STRONG MAGNETIC FIELD INDUCED CHANGES OF GENE EXPRESSION IN ARABIDOPSIS

Anna-Lisa Paul¹, Robert J. Ferl¹, Bernhard Klingenberg², James S. Brooks³,
A. Nicole Morgan⁴, June Yowtak⁴, and Mark W. Meisel⁴

¹*Department of Horticultural Sciences and The Biotechnology Program,
University of Florida, Gainesville, FL 32611-0690, USA*

²*Department of Statistics, Institute of Food and Agricultural Sciences,
University of Florida, Gainesville, FL 32611-0339, USA*

³*Department of Physics and National High Magnetic Field Laboratory,
Florida State University, Tallahassee, FL 32306-4005, USA*

⁴*Department of Physics and National High Magnetic Field Laboratory,
University of Florida, Gainesville, FL 32611-8440, USA*

We review our studies of the biological impact of magnetic field strengths up to 30 Tesla on transgenic arabidopsis plants engineered with a stress response gene consisting of the alcohol dehydrogenase (Adh) gene promoter driving the β -glucuronidase (GUS) gene reporter. Field strengths in excess of 15 Tesla induce expression of the Adh/GUS transgene in the roots and leaves. Microarray analyses indicate that such field strengths have a far reaching effect on the genome. Wide spread induction of stress-related genes and transcription factors, and a depression of genes associated with cell wall metabolism are prominent examples.

1 Motivation

Since earth-based, low-gravity environments are restricted to durations of less than several seconds [1], we have investigated the possibility of using magnetic levitation as a reduced gravity environment appropriate for long-duration (i.e. up to several hours) studies of plant gene expression [2,3]. The possibility of using magnetic levitation to mimic a reduced gravity environment has been explored in a variety of systems [3-7]. During our preliminary studies involving transgenic arabidopsis, we discovered that the plants were stressed by the presence of a strong, static, non-gradient magnetic field [2]. These preliminary, qualitative observations have now been corroborated by a series of systematic, quantitative studies [8].

The possibility that strong, static (non-gradient) magnetic fields might have an influence on biological processes has been discussed for many years [9-11], including a recent report that implicates high magnetic fields in alterations of the cleavage plane during cell division [12]. Nevertheless, the common viewpoint is that presently achievable static magnetic fields do not have a lasting effect on biological systems [11]. Indeed, magnetic resonance imaging (MRI), utilizing static magnetic fields up to 12 Tesla, is a powerful tool for non-invasive in vivo imaging at the molecular level [13-15]. The demands for more precise in vivo imaging have driven the field strengths progressively higher (approaching 20 Tesla) [16], yet information regarding the biological impact of exposing metabolically active cells to fields of this magnitude is limited. We have recently reported about the effect of high magnetic fields on the gene expression profile of the plant *Arabidopsis thaliana* [8].

2 The Plants

Our research efforts employ transgenic Arabidopsis that had been engineered with a gene reporter shown to be induced by a spaceflight environment (TAGES - Transgenic Arabidopsis Gene Expression System) [17]. The TAGES Arabidopsis plants are engineered with the GUS (β -glucuronidase) reporter gene driven by the alcohol dehydrogenase (Adh) gene promoter [17]. The Adh promoter responds to a variety of environmental stresses (e.g. hypoxia, cold, abscisic acid) [18], which in turn initiates transcription of the GUS reporter gene. GUS expression can be monitored qualitatively, by histochemical staining, and quantitatively, by biochemical assays of the gene product. The magnetically levitated plants showed evidence of reporter gene activation, however, the control plants placed in the static magnetic field (19 Tesla) showed similar patterns of transgene expression [2].

These observations lead to the design of additional experiments using transgenic plants as biomonitors of the effects of high magnetic fields in metabolically active tissues. The evaluation of global changes in the Arabidopsis genome in response to exposure to high magnetic fields was facilitated with the Affymetrix® GeneChip® arrays. These arrays allow for the survey of over 8000 genes at a time and were used for genome-wide characterization of the effects of exposing Arabidopsis plants to a field strength of 21 Tesla. The resulting differential patterns of gene expression from the array data were then used to guide quantitative analyses of gene expression with Real-Time, quantitative RT PCR. This approach is an effective means of characterizing an abiotic stress response [19].

3 Results and Discussion

Of the 8000 genes surveyed, there were 112 genes that were differentially expressed to a degree greater than 2.5 fold over the control, Figure 1. Many genes associated with a variety of stress responses were induced (heat, cold, drought, touch), as were genes encoding proteins that are involved with ion transport functions (chloride, sulfate, ammonium). The down regulated set included a number of genes involved in cell wall biosynthesis (e.g. Xtr7). A final, large category is populated by genes that encode transcription factors (e.g. Athb12). Additional experiments are under development to address the mechanism by which high magnetic fields induce changes in gene expression patterns in Arabidopsis.

In conclusion, magnetic fields above 15 Tesla induce gene expression responses in Arabidopsis plants, and a detailed presentation of our work is given elsewhere [8]. These data provide evidence for the perturbation of metabolic processes in the presence of strong magnetic fields and may be useful for guiding future research aimed at determining safe exposure standards for living organisms.

Acknowledgements

This work was supported, in part, by the National Science Foundation and the State of Florida through support and operation of the National High Magnetic Field Laboratory (NHMFL), the NHMFL In-House Research Program, and the NHMFL Research Experience

for Undergraduates. We acknowledge useful conversations with S. J. Hagen, T. H. Mareci, and A. S. Edison.

Figure 1. Levels of differentiated gene expression between the control and treatment at 21 Tesla. The bars represent fold-change differences for representative genes from the Affymetrix® AtH1 array, and genes are clustered with regard to metabolic function.

References

1. "NASA Reduced-Gravity Carriers for Experiment Operations", Microgravity Research Program Office, NASA, Marshall Space Flight Center, http://microgravity.nasa.gov/NASA_Carrier_User_Guide.pdf.
2. Stalcup T.F., Reavis J.A., Brooks J.S., Paul A.-L., Ferl R.J., Meisel M.W. 1999. Transgenic arabidopsis plants as monitors of low gravity and magnetic field effects. In: Fisk Z., Gor'kov L., Schrieffer R., editors. *Physical Phenomena in High Magnetic Fields - III*. Singapore: World-Scientific, pp. 659-662.
3. Brooks J.S., Reavis J.A., Medwood R.A., Stalcup T.F., Meisel M.W., Steinberg E., Arnowitz L., Stover C.C., Perenboom J.A.A.J. 2000. New opportunities in science, materials, and biological systems in the low-gravity (magnetic levitation) environment. *Appl. Phys.* **87**(9), pp.6194-6199.
4. Beaugnon E., Tournier R. 1991. Levitation of organic materials. *Nature* **349**, p.470.
5. Valles J.M., Jr., Lin K., Denegre J.M., Mowry K.L. 1997. Stable magnetic field gradient levitation of *Xenopus laevis*: toward low-gravity simulation. *Biophys. J.* **73**(2), pp. 1130-1133.
6. Geim A.K., Simon M.D., Boamfa M.I., Heflinger L.O. 1999. Magnetic levitation at your fingertips. *Nature* **400**, pp. 323-324.
7. Valles J.M., Jr., Guevorkian K. 2002. Low gravity on earth by magnetic levitation of biological material. *J. Gravit. Physiol.* **9**(1), pp. 11-14.

8. Paul, A.-L., Ferl, R.J., Klingenberg, B., Brooks, J.S., Morgan, A.N., Yowtak, J., Meisel, M.W., preprint.
9. Maret G., Dransfeld K. 1985. Biomolecules and polymers in high steady magnetic fields. In: Herlach F., editor. Strong and Ultrastrong Magnetic Fields and Their Applications - Topics in Applied Physics. Berlin: Springer-Verlag, pp. 143-204.
10. Maret G. 1990. Recent biophysical studies in high magnetic fields. *Physica B* **164**(1-2), pp. 205-212.
11. Schenck J.F. 1998. MR safety at high magnetic fields. *Magn. Reson. Imaging Clin. N. Am.* **6**(4), pp. 715-730.
12. Denegre J.M., Valles J.M., Jr., Lin K., Jordan W.B., Mowry K.L. 1998. Cleavage planes in frog eggs are altered by strong magnetic fields. *Proc. Natl. Acad. Sci. USA* **95**(25), pp. 14729-14732.
13. Ichikawa T., Hogemann D., Sacki Y., Tyminski E., Terada K., Weissleder R., Chiocca E.A., Basilion J.P. 2002. MRI of transgene expression: correlation to therapeutic gene expression. *Neoplasia* **4**(6), pp. 523-530.
14. Louie A.Y., Huber M.M., Ahrens E.T., Rothbacher U., Moats R., Jacobs R.E., Fraser S.E., Meade T.J. 2000. In vivo visualization of gene expression using magnetic resonance imaging. *Nat. Biotechnol.* **18**(3), pp. 321-325.
15. Weissleder R., Moore A., Mahmood U., Bhorade R., Benveniste H., Chiocca E.A., Basilion J.P. 2000. In vivo magnetic resonance imaging of transgene expression. *Nat. Med.* **6**(3), pp. 351-355.
16. Lin Y., Ahn S., Murali N., Brey W., Bowers C.R., Warren W.S. 2000. High-resolution, >1 GHz NMR in unstable magnetic fields. *Phys. Rev. Lett.* **85**(17), pp. 3732-3735.
17. Paul A.-L., Daugherty C.J., Bihn E.A., Chapman D.K., Norwood K.L., Ferl R.J. 2001. Transgene expression patterns indicate that spaceflight affects stress signal perception and transduction in arabidopsis. *Plant Physiol.* **126**(2), pp. 613-621.
18. Dolferus R., Jacobs M., Peacock W.J., Dennis E.S. 1994. Differential interactions of promoter elements in stress responses of the Arabidopsis Adh gene. *Plant Physiol.* **105**(4), pp. 1075-1087.
19. Paul A.-L., Schuerger A.C., Popp M.P., Richards J.T., Manak M.S., Ferl R.J. 2004. Hypobaric biology: Arabidopsis gene expression at low atmospheric pressure. *Plant Physiol.* **134**(1), pp. 215-223.