A* gene product is solely responsible for traits specific to the *ars27A* mutant.

Discussion

In a screen for *Arabidopsis* mutants hypersensitive to genotoxic treatments, we recovered a T-DNA-tagged line

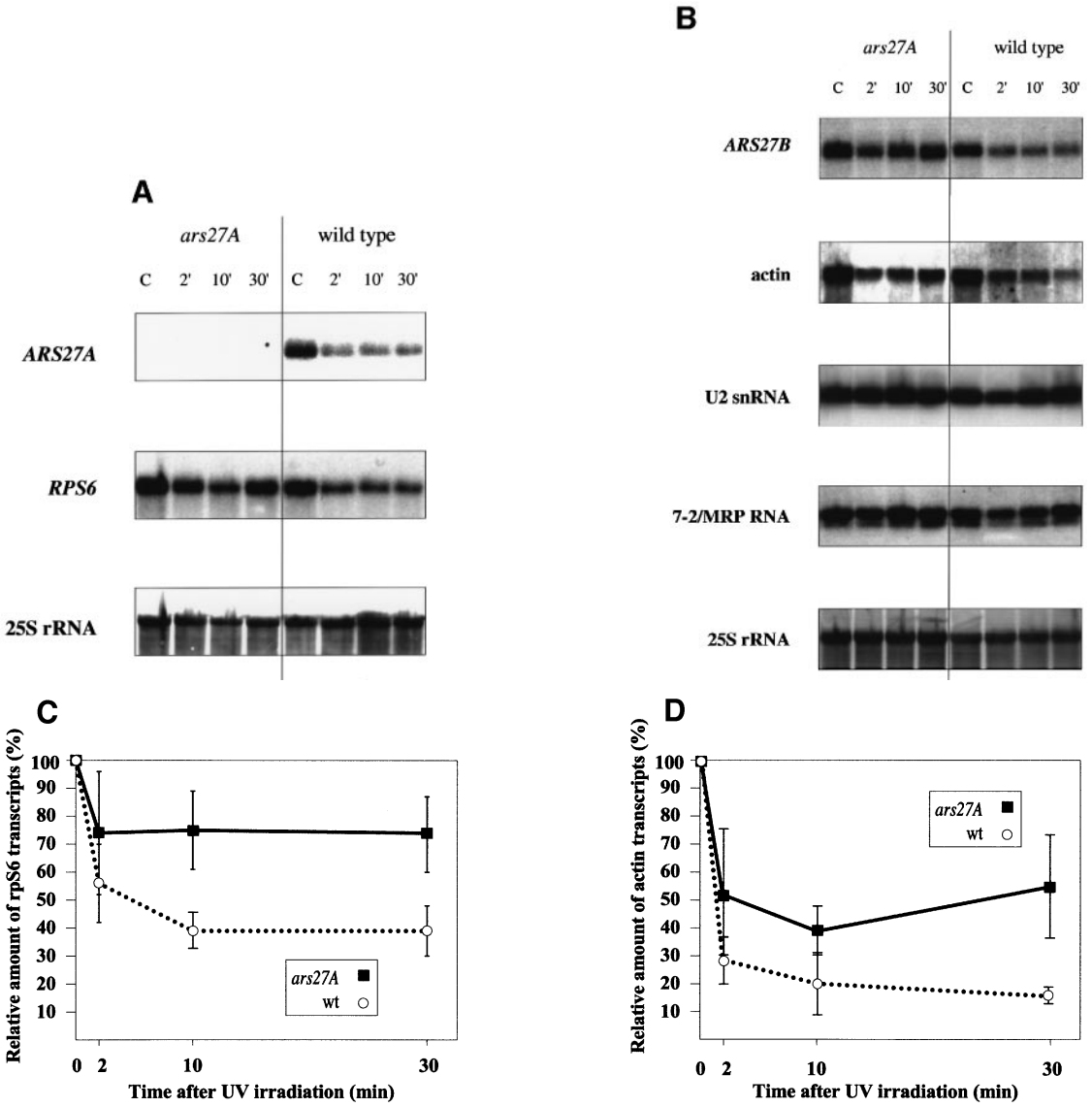

Fig. 6. Reduction of transcript levels after irradiation with 1 kJ/m^2 of UV-C. Seven-day-old seedlings were collected immediately (2 min), 10 and 30 min after irradiation. Times of harvest are indicated above the lanes; lane C represents non-irradiated seedlings. The first membrane (**A**) was hybridized with ARS27A-, ribosomal protein S6- and 25S rRNA-specific probes and the second membrane (**B**) with ARS27B-, actin-, U2 snRNA-, 7-2/MRP-like snoRNA- and 25S rRNA-specific probes. (C and D) Quantification of mRNA levels after 1kJ/m^2 of UV-C; (**C**) ribosomal protein S6 and (**D**) actin mRNA. Data were quantified using a PhosphorImager (Molecular Dynamics), standardized using the 25S rRNA signal and normalized to the amount of mRNA in non-irradiated seedlings (100%). Means and standard deviations of three independent experiments are presented.

hypersensitive to UV-C radiation and MMS. The T-DNA insertion disrupted the gene coding for ribosomal protein S27 (*ARS27A*), resulting in the absence of its transcript. Considering the high level of expression of the knocked-out gene in the wild type, the normal development and fertility of mutant plants appeared to be rather unusual, when compared with mutants of ribosomal protein genes recovered in various experimental systems. This may be explained in part by the existence of two additional active genes coding for other isoforms of S27 (*ARS27B* and *ARS27C*), although the unaltered expression level of *ARS27B* in the *ars27A* background suggests the absence of transcriptional compensation for the lack of the S27A transcript. Significantly, growth and development of *ars27A* as well as its resistance to various non-genotoxic stresses were not different to the wild type. This is in contrast to the specific hypersensitivity of *ars27A* seedlings to UV or MMS treatments. An MMS dose well tolerated by the wild type led to induction of tumors in the meristematic areas of mutant roots, followed by chlorosis and death of the entire plant. It is plausible, therefore, that this particular sensitivity of the mutant reflects a distinct function of *ARS27A*.

The S27 protein is a ribosomal protein with rather unusual structural features (Wool, 1993). It contains a C_2–C_2 zinc finger structure reminiscent of transcription factors. In fact, a computer database search revealed structural similarities of S27 to proteins such as LSD1, a putative transcription factor involved in the regulation of programed cell death in plants (Dietrich *et al.*, 1997), and VFBZIPZF protein, a *V.faba* transcription factor containing a leucine zipper and a zinc finger (DDBJ/EMBL/GenBank accession No. X97904, direct submission). In a search for proteins containing the motif VCHNCQNILCHP

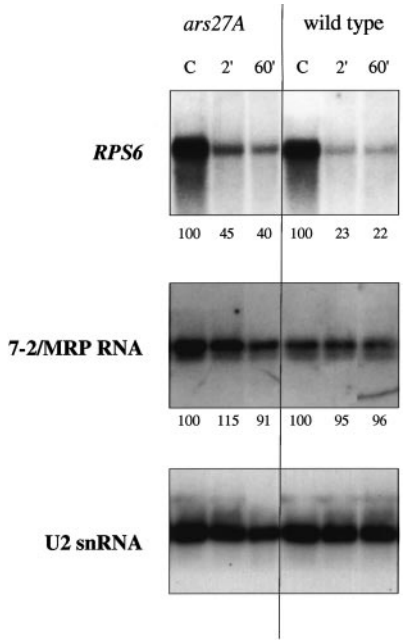

Fig. 7. Reduction of transcript levels after irradiation with 5 kJ/m^2 of UV-C. Seven-day-old seedlings were collected immediately (2 min) and 60 min after irradiation. Times of harvest are indicated above the lanes; lane C represents non-irradiated seedlings. The same membrane was hybridized with ribosomal protein S6-, U2 snRNA- and 7-2/MRP-like snoRNA-specific probes. Data were quantified using a PhosphorImager (Molecular Dynamics), standardized using the U2 snRNA signal and normalized to the amount of RNA in non-irradiated seedlings (100%). The relative amounts are shown below the lanes.

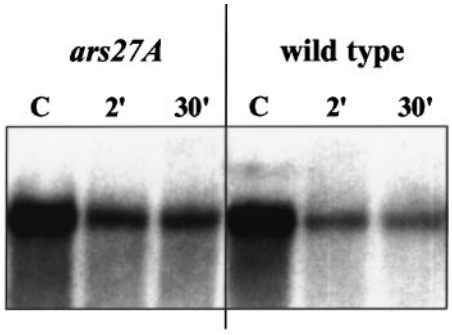

Fig. 8. S6 transcript levels after UV irradiation in *ars27A* and wild-type seedlings pre-treated with cordycepin. Seven-day-old seedlings were incubated in 600 μM cordycepin for 2 h prior to irradiation with 5 kJ/m^2 of UV-C. Times after the irradiation are indicated above the lanes; lane C represents non-irradiated seedlings.

(Figure 2B), two core subunits (p34 and p44) of the human transcription factor TFIIH (BTF2) (Humbert *et al*., 1994) were detected. Apart from transcription, both subunits are involved in nucleotide excision repair and cell-cycle regulation. The gene coding for p44 is the human homolog of yeast *SSL1* (Humbert *et al*., 1994), mutant alleles of which initially were detected as supressors of a translation block caused by a stem–loop at the 5′ end of mRNA (Yoon *et al*., 1992). Later, SSL1 was identified as a subunit of transcription factor TFIIH (Feaver *et al*., 1993) and has been shown to be involved in DNA repair (Wang *et al*., 1995). Proteins p34, p44 and SSL1 contain zinc finger-like motifs with conserved cysteines, supporting the idea that these proteins have a common function (Humbert *et al*., 1994). The motif VCHNCQNILCHP

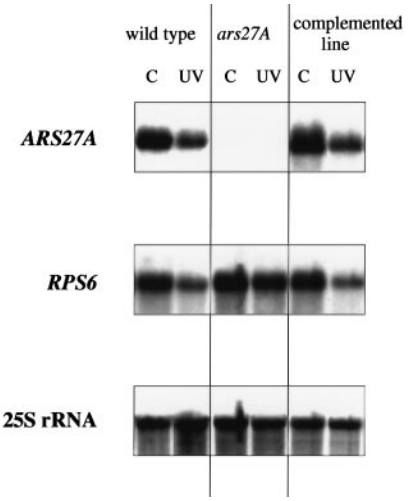

Fig. 9. UV-induced reduction of transcript levels in a line obtained after the transformation of the *ars27A* mutant with the genomic fragment containing the *ARS27A* gene. Seven-day-old seedlings were irradiated with 1 kJ/m^2 of UV-C and collected 30 min after irradiation. Lane C represents non-irradiated seedlings and lane UV, irradiated seedlings. The same membrane was hybridized with ARS27A-, ribosomal protein S6- and 25S rRNA-specific probes.

overlaps with zinc finger-like structures in ARS27A, p34 and p44. It is attractive to speculate that this particular motif, in combination with a zinc finger, may be a signature of proteins capable of both DNA and RNA interactions.

The three isoforms of S27 in *Arabidopsis* are encoded by three closely related genes. Adjacent to the zinc finger motif is a position in which every isoform has a different amino acid (ARS27A has isoleucine, ARS27B has valine and ARS27C has leucine) (Figure 2, position marked by the vertical arrow). Strikingly, *S.cerevisiae* has two S27 genes, differing in only one amino acid at the corresponding position (RPS27A contains isoleucine and RPS27B contains valine). Yeast mutants with the deletion of either of the two genes are viable and display distinct phenotypes (Baudin-Baillieu *et al*., 1997). Deletion of *RPS27B* affects biogenesis of 40S ribosomal subunits, resulting in a weak, slow-growing strain with impaired processing of pre-rRNA. In contrast, deletion of *RPS27A* has no significant effects on these processes and results in wild-type viability under standard growth conditions, which is consistent with the phenotype of *Arabidopsis ars27A*. Significantly, yeast *RPS27A* seems to be involved in RNA metabolism, as indicated by its ability to suppress a lethal mutation affecting rRNA processing (Baudin-Baillieu *et al*., 1997). Double mutants of *RPS27A* and *RPS27B* are lethal, suggesting a possible functional cross-complementation of the two S27 isoforms in yeast. This arrangement resembles the status of bifunctional ribosomal protein S3 (UV endonuclease III) present as two isoforms with different molecular masses (Louie *et al*., 1996).

Genotoxic treatments not only damage chromosomal DNA but have various effects on other cell components, including RNA. For example, UV treatment also produces RNA pyrimidine dimers (Gordon *et al*., 1976), and treatment with alkylating agents such as MMS should lead to modification of both DNA and RNA. It is possible that such damaged RNAs are recognized and degraded or, less probably, repaired. Although RNA photolyase activity has

been described (Gordon *et al*., 1976), the pathways of damaged RNA surveillance are not known. It has been observed that rRNA damage elicits a specific ribotoxic stress provoking activation of selected cellular signals (Iordanov *et al*., 1998). The system of recognition and accelerated turnover of aberrant transcripts is present in all eukaryotes and seems to be associated with translation (for reviews, see Maquat, 1995; Ruiz-Echevarria *et al*., 1996). It has been postulated that RNase complexes similar to those involved in the processing of pre-ribosomal transcripts to 25S, 18S and 5.8S RNA can also function in turnover regulation of cytoplasmic RNA (Mitchell *et al*., 1997). The necessity for removal of aberrant transcripts has been demonstrated in *C.elegans*. In *smg* mutants, steady-state levels of aberrant mRNAs are increased. Accumulation of transcripts of the myosin heavy chain *unc-54* gene with introduced nonsense mutations in *smg* mutants leads to disruption of muscles, most probably due to the presence of N-terminal polypeptide fragments (Pulak and Anderson, 1993). Thus, it has been proposed that *smg* genes are part of an mRNA surveillance system which protects cells from the deleterious effects of aberrant transcripts (Pulak and Anderson, 1993). In yeast, a similar function has been assigned to *UPF* genes (Ruiz-Echevarria *et al*., 1996). Unfortunately, the sensitivity of these mutants to genotoxic treatments was not investigated. On the other hand, there is an example of the implication of an RNase in UV resistance. *Saccharomyces cerevisiae* cells lacking the major cytoplasmic 5′→3′ exoribonuclease XRN1 (known also as SEP1) are moderately sensitive to UV with delayed recovery from irradiation (Tishkoff *et al*., 1991). The mutations of *XRN1* result in pleiotropic phenotypes including slow growth, increased cell size, defective sporulation and altered mitotic and meiotic recombination properties (for reviews, see Kearsley and Kipling, 1991; Heyer, 1994). The connections of all these traits to the process of RNA turnover still have to be elucidated. In our experiments, we observed that UV irradiation induced a rapid decrease of the mRNA level, which indicated the involvement of RNase activities in immediate response to nucleic acid damage. Since mRNA turnover is tightly linked to translation (for a review, see Jacobson and Peltz, 1996), S27 might be directly involved in providing such a link.

The elevated stability of transcripts in *ars27A* after genotoxic treatments suggests inhibition of their decay. It is not clear whether this defect directly results in hypersensitivity to genotoxic treatments and triggers tumorous growth, but it is certain that the *ars27A* mutation is responsible for the observed phenotypes, since the ectopic copy of the *ARS27A* gene restores wild-type responses. It is plausible that the S27A protein involved in rRNA maturation (Baudin-Baillieu *et al*., 1997) is also involved in the degradation of damaged transcripts. Such a function might require the zinc finger motif present in S27 as a potential nucleic acid-binding domain and thus its conservation.

Materials and methods

Plant growth conditions

Arabidopsis thaliana ecotype Columbia plants were grown in sterile culture or in soil under conditions described previously (Masson *et al*., 1997).

Mutant isolation

The T3 families of *A.thaliana* transgenic for T-DNA inserts conferring hygromycin resistance (Koncz *et al*., 1989) were examined for hypersensitivity to UV-C and MMS (Fluka). Twenty 5-day-old seedlings, pregerminated on vertical agar plates (Masson *et al*., 1997), were irradiated with UV-C at 1 kJ/m^2 (254 nm, Osram HNS 55W ORF). After irradiation, plates were kept in the dark for 1 day and then transferred to standard growth conditions. The growth of the main roots was monitored daily for 1 week. The families containing seedlings with arrested growth of the main root after UV irradiation were re-screened by assaying another 40 seedlings for UV sensitivity and 20 for control growth.

The assay for MMS sensitivity was also performed on twenty 5-day-old seedlings transferred individually from vertical agar plates to multivial plates containing 0.5 ml aliquots of liquid germination medium with 100 p.p.m. of MMS. This dose was determined to be permissive for the wild type. Results were evaluated after 3 weeks. Families containing sensitive (dead) individuals were re-screened using an additional 40 seedlings on MMS plates and 20 on control medium.

Determination of stress tolerance

Five-day-old seedlings were transferred to multivial plates for further growth on media supplemented with various concentrations of stress-provoking compounds. The minimal lethal dose of the different agents for wild-type seedlings was used as the maximal concentration in each sensitivity test. Responses to the following treatments were tested: (i) oxidative stress provoked by a reagent directly inducing reactive oxygen species (ROS) (4,5,6,7-tetrachloro-2′,4′,5′,7′-tetraiodofluorescein; Rose Bengal) at concentrations of 0.1, 0.5, 1, 2 and 4 μM; (ii) scavenger of ROS (*N*-acetyl-L-cysteine) at 0.3, 1, 3, 6 and 12 mM; (iii) osmotic stress, mannitol at 0.1, 0.2, 0.4, 0.8 and 1.0 M; (iv) salinity stress, NaCl at 0.04, 0.08 and 0.12 M. The influence of heat stress was examined by subjecting seedlings to 32°C for different time periods (1, 3 and 7 days) followed by further growth in standard growth conditions (22°C).

Cloning of the ARS27A locus and corresponding cDNA

Plant genomic DNA from *ars27A/ars27A* plants was isolated according to Dellaporta *et al*. (1983). The DNA was digested with *Eco*RI or *Hin*dIII (Figure 3). A plasmid rescue procedure (Koncz *et al*., 1989) was used to clone flanking plant sequences, resulting in two plasmids pRescE and pRescH (Figure 3). The 2.8 kb *Bgl*II fragment of plasmid pRescE (Figure 3) was labeled with [α-^{32}P]ATP (Feinberg and Vogelstein, 1983) and was used as a probe to screen an *Arabidopsis* cDNA library (Minet *et al*., 1992) and an *Arabidopsis* (ecotype Columbia) genomic library (Stratagene) using a standard procedure (Sambrook *et al*., 1989). For genomic clones, plasmids were excised from positive bacteriophages according to the supplier's instructions. The DNA sequences of cDNA and genomic clones were determined by automatic sequencing (DDBJ/EMBL/GenBank accession Nos AF083336 and AF083337).

Southern and Northern blot analysis

For Southern blot analysis, genomic DNA from *ars27A* and wild-type plants was isolated according to Dellaporta *et al*. (1983) and separated electrophoretically after endonucleolytic digestion. DNA fragments were transferred to nylon membranes (Hybond N, Amersham) using standard protocols (Sambrook *et al*., 1989). For Northern blot analysis, total RNA was isolated with the RNeasy Plant Mini Kit (Qiagen) according to the supplier's instructions, and 5 μg aliquots of RNA were separated elecrophoretically and blotted to nylon membranes (Hybond N, Amersham) using standard protocols (Sambrook *et al*., 1989). Hybridization and washing of all blots was performed according to Church and Gilbert (1984). The ARS27A probe corresponded to a 0.3 kb *Not*I–*Eco*NI fragment of the *ARS27A* cDNA clone (DDBJ/EMBL/GenBank accession No. AF083336) from the library constructed in the vector pFL61 (Minet *et al*., 1992) and the ARS27B probe to a 0.5 kb *Not*I–*Sal*I fragment of the expressed sequence tag clone 110J16T7 (DDBJ/EMBL/GenBank accession No. T42115). The S6 probe corresponded to the 0.7 kb *Xho*I–*Xba*I fragment of *Arabidopsis* ribosomal protein S6 cDNA (Turck *et al*., 1998; DDBJ/EMBL/GenBank accession No. Y14052), and the actin probe to a 1.8 kb *Bam*HI fragment containing the *Arabidopsis AAc*1 gene (Nairn *et al*., 1988; DDBJ/EMBL/GenBank accession No. M20016). The *Arabidopsis* U2 snRNA gene probe corresponded to the *Hin*dIII–*Eco*RI of pU2PCR (Connelly and Filipowicz, 1993). The *Arabidopsis* 7-2/MRP-like snoRNA gene probe corresponded to the *Bam*HI–*Eco*RI fragment of the plasmid pAt7-2 (Kiss *et al*., 1992). In order to standardize the signals, a hybridization was performed using a tomato 25S rRNA gene probe (Kiss *et al*., 1989;

DDBJ/EMBL/GenBank accession No. X13557). In experiments with UV-irradiated seedlings, different doses of UV-C were applied to 7-day-old seedlings grown on vertical plates. After irradiation, seedlings were collected immediately or incubated in the dark for the times indicated. In experiments with the transcription inhibitor, 7-day-old seedlings were transferred to a plastic dish with incubation buffer (Seeley *et al*., 1992) and 600 μM cordycepin (Sigma) and incubated under standard growth conditions with gentle agitation. After 2 h, the seedlings were collected, spread on wet Whatman 3MM paper and immediately irradiated with 5 kJ/m^2 of UV-C.

Complementation of the ars27A mutation

A genomic *Nco*I–*Bam*HI 2.7 kb fragment containing the *ARS27A* gene (Figure 3) was inserted into an *Spe*I site of the binary vector p35Sbarbi (Mengiste *et al*., 1997). In the resulting plasmid p35Sbarbi::S27A, the *ARS27A* gene was situated proximal to the right T-DNA border. p35Sbarbi::S27A was introduced into *A.tumefaciens* strain C58CIRif ^{R} containing the pGV3101 Ti plasmid (Van Larebeke *et al*., 1974). The *ARS27A* gene was transferred to *ars27A* plants by *in planta* infiltration of *Agrobacterium* (Bechtold *et al*., 1993). Screening for transformants and analysis of the T2 generation were performed as described previously (Mengiste *et al*., 1997).

Acknowledgements

We thank O.Mittelsten Scheid, W.Filipowicz, Y.Nagamine, G.Thomas and P.King for helpful comments on the manuscript. We acknowledge A.Bogucki for technical assistance. We also thank all the members of our laboratory for continuous support. E.R. is also a member of Center for Bioengineering, Russian Academy of Sciences.

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Received September 10, 1998; revised November 5, 1998; accepted November 16, 1998