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

Ekaterina Revenkova¹, Jean Masson, Csaba Koncz², Karin Afsar, Ludmila Jakovleva and Jerzy Paszkowski

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

¹Corresponding author

e-mail: revenek@fmi.ch

A recessive *Arabidopsis* mutant with elevated sensitivity to DNA damaging treatments was identified in one 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 TFIID; 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) (Saebøe-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 C2−C2 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-β, 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). 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 isofrom 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 isofrom 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).

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 C2−C2 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. X97904);
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).

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 chromosomme (YAC) clones (provided by Arabidopsis Biological Resource Center,
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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 HindIII (plasmid pRescH) or EcoRI (plasmid pRescE). The BglII fragment of pRescE was used as a probe for screening the cDNA library. The NcoI–BamHI 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 NcoI–BamHI 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
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/m²) 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/m²) 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/m², 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/m²), the decrease of S6 mRNA was even more drastic compared with at 1 kJ/m². U2 snRNA and 7-2/MRP snoRNA appeared to be stable also under these conditions (Figure 7). Quantification 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 cytoplasmic 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.
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Fig. 6. Reduction of transcript levels after irradiation with 1 kJ/m² 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/m² 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.

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 programmed 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 VCHNCQNILCYP
Fig. 7. Reduction of transcript levels after irradiation with 5 kJ/m² 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/m² of UV-C. Times after the irradiation are indicated above the lanes; lane C represents non-irradiated seedlings.

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/m² 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.

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 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 prerRNA. 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).
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 S′→3′ exoribonuclease XRN1 (known also as SEPI) are moderately sensitive to UV with delayed recovery from irradiation (Tishkoff et al., 1991). The mutations of XRN1 result in pleiotrophic 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 RNA 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, pre-germinated on vertical agar plates (Masson et al., 1997), were irradiated with UV-C at 1 kJ/m² (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′,3′,4′,5′-tetrachlorofluorescein; Rose Bengal) at concentrations of 0.1, 0.5, 1, 2 and 4 μM; (ii) scavenger of ROS (V-amino-cyclo-(6-carboxy-tetrahydroxyestrane)) at 0.3, 1, 3, 6 and 12 nM; (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 EcoRI or HindIII (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 EcoRI fragment of plasmid pRescE (Figure 3) was labeled with [α-32P]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 electrophoretically 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 NotI–EcoNI fragment of the ARS27A cDNA clone (DDBJ/EMBL/GenBank accession No. AF083336) from the library constructed in the vector pU2PCR (Minet et al., 1992) and the ARS27B probe to a 0.5 kb NotI–SalI fragment of the expressed sequence tag clone 1101617D1 (DDBJ/EMBL/GenBank accession No. T42115). The S6 probe corresponded to the 0.7 kb XhoI–XbaI 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 BamHI fragment containing the Arabidopsis ActI gene (Nairn et al., 1988; DDBJ/EMBL/GenBank accession No. M20016). The Arabidopsis U2 snRNA gene probe corresponded to the HindIII–EcoRI fragment of pU2PCR (Connelly and Filipowicz, 1993). The Arabidopsis 7-2-MRP-like snoRNA gene probe corresponded to the BamHI–EcoRI fragment of the plasmid pA7-2 (Kiss et al., 1992). In order to standardize the signals, a hybridization was performed using a tomato 258 RNA gene probe (Kiss et al., 1989);
DDU/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 kJm² of UV-C.

Complementation of the ars27A mutation

A genomic Ncol–BamHI 2.7 kb fragment containing the ARS27A gene (Figure 3) was inserted into an Spel 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.nicotianae strain CS8CTIFR8 containing the pGV3101 Ti plasmid (Van Larebeke et al., 1974). The ARS27A gene was transferred to ars27A plants by in planta infection of Agrobacterium (Bechtold et al., 1993). Screening for transformants and analysis of the T2 generation were performed as described previously (Mengiste et al., 1997).

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References


Ribosomal protein S27 in genotoxic stress response


