

A UVB-hypersensitive mutant in *Arabidopsis thaliana* is defective in the DNA damage response

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Abstract: To investigate UVB DNA damage response in higher plants, we used a genetic screen to isolate *Arabidopsis thaliana* mutants that are hypersensitive to UVB irradiation, and isolated a UVB-sensitive mutant, termed *suv2* (for sensitive to UV 2) that also displayed hypersensitivity to ??-radiation and hydroxyurea. This phenotype is reminiscent of the *Arabidopsis* DNA damage-response mutant *atr*. The *suv2* mutation was mapped to the bottom of chromosome 5, and contains an insertion in an unknown gene annotated as MRA19.1. RT-PCR analysis with specific primers to MRA19.1 detected a transcript consisting of 12 exons. The transcript is predicted to encode a 646 amino acid protein that contains a coiled-coil domain and two instances of predicted PIKK target sequences within the N-terminal region. Fusion proteins consisting of the predicted MRA19.1 and DNA-binding or activation domain of yeast transcription factor GAL4 interacted with each other in a yeast two-hybrid system, suggesting that the proteins form a homodimer. Expression of CYCB1;1:GUS gene, which encodes a labile cyclin:GUS fusion protein to monitor mitotic activity by GUS activity, was weaker in the *suv2* plant after ??-irradiation than in the wild-type plants and was similar to that in the *atr* plants, suggesting that the *suv2* mutant is defective in cell-cycle arrest in response to DNA damage. Overall, these results suggest that the gene disrupted in the *suv2* mutant encodes an *Arabidopsis* homologue of the ATR-interacting protein ATRIP. ?? 2009 Blackwell Publishing Ltd.

Author Keywords: ATR; Cell cycle; Checkpoint; DNA damage; Hydroxyurea; Ultraviolet light

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References:

1. Ahmad, M., Jarillo, J.A., Klimczak, L.J., Landry, L.G., Peng, T., Last, R.L., Cashmore, A.R., An enzyme similar to animal type II photolyases mediates photoreactivation in *Arabidopsis* (1997) *Plant Cell*, 9, pp. 199-207
2. Bao, S., Tibbetts, R.S., Brumbaugh, K.M., Fang, Y., Richardson, D.A., Ali, A., Chen, S.M., Wang, X.F., ATR/ATM-mediated phosphorylation of human Rad17 is required for genotoxic stress responses (2001) *Nature*, 411, pp. 969-974
3. Bell, C.J., Ecker, J.R., Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis* (1994) *Genomics*, 19, pp. 137-144
4. Bentley, N.J., Holtzman, D.A., Flaggs, G., Keegan, K.S., Demaggio, A., Ford, J.C., Hoekstra, M., Carr, A.M., The *Schizosaccharomyces pombe* rad3 checkpoint gene (1996) *EMBO J.*, 15, pp. 6641-6651

5. Britt, A.B., Molecular genetics of DNA repair in higher plants (1999) *Trends Plant Sci.*, 4, pp. 20-25
6. Britt, A.B., Chen, J.-J., Wykoff, D., Mitchell, D., A UV-sensitive mutant of *Arabidopsis* defective in the repair of pyrimidine-pyrimidinone(6-4) dimers (1993) *Science*, 261, pp. 1571-1574
7. Col?n-Carmona, A., You, R., Haimovitch-Gal, T., Doerner, P., Spatio-temporal analysis of mitotic activity with a labile cyclin-GUS fusion protein (1999) *Plant J.*, 20, pp. 503-508
8. Cortez, D., Guntuku, S., Qin, J., Elledge, S.J., ATR and ATRIP: Partners in checkpoint signalling (2001) *Science*, 294, pp. 1713-1716
9. Culligan, K., Tissier, A., Britt, A., ATR regulates a G2-phase cell-cycle checkpoint in *Arabidopsis thaliana* (2004) *Plant Cell*, 16, pp. 1091-1104
10. Culligan, K.M., Robertson, C.E., Foreman, J., Doerner, P., Britt, A.B., ATR and ATM play both distinct and additive roles in response to ionizing radiation (2006) *Plant J.*, 48, pp. 947-961
11. Curtis, M.J., Hays, J.B., Tolerance of dividing cells to replication stress in UVB-irradiated *Arabidopsis* roots: Requirements for DNA translesion polymerases ? and ?? (2007) *DNA Repair*, 6, pp. 1341-1358
12. De Schutter, K., Joub??s, J., Cools, T., *Arabidopsis* WEE1 kinase controls cell cycle arrest in response to activation of the DNA integrity checkpoint (2007) *Plant Cell*, 19, pp. 211-225
13. Edward, R.J., Bentlay, N.J., Carr, A.M., A Rad3-Rad26 complex responds to DNA damage independently of other checkpoint proteins (1999) *Nature Cell Biol.*, 1, pp. 393-396
14. Gallego, F., Fleck, O., Li, A., Wyrzykowska, J., Tinland, B., AtRAD1, a plant homologue of human and yeast nucleotide excision repair endonucleases, is involved in dark repair of UV damages and recombination (2000) *Plant J.*, 21, pp. 507-518
15. Gao, M.-J., Murphy, T.M., Alternative forms of formamidopyrimidine-DNA glycosylase from *Arabidopsis thaliana* (2001) *Photochem. Photobiol.*, 73, pp. 128-134
16. Garcia, V., Salanoubat, M., Choisne, N., Tissier, A., An ATM homologue from *Arabidopsis thaliana*: Complete genomic organization and expression analysis (2000) *Nucleic Acids Res.*, 28, pp. 1692-1699
17. Garcia, V., Bruchet, H., Comescasse, D., Granier, F., Bouchez, D., Tissier, A., AtATM is essential for meiosis and the somatic response to DNA damage in plants (2003) *Plant Cell*, 15, pp. 119-132
18. Garc??a-Ortiz, M.V., Ariza, R.R., Rold?n-Arjona, T., An OGG1 orthologue encoding a functional 8-oxoguanine DNA glycosylase/lyase in *Arabidopsis thaliana* (2001) *Plant Mol. Biol.*, 47, pp. 795-804
19. Heffernan, T.P., Simpson, D.A., Frank, A.R., Heinloth, A.N., Paules, R.S., Cordeiro-Stone, M., Kaufmann, W.K., An ATR- and Chk1-dependent S checkpoint inhibits replicon initiation following UVC-induced DNA damage (2002) *Mol. Cell. Biol.*, 22, pp. 8552-8561
20. Heitzeberg, F., Chen, I.P., Hartung, F., Orel, N., Angelis, K.J., Puchta, H., The Rad17 homologue of *Arabidopsis* is involved in the regulation of DNA damage repair and homologous recombination (2004) *Plant J.*, 38, pp. 954-968
21. Itakura, E., Takai, K.K., Umeda, K., Kimura, M., Ohsumi, M., Tamai, K., Matsuura, A., Amino-terminal domain of ATRIP contributes to intranuclear relocation of the ATR-ATRIP complex following DNA damage (2004) *FEBS Lett.*, 577, pp. 289-293
22. Itakura, E., Umeda, K., Sekoguchi, E., Takata, H., Ohsumi, M., Matsuura, A., ATR-dependent phosphorylation of ATRIP in response to genotoxic stress (2004) *Biochem. Biophys. Res. Commun.*, 323, pp. 1197-1202
23. Itakura, E., Sawada, I., Matsuura, A., Dimerization of the ATRIP protein through the coiled-coil motif and its implication to the maintenance of stalled replication forks (2005) *Mol. Biol. Cell*, 16, pp. 5551-5562
24. Karimi, M., Inze, D., Depicker, A., Gateway vectors for *Agrobacterium*-mediated plant transformation (2002) *Trends Plant Sci.*, 7, pp. 193-195

25. Kitamura, S., Shikazono, N., Tanaka, A., TRANSPARENT TESTA 19 is involved in the accumulation of both anthocyanins and proanthocyanidins in Arabidopsis (2004) *Plant J.*, 37, pp. 104-114
26. Konieczny, A., Ausubel, F.M., A procedure for mapping Arabidopsis mutations using co-dominant ecotype specific PCR-based markers (1993) *Plant J.*, 4, pp. 403-410
27. Liu, Z., Hall, J.D., Mount, D.W., Arabidopsis UVH3 gene is a homolog of the *Saccharomyces cerevisiae* RAD2 and human XPG DNA repair genes (2001) *Plant J.*, 26, pp. 329-338
28. Lupas, A., Van Dyke, M., Stock, J., Predicting coiled coils from protein sequences (1991) *Science*, 252, pp. 1162-1164
29. Nakajima, S., Sugiyama, M., Iwai, S., Hitomi, K., Otoshi, E., Kim, S.-T., Jiang, C.-Z., Yamamoto, K., Cloning and characterization of a gene (UVR3) required for photorepair of 6-4 photoproducts in *Arabidopsis thaliana* (1998) *Nucleic Acids Res.*, 26, pp. 638-644
30. Orren, D.K., Petersen, L.N., Bohr, V.A., A UV-responsive G2 checkpoint in rodent cells (1995) *Mol. Cell. Biol.*, 15, pp. 3722-3730
31. Paciotti, V., Clerici, M., Lucchini, G., Longhese, M.P., The checkpoint protein Ddc2, functionally related to *S. pombe* Rad26, interacts with Mec1 and is regulated by Mec1-dependent phosphorylation in budding yeast (2000) *Genes Dev.*, 14, pp. 2046-2059
32. Rahman, A., Nakasone, A., Chhun, T., Ooura, C., Biswas, K.K., Uchimiya, H., Tsurumi, S., Oono, Y., A novel small acidic protein 1 (SMAP1) mediates responses of the *Arabidopsis* root to the synthetic auxin 2,4-dichlorophenoxyacetic acid (2006) *Plant J.*, 47, pp. 788-801
33. Sakamoto, A., Lan, V.T.T., Hase, Y., Shikazono, N., Matsunaga, T., Tanaka, A., Disruption of the AtREV3 gene causes hypersensitivity to ultraviolet B light and ??-rays in *Arabidopsis*: Implication of the presence of a translesion synthesis mechanism in plants (2003) *Plant Cell*, 15, pp. 2042-2057
34. Schiffer, M., Edmundson, A.B., Use of the helical wheels to represent the structure of protein and to identify segments with helical potential (1967) *Biophys. J.*, 7, pp. 121-135
35. Shikazono, N., Suzuki, C., Kitamura, S., Watanabe, H., Tano, S., Tanaka, A., Analysis of mutations induced by carbon ions in *Arabidopsis thaliana* (2005) *J. Exp. Bot.*, 56, pp. 587-596
36. Siede, W., Friedberg, A.S., Dianova, I., Friedberg, E.C., Characterization of G1 checkpoint control in the yeast *Saccharomyces cerevisiae* following exposure to DNA-damaging agents (1994) *Genetics*, 138, pp. 271-281
37. Sweeney, T.R., Britt, A.B., Culligan, K.M., The *Arabidopsis* ATRIP ortholog is required for programmed response to replication inhibitors (2009) *Plant J.*, , doi:
38. Takahashi, S., Sakamoto, A., Sato, S., Kato, T., Tabata, S., Tanaka, A., Roles of *Arabidopsis* AtREV1 and AtREV7 in translesion synthesis (2005) *Plant Physiol.*, 138, pp. 870-881
39. Waterworth, W.M., Altun, C., Armstrong, S.J., Roberts, N., Dean, P.J., Young, K., Weil, C.F., West, C.E., NBS1 is involved in DNA repair and plays a synergistic role with ATM in mediating meiotic homologous recombination (2007) *Plant J.*, 52, pp. 41-52
40. Zhao, H., Piwnica-Worms, H., ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1 (2001) *Mol. Cell. Biol.*, 21, pp. 4129-4139

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