Radioprotection, vol. **44**, n° 5 (2009) 887–890

DOI: 10.1051/radiopro/20095158

Genomic and proteomic analyses of plant response to radiation in the environment – an abiotic stress context

N.J. Willey, Y.-J. Heinekamp and A. Burridge

Centre for Research in Plant Science, Faculty of Health and Life Sciences, University of the West of England, Coldharbour Lane, Frenchay, Bristol BS16 1QY, UK
e-mail: Neil.Willey@uwe.ac.uk

Abstract. Genomic and proteomic techniques provide the opportunity to investigate plant response to ionising radiation in unprecedented detail. Understanding plant molecular responses to ionising radiation might be useful for radioprotection but also for understanding plant stress responses. This is because radioactivity was a primordial stressor to cells and many stress responses are highly conserved through evolution. DNA microarrays for *Arabidopsis* plants exposed to 40 μGy h^{-1} through a hydroponic solution revealed that, after 14 days, there are changes in gene expression primarily in roots. The genes that change are not associated with DNA repair, and correlations with responses to other stressors in public databases suggest that there are elements of plant stress response being activated. The number of genes and their fold changes are lower than those reported for many other stressors but have particular overlaps with oxidative stress responses. Proteomic analyses form similar experiments are ongoing but similarly show no change of abundance in proteins associated with DNA repair and more changes in roots than shoots at these exposures.

1. INTRODUCTION

Environmental radioactivity was a primordial stressor to living cells. Earth's early environment produced β/γ doses to living organisms about 5 times higher than today [1]. In addition, at various periods of Earth's history other sources of radiation such as cosmic radiation, cosmic γ-ray bursts and UV-C have probably all contributed to background ionising radiation doses [2]. Assuming that background doses of ionising radiation have always been spatially variable, at various times and places during the evolution of life background radiation probably produced doses equivalent to those at sites now regarded as radioactively contaminated.

Cells from across all the kingdoms of organisms have an identifiable cellular stress response in common – a vestige of their shared ancestry [3]. Driven by concern about anthropogenic changes in the global environment, the genomic and proteomic response of cells to abiotic stress is now an active area of research. Techniques are now readily available for analyses of whole genome and whole proteome responses to environmental variables. This provides an opportunity not only to dissect genomic and proteomic responses to ionising radiation but also to assess whether radioactivity might have had a role in the evolution of cellular stress responses. If there are links between response to radiation stress and those induced by other abiotic factors it might be very revealing for studies investigating the response of organisms to multiple stressors. Such knowledge might be useful for assessing the risk that ionising radiation poses to flora in the context of other stress responses.

Here we report some results from genomic and proteomic analyses of roots and shoots of *Arabidopsis* exposed to doses of radiation in the range of those at contaminated sites and the environment of the early Earth. We reveal similarities in response to other stressors, especially those with responses mediated via oxidative signalling, and propose a stress-response context within which to view response to ionising radiation. We note that many genes with altered expression in response to radiation are part of fundamental stress responses.

2. MATERIALS AND METHODS

A. thaliana Col-0 (NASC N1092) wild-type plants were grown in non-aerated hydroponics for 14 days in 1/4 strength nutrient solution [4] then for 14 d in nutrient solution plus 10 μM CsCl (control) or 10 μM CsCl + 90 kBq L^{-1} ^{137}Cs (irradiated). Plants were grown in growth cabinets with 16/8 h day/night, *ca*. 250 μ mol m^2 s^{-1} PAR, 23 °C and 80% humidity. Roots and shoots were harvested separately and pooled from different tanks and blocks to provide three root and three shoot samples for controls and treatments. RNA was extracted using standard Trireagent protocols. Gene expression was analysed using the NASC Affymetrix 'ATH1' Microarray service (http://arabidopsis.info/). The ATH1 micorarray has probes for $> 24,000$ genes. Significance Analysis of Microarrays (SAM 2.2; http://www-stat.stanford.edu/~tibs/SAM/; [5]) was used to describe differences in gene expression between shoot and root. GeneSpring GX was used to analyse changes in individual gene expression with thresholds of < 0.67 and > 1.5 and $P < 0.05$ for T-test.

3. RESULTS

Background radiation dose for control plants was *ca.* 0.5 μGy h^{-1} and external dose to irradiated plant roots *ca.* 40 μGy h^{-1}. Of the approximately entire genome of 24,000 genes SAM revealed differences in expression between shoot and root in 11, 096 genes, 7,393 greater in shoot than root, 3,703 less in shoot than root, at a false discovery rate of 1.1% (Figure 1).

Analysis of individual gene expression using Genespring revealed that the 14 d dose at 40 μGy h^{-1} had significantly changed the expression of some genes, primarily in the roots (Figure 2). More genes increased in expression than decreased. Of the genes associated with known proteins some of those that decreased on exposure to 40 μGy h^{-1} are genes ordinarily associated with increased expression in response to anoxia (e.g. pyruvate decarboxylase, ACC oxidase), whilst a number of genes associated with oxidative stress (e.g. peroxidases) had increased expression.

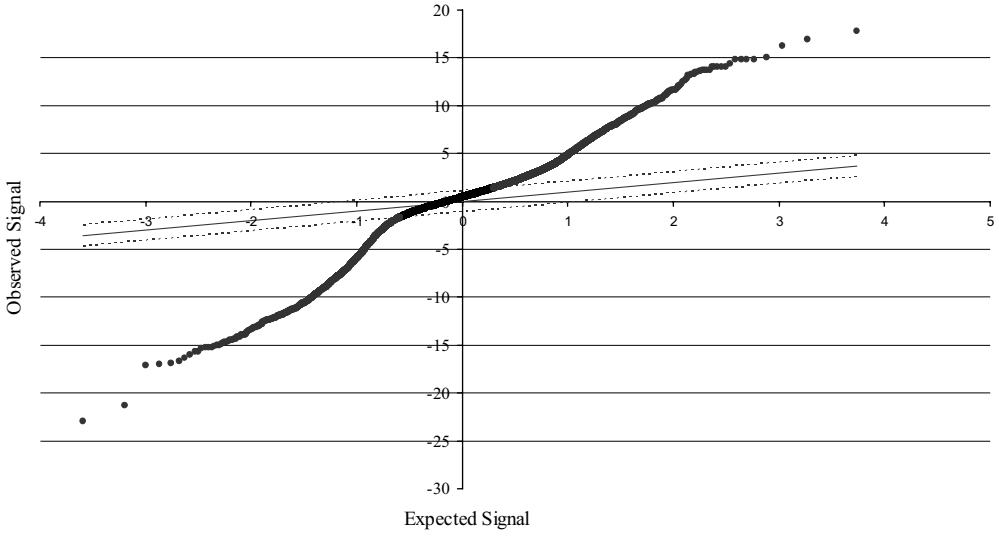

Figure 1. Statistical Analysis of Microarray (SAM) output for differences in gene expression between root and shoot of control plants. Each of $> 24,000$ genes is represented, many having observed expression in shoots that is higher or lower than expected when compared to roots.

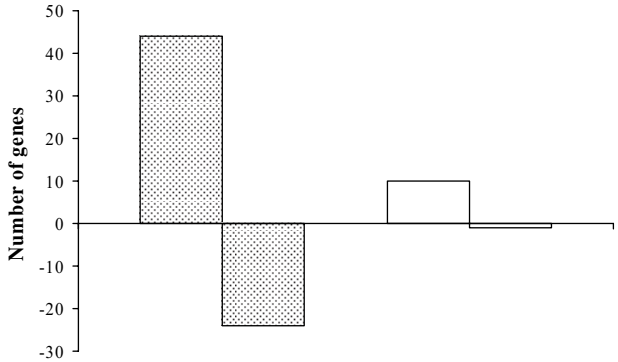

Figure 2. Number of genes with changed expression out of 24,000 after 14 d exposure to 40 μGy h^{-1} ionising radiation dose. Positive values = increase in expression, negative values = decrease in expression. Shaded bars = roots, non-shaded bars = shoots.

Analysing expression using gene ontological (GO) categories related to DNA repair plus genes previously reported to be responsive to high doses of ionising radiation revealed that at 40 μG h^{-1} there were no detectable changes in the expression of genes associated with DNA repair (Figure 3) in roots or shoots.

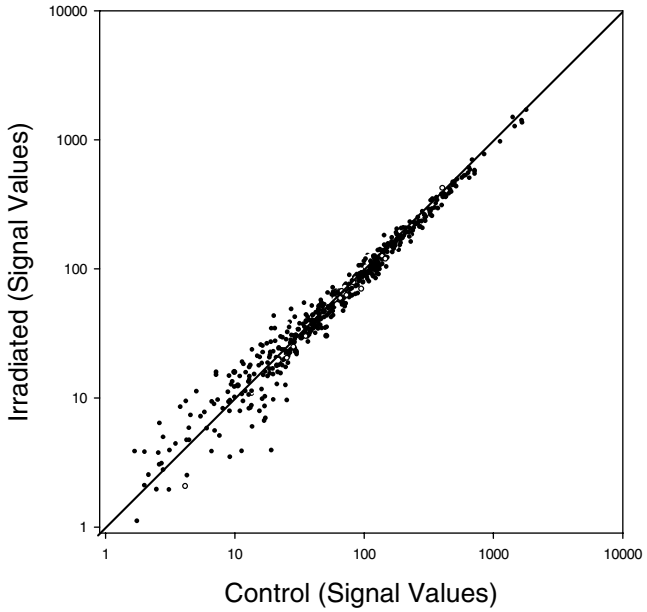

Figure 3. Mean signal values for gene ontologies (GO) associated with DNA repair [GO:0000074 (regulation of cell cycle), n = 44; GO:0000166 (nucleotide binding), n = 120, GO:0003676 (nucleic acid binding), n = 131, GO:0003677 (DNA binding), n = 319, GO:0006281 (DNA repair), n = 13] or genes reported to be responsive to radiation and involved in DNA repair [poly(ADP-ribose)polymerases (PARPs) (At2g31320, At4g02390, At5g22470); ataxia-telangiectasia mutated protein (ATM) (At3g48190); DNA ligases (At5g57160, At1g49250, At1g08130, At1g66730); RAD proteins (At2g31970, At5g16270, At5g16630, At1g16190, At1g79650, At5g38470), n = 14] in controls and plants irradiated with chronic low-level radiation (40 μGy h^{-1} external dose).

The Genevestigator database of microarrays (https://www.genevestigator.ethz.ch) includes *A. thaliana* with many 100s for stress responses. Using the genes that changed in expression in

our irradiation experiments, correlations of expression changes from more than 800 microarrays in 6 different stress categories (chemical, nutrient, biotic, abiotic, hormonal, light) showed the most similarity to abiotic stress ($R = 0.59$, $P = 0.00$).

Proteomic investigations with *A. thaliana* similarly exposed to ionising radiation have now indicated that changes in the proteome of similarly exposed plants occur primarily in the root rather than shoot. Further proteomic experiments with time courses of exposure of up to 40 μGy h^{-1} indicate that at this exposure changes in the proteome are detectable after a few hours.

4. DISCUSSION AND CONCLUSIONS

A chronic 14 d exposure to 40 μGy h^{-1} ionising radiation in nutrient solution from the β/γ emitter ^{137}Cs results in detectable changes in gene and protein expression in *A. thaliana*. These occur primarily in the roots. None of the genes with changed expression or proteins with changed abundance have ontologies associated with DNA repair but some are genes normally associated with plant response to altered oxygen status. There is similarity between how these altered genes change with ionising radiation and abiotic stress. Some of these changes and similarities seem likely to be detectable at the proteomic as well as the genomic level. The snapshot of gene expression provided by microarrays therefore indicates that after 14 d of 40 μGy h^{-1}the gene expression profile might best be viewed within the context abiotic stress responses in plants.

Plant genomic and proteomic responses to abiotic stress are rapidly being described, not just for *A. thaliana* but numerous other species too. Some of the genes that respond to low level ionising radiation in *A. thaliana* are also involved in responses to abiotic stresses. However, the number of genes with changed expression in response to low level irradiation seems much less than the many 100s commonly reported in response to other abiotic stresses – although many microarray stress experiments use quite extreme exposures. Gene expression can differ greatly between plant parts (Figure 1) and with conditions. Further, the fold changes in gene expression produced by exposure to 40 μGy h^{-1} seem lower than those frequently lodged in databases for other stressors. So, chronic low level irradiation has a detectable affect on gene expression and abiotic stress, rather than DNA repair, might be the most appropriate physiological context within which to understand it. It seems unlikely that, in general, background radiation has been a major driver in the evolution of stress responses although much higher than average background doses which have occurred at particular times or places might have played a part.

These results provide: a) a useful perspective on the effects of doses to flora, especially in an era of environmental change, b) some of the molecular data necessary for attempts to integrate effects of radiation from the molecular to the ecological scale, and c) an expanded context in which to view the environmental risks of radiation.

References

[1] Karam P.A. and Leslie S.A. (1999). Calculations of background β-γ radiation dose through geologic time. *Health Physics*. **77**, 662–667.

[2] Karam P.A. (2003) Inconstant sun: How solar evolution has affected cosmic and ultraviolet radiation exposure over the history of life on Earth. *Health Physics* **84**, 322–333.

[3] Kultz D. (2005) Molecular and evolutionary basis of the cellular stress response. *Annual Review of Physiology*. **67**, 225–257.

[4] Arteca R.N. and Arteca J.M. (2000) A novel method for growing *Arabidopsis thaliana* plants hydroponically. *Physiologia Plantarum* **108**, 188–93.

[5] Tusher V.G., Tibshirani R. and Chu G. (2001) Significance analysis of microarrays applied to the ionising radiation response. *Proceedings of the National Academy of Sciences on the USA* **98**, 5116–5121.