SUPPLEMENTARY DATA

Plant mitochondria possess a short-patch base excision DNA repair pathway

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SUPPLEMENTARY MATERIALS AND METHODS

Construction of the pGII35S-GFP and pGII35SNUDG-GFP plasmids

The pGreenII0000 vector (http://www.pgreen.ac.uk/JIT/pGreen0000_fr.htm) (Hellens *et al.*, 2000) was linearized following cleavage of the *BamHI* site in the cloning cassette. To eliminate this site, the sticky ends were filled with the Klenow fragment of *E. coli* DNA polymerase I and the plasmid was religated before transformation into DH5-α bacterial cells. In parallel, the fragment corresponding to the complete plant expression cassette [double *Cauliflower mosaic virus* (CaMV) 35S promoter, *Tobacco etch virus* (TEV) translation leader, enhanced green fluorescent protein (eGFP) gene, CaMV 35S terminator] was cut out with *HindIII* from the pUCAP plasmid (van Engelen *et al.*, 1995) carrying the eGFP gene (pUCAP-GFP, a gift from J. Gualberto). The recovered fragment was cloned into the *HindIII* site in the cloning cassette of the *BamHI*-deprived pGreenII0000, yielding the pGII35S-GFP plasmid. Finally, the complete coding sequence of the At3g18630 gene was amplified by PCR with the direct primer AtUDG5d and the reverse primer AtUDGfull3r (see below), using as a template an *A. thaliana* cDNA library generated with random hexanucleotides (a gift from D. Gagliardi). The PCR products were cloned into the pGEM-T Easy vector (Promega) and the sequence corresponding to the N-terminal part of the gene (nucleotides 1-521 of the coding sequence) was cut out with *KpnI* (brought by the PCR primer) and *BamHI* (present in the gene sequence). The fragment obtained was inserted downstream of the TEV translation leader, upstream of and in-frame with the eGFP gene in the plant expression cassette of pGII35S-GFP digested with the same enzymes, yielding the pGII35SNUDG-GFP plasmid.

AtUDG5d (*KpnI* restriction site underlined):
5'-AGCAAGGTACCATGGCTTCGTCGACACCTAAA-3'
AtUDGfull3r (*EcoRI* restriction site underlined):
5'-AGCAAGAATTCCAAGTTAAAGTTGCCAGTCTATG-3'

Construction of the pBSNUDG-GFP plasmid

The sequence corresponding to the N-terminus of the At3g18630 gene (nucleotides 1-285 of the coding sequence, *i.e.* the first exon) was amplified by PCR with the *KpnI* site-containing direct primer AtUDG5d, as above, and the *BamHI* site-containing reverse primer AtUDG1ex3r (see below), using *A. thaliana* total DNA as a template. Following digestion with *KpnI* and *BamHI*, the PCR products were cloned into the same sites in the pUCAP-GFP plasmid, upstream of and in-frame with the eGFP gene. Finally, the fragment carrying the

fusion between the partial UDG and the eGFP sequence was cut out with *KpnI* and *XbaI* and cloned into the same sites in pBlueScript-SK (Stratagene), yielding the pBSNUDG-GFP plasmid.

AtUDG1ex3r (*BamHI* restriction site underlined):
5'-GTACAGGATCCTCTTTTGCTTTTGTGACCCTCTCG-3'

In vitro protein import into isolated mitochondria

The pBSNUDG-GFP plasmid served for *in vitro* synthesis of the eGFP fused to the N-terminal part of the At3g18630-encoded protein. As a positive control, we used a previously described construct (Pujol *et al.*, 2007) (a gift from A.M. Duchêne) carrying the sequence for the first 81 amino acids of *A. thaliana* glutamyl-tRNA synthetase (At5g64050) fused to the GFP gene in the pCRII-Topo vector (Invitrogen). The latter fusion protein is known to be dual-targeted to mitochondria and chloroplasts (Pujol *et al.*, 2007). The constructs were used as templates for *in vitro* transcription/translation in a TNT^{TM} coupled reticulocyte lysate system (Promega) in the presence of [^{35}S]methionine. Import of [^{35}S]-labeled fusion proteins into purified potato (*S. tuberosum*) mitochondria was performed as described (Wischmann and Schuster, 1995) and analyzed by SDS-polyacrylamide gel electrophoresis.

References

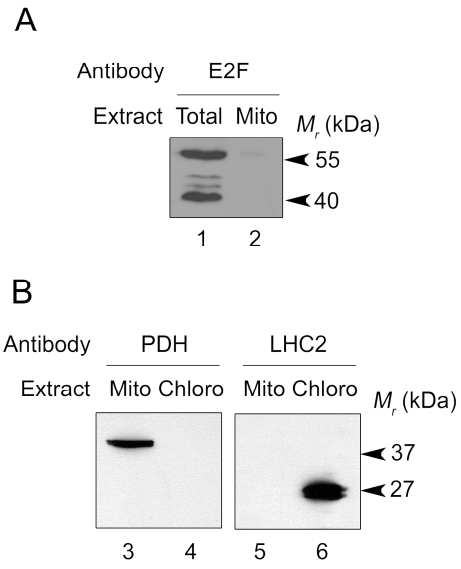
Hellens,R.P., Edwards,E.A., Leyland,N.R., Bean,S., and Mullineaux,P.M. (2000) pGreen: a versatile and flexible binary Ti vector for *Agrobacterium*-mediated plant transformation. *Plant Mol. Biol.*, **42**, 819-832.

Pujol,C., Maréchal-Drouard,L. and Duchêne,A.M. (2007) How can organellar protein N-terminal sequences be dual targeting signals? *In silico* analysis and mutagenesis approach. *J. Mol. Biol.*, **369,** 356-367.

van Engelen,F.A., Molthoff,J.W., Conner,A.J., Nap,J.P., Pereira,A., and Stiekema,W.J. (1995) pBINPLUS: an improved plant transformation vector based on pBIN19. *Transgenic Res.*, **4,** 288-290.

Wischmann,C. and Schuster,W. (1995) Transfer of *rps10* from the mitochondrion to the nucleus in *Arabidopsis thaliana*: evidence for RNA-mediated transfer and exon shuffling at the integration site. *FEBS Lett.*, **374,** 152-156.

SUPPLEMENTARY FIGURES

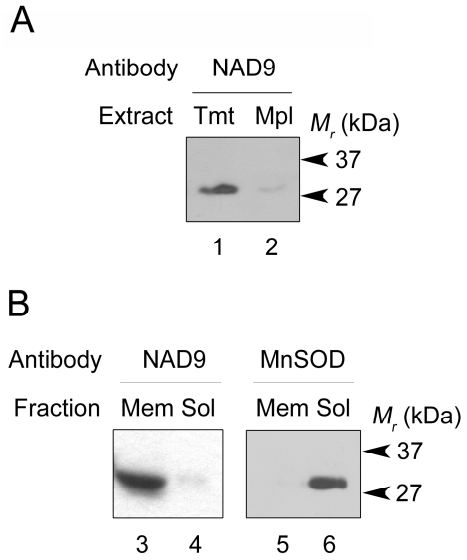


Supplementary Figure 1. Absence of significant cross-contamination in *A. thaliana* organelle extracts. Panel A: total protein extract from *A. thaliana* cells (Total) and enriched mitochondrial enzymatic extract (Mito) were submitted to western blot analysis with a polyclonal antiserum (E2F) raised against the DNA-binding domain of the human E2F5 nuclear transcription factor (Santa Cruz Biotechnologies). The antiserum reveals the different forms of plant E2F and E2F-like (DEL) nuclear factors. Panel B: *A. thaliana* enriched mitochondrial (Mito) and chloroplast (Chloro) enzymatic extracts were submitted to western blot analysis with a monoclonal antibody raised against the maize mitochondrial pyruvate dehydrogenase (PDH) or a polyclonal antiserum against the *Chlamydomonas reinhardtii* chlorophyll a/b-binding protein of the photosystem II light-harvesting complex (LHC2). Total *A. thaliana* protein extracts were prepared as described by Lincker *et al.* (2006). Preparation of enriched organelle enzymatic extracts is described in the "Materials and Methods" section of the article. *A. thaliana* chloroplasts were purified as described by Sakamoto *et al.* (2000) from cell suspensions grown under constant light. Protein samples (25 µg per lane) were fractionated by SDS-polyacrylamide gel electrophoresis and transferred to Immobilon-P membranes (Millipore). Blots were incubated with the primary and secondary antibodies and revealed using ECL chemiluminescence reagents (GE Healthcare). The migration of size marker proteins is indicated by arrows.

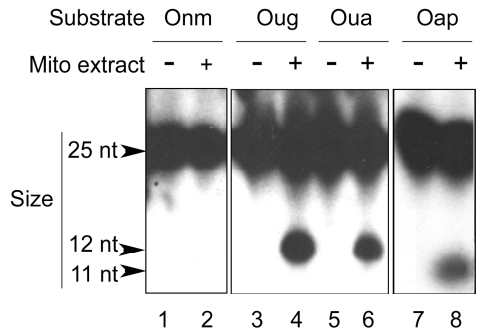
References

Lincker,F., Messmer,M., Houlne,G., Devic,M., and Chaboute,M.E. (2006) E2F factors rate controls the dual role of CDE/E2F composite element: a model of E2F-regulated gene expression in plant development. *FEBS Lett.*, **580,** 5167-5171.

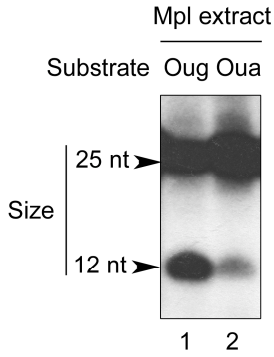
Sakamoto,W., Spielewoy,N., Bonnard,G., Murata,M. and Wintz,H. (2000) Mitochondrial localization of AtOXA1, an *Arabidopsis* homologue of yeast Oxa1p involved in the insertion and assembly of protein complexes in mitochondrial inner membrane. *Plant Cell Physiol.*, **41**, 1157-1163.



Supplementary Figure 2. Absence of significant cross-contamination in *A. thaliana* submitochondrial fractions. Panel A: a total fraction from *A. thaliana* mitochondria (Tmt) and an enriched soluble enzymatic extract prepared from mitoplasts (Mpl) were submitted to western blot analysis with a polyclonal antiserum (NAD9) raised against subunit 9 of wheat mitochondrial complex I. The NAD9 protein is a marker for the mitochondrial inner membrane. Panel B: a membrane fraction (Mem) and a soluble fraction (Sol) from *A. thaliana* mitochondria were submitted to western blot analysis with the antiserum against NAD9 (NAD9) or with a polyclonal antiserum against the *Nicotiana plumbaginifolia* mitochondrial manganese superoxide dismutase (MnSOD), a matrix marker. Preparation of enriched organelle enzymatic extracts and submitochondrial fractions is described in the "Materials and Methods" section of the article. Protein samples (25 µg per lane) were fractionated by SDS-polyacrylamide gel electrophoresis and transferred to Immobilon-P membranes (Millipore). Blots were incubated with the primary and secondary antibodies and revealed using ECL chemiluminescence reagents (GE Healthcare). The migration of size marker proteins is indicated by arrows.



Supplementary Figure 3. UDG and AP endonuclease activities in *S. tuberosum* mitochondrial extracts. Double-stranded oligodeoxyribonucleotides Onm, Oug, Oua and Oap, [^{32}P]-labeled at the 5' end of one of the strands as indicated in Figures 1 and 2 of the article, were incubated in the absence of extract (lanes 1, 3, 5 and 7) and in the presence of enriched *S. tuberosum* mitochondrial (Mito) enzymatic extract (lanes 2, 4, 6 and 8) for 1 h. To evaluate the extent of lesion-specific incision, the samples were subsequently denatured and analyzed by electrophoresis on polyacrylamide gels in the presence of 7 M urea. The migration of size markers is indicated by arrows.



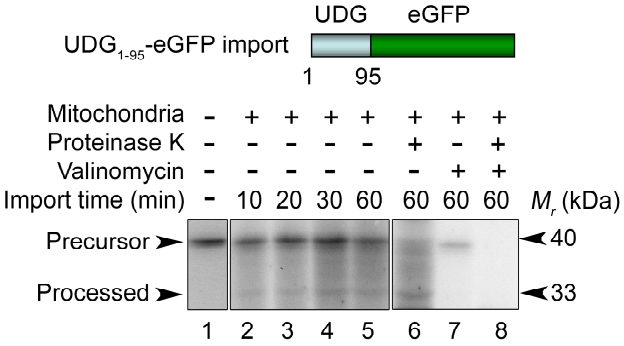
Supplementary Figure 4. UDG activity is present in a soluble mitoplast fraction. Double-stranded oligodeoxyribonucleotides Oug (lane 1) and Oua (lane 2), [^{32}P]-labeled at the 5' end of the strand carrying the lesion, were incubated in the presence of enriched enzymatic extract from *A. thaliana* mitoplasts (Mpl) for 1 h. To evaluate the extent of lesion-specific incision, the samples were subsequently denatured and analyzed by electrophoresis on polyacrylamide gels in the presence of 7 M urea. The migration of size markers is indicated by arrows.

SUPPLEMENTARY RESULTS

The mitochondrial targeting properties of the N-terminal region of the *A. thaliana* At3g18630-encoded UDG were further tested through *in vitro* import into isolated organelles. For these assays, a UDG_{1-95}-eGFP fusion protein was synthesized in a [^{35}S]methionine-containing cell-free system from a gene construct inserted into the pBluescript plasmid (pBSNUDG-GFP, see the Supplementary Materials and Methods for details). *In vitro* transcription/translation of the chimeric gene yielded the expected 37 kDa polypeptide. When incubated with *S. tuberosum* mitochondria in regular protein import conditions, this product was partially processed into a polypeptide of about 32 kDa (Supplementary Figure 5, lanes 2 to 5), consistent with the M_r of the fusion protein upon cleavage of the targeting sequence predicted by the MitoProt programme (http://ihg2.helmholtz-muenchen.de/ihg/mitoprot.html) (Claros and Vincens, 1996). The addition of proteinase K to the medium after the import step led to a strong reduction of the signal corresponding to the original translation product, but did not affect the signal corresponding to the putative processed protein which was protected (Supplementary Figure 5, lane 6). Valinomycin is known to inhibit mitochondrial protein import by dissipating the membrane potential. The addition of valinomycin to the mitochondria before the import step prevented the formation of the processed polypeptide (Supplementary Figure 5, lane 7). Finally, when both treatments were applied, neither the original translation product, nor the processed protein was recovered in the mitochondrial fraction (Supplementary Figure 5, lane 8).

Reference

Claros,M.G. and Vincens,P. (1996) Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur. J. Biochem.*, **241,** 779-786.



Supplementary Figure 5. The N-terminal region of the UDG encoded by the At3g18630 locus drives mitochondrial import *in vitro*. *In vitro* import of the eGFP, fused in-frame at its N-terminus to the first 95 amino acids of the At3g18630-encoded protein, into isolated *S. tuberosum* mitochondria. The UDG_{1-95}-eGFP fusion protein was *in vitro* translated (lane 1) and incubated for increasing times at 25°C with the mitochondria (lanes 2 to 5). Control assays (lanes 6 to 8) were run for the longest incubation time. Mitochondria were submitted to proteinase K digestion after import (lane 6), treated with valinomycin prior to import (lane 7) or both treated with valinomycin prior to import and submitted to proteinase K digestion after import (lane 8). Samples were analyzed by SDS-polyacrylamide gel electrophoresis. Migration of the initial fusion protein (Precursor) and of the processed polypeptide generated upon import (Processed) is indicated. Migration of marker proteins is given on the right side of the panel.