[Plant Signaling & Behavior 3:6, 383-385; June 2008]; ©2008 Landes Bioscience

Article Addendum

A possible role for extra-cellular ATP in plant responses to high frequency, low amplitude electromagnetic field

David Roux,[1] Catherine Faure,[1] Pierre Bonnet,[2] Sébastien Girard,[2] Gérard Ledoigt,[1] Eric Davies,[3] Michel Gendraud,[4] Françoise Paladian[2] and Alain Vian[1,*]

[1]EA 3296 ERTAC; [2]UMR CNRS 6602 LASMEA; Université Blaise Pascal; Aubrière, France; [3]North Carolina State University; Deptartment of Plant Biology; Raleigh, North Carolina USA; [4]Université Blaise Pascal; Aubrière, France

Abbreviations: AEC, adenylate energy charge; CCCP, carbonyl cyanine 3-chlorophenylhydrazone; eATP, extra-cellular ATP; EMF, electromagnetic field

Key words: EMF, ATP, eATP, tomato, stress

In parallel to evoking the accumulation of stress-related transcripts, exposure to low level 900 MHz EMF affected the levels of ATP, the main energy molecule of the cell. Its concentration dropped rapidly (27% after 30 min) in response to EMF exposure, along with a 18% decrease in the adenylate energy charge (AEC), a good marker of cell energy status. One could interpret this decrease in ATP and AEC in a classical way, i.e., as the result of an increase in cellular energy usage, but recent work brings exciting new insights in pointing out a signalling function for ATP, especially in the stress physiology context where it could trigger both reactive oxygen species and calcium movement (this latter being involved in plant responses to EMF exposure). In this addendum, we discuss our results within this new perspective for ATP function.

The use of wireless devices has caused a massive increase in high frequency EMF (900–2400 MHz) in the environment that has long been suspected for its possible impact on living organisms.[1] Plants have proven to be very useful model systems that undergo physiological modification after exposure to various kinds of EMF.[2,3] Recent publications from our group have pointed out that plants exposed for as little as 10 min to a low-level, high frequency isotropic and homogeneous electromagnetic field (5 $V.m^{-1}$, 900 MHz produced in a Mode Stirred Reverberation Chamber) displayed a rapid and major increase (3–7 fold) in accumulation of stress-related transcripts (bZIP, calmodulin, Pin2, CDPK, etc.).[4,5] The pattern, the amplitudes and the kinetics of these responses were quite similar to those evoked by tissue flaming or crushing.[6,7] These responses occurred systemically (in distant, non-exposed tissue) after local exposure, and were highly dependent on both abscisic and jasmonic acids metabolisms.[8] Furthermore, treatment of plants with calcium counteracting drugs (EGTA, BAPTA and $LaCl_3$) abolished the normal responses of plants to EMF exposure.[9] Taken together, these results strongly suggest that low level, high frequency EMF actually acts as an environmental stimulus able to evoke, in plants, molecular changes that are similar to those induced by injurious treatments.

Of particular importance here and what makes plants fundamentally different from, and more sensitive to EMF than, animals is their high surface to volume ratio. Plants have a high proportion of their above-ground (leaf) cells at the surface, with a direct interface to EMF,[10] and which produce abundant amounts of ATP.

Indeed, most pertinent to this discussion arise from EMF-induced changes in the status of ATP, the major energy-related molecule of the cell that is directly or indirectly involved in most physiological process. In our culture conditions, the terminal leaf of un-stimulated tomato plants contained normal ATP concentration (12–15 $pmol.mg^{-1}$ protein). However, exposure of the plant to EMF caused a large drop (30%) within 30 minutes, leading to an average ATP content of 8–9 $pmol.mg^{-1}$ protein (Fig. 1, left). In parallel, the adenylate energy charge (AEC) also decreased 23% from 0.82 to near 0.63 (60 min), with the latter value being extremely low for an actively growing tissue (Fig. 1, left).

Plant treatment with the uncoupling agent CCCP, that dissipates proton gradients across membranes (thus perturbing the biosynthesis of ATP), totally inhibited the molecular responses that normally occur after irradiation.[9] Thus, it is highly likely that ATP is a critical parameter for processing the plant response to EMF exposure. In this context, it is worth noting that transcript recruitment into polysomes occurred later in the response cascade than changes in ATP, strongly implying that ATP is indeed involved in earlier events.

Similar decreases (about 30%) in ATP content have been noted after different kinds of environmental stimulus in various organisms such as slime-molds[11] or mammals[12] after EMF exposure, or in plants after wounding.[13] These decreases were interpreted as an EMF-induced reduction in ATP synthesis and/or an increase in cell

*Correspondence to: Alain Vian; UMR CNRS 6602 LASMEA; Université Blaise Pascal; Campus Universitaire des Cézeaux, 63177; Aubière cedex, France; Tel.: 33.473.407.918; Fax: 33.473.407.340; Email: Alain.VIAN@univ-bpclermont.fr

Submitted: 12/03/07; Accepted: 12/07/07

Previously published online as a *Plant Signaling & Behavior* E-publication: http://www.landesbioscience.com/journals/psb/article/5385

Addendum to: Roux D, Vian A, Girard S, Bonnet P, Paladian F, Davies E, Ledoigt G. High frequency (900 MHz) low amplitude (5 V m^{-1}) electromagnetic field: a genuine environmental stimulus that affects transcription, translation, calcium and energy charge in tomato. Planta 2008; 227:883–91; PMID: 18026987; DOI: 10.1007/s00425-007-0664-2.

©2008 Landes Bioscience. Do not distribute.

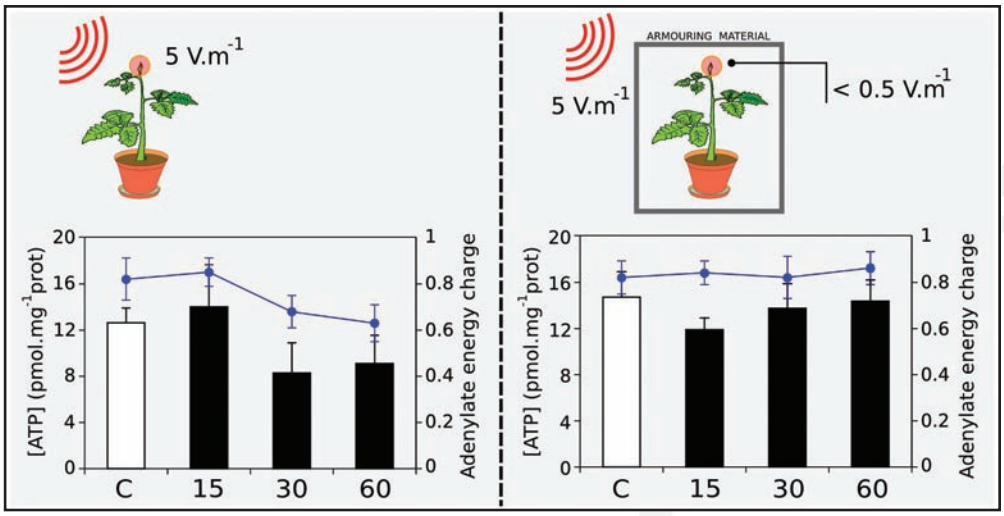


Figure 1. Changes in ATP (boxes) and Adenylate Energy Charge (AEC, lines) after low intensity (10 min, 5 V m^{-1}) high frequency (900 MHz) EMF exposure in a Mode Stirred Reverberation Chamber. Plants were directly exposed to the EMF radiation (left) or protected in an EMF-proof, armoured culture chamber (right), where the EMF amplitude was lower than 0.5 V m^{-1}. The terminal leaf (red circle) was harvested before EMF application (C, white boxes) or 15, 30 and 60 min after the end of the 10 min EMF exposure (15, 30, 60, black boxes) and assayed for energy-related compounds.[9]

energy needs (resulting in more ATP consumption). However, new functions assigned to ATP have brought new insight. In several recent publications, this energy-related nucleoside has been proposed to play an important role as an informative molecule outside the plant cell (i.e., extra-cellular ATP),[14,15] leading to the "eATP" concept that has been long known in animal physiology.[16,17] This eATP was proposed to act as a major signalling molecule implicated in many aspects of local and systemic stress signaling including calcium movement and induction of reactive oxygen species.[18,19] The intrinsic polar characteristics of ATP make the plasmalemma highly impermeable to this molecule, and strongly question the mechanism of its externalization. Proposed explanations include leakage out of damaged cells at the wound sites and exocytosis.[19] This latter explanation is of great interest in the context of EMF, since exo- and/or endo-cytosis appear to be affected by exposure to electromagnetic radiation.[20] External ATP has been reported to play a role in membrane potential,[21] which may be involved in long distance signalling after a local EMF stimulus in tomato.[8] Thus, it is very tempting to propose an EMF-induced release of ATP from cells and this may account for at least part of the rapid reduction in ATP induced by EMF exposure, since the eATP can be rapidly degraded by ecto-apyrases.[19,22] Extracellular ATP (1–3 mM) has also been implicated in another instance of environmental perception/response, in this case gravity.[23] We propose eATP as a potential player in the plant response to EMF exposure (Fig. 2).

Taken together, these data raise the question of whether ATP is released after EMF exposure. Its demonstration would bring exciting new ideas to better understand interactions of living organisms with low amplitude EMF, which is of increasing importance nowadays because of the vast increase in usage of wireless communication devices.

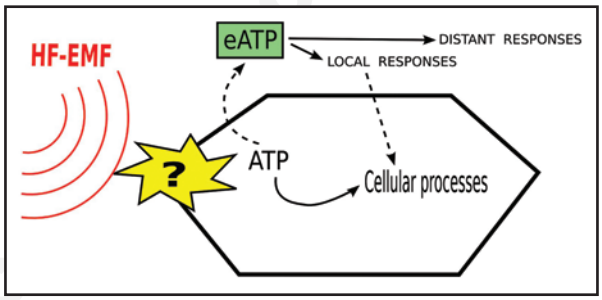


Figure 2. A general scheme proposing a role for external ATP (eATP) in the response pathways to low level, high frequency EMF exposure. The mechanism for the externalization of the polar ATP molecule has been recently extensively discussed.[19]

©2008 Landes Bioscience. Do not distribute.

References

1. Lacy Hulbert A, Metcalfe J, Hesketh R. Biological responses to electromagnetic fields. FASEB J 1998; 12:395-420.
2. Tafforeau M, Verdus MC, Norris V, White G, Demarty M, Thellier M, Ripoll C. SIMS study of the calcium-deprivation step related to epidermal meristem production induced in flax by cold shock or radiation from a GSM telephone. J Trace Microprobe Techn 2002; 20:611-23.
3. Tafforeau M, Verdus MC, Norris V, White GJ, Cole M, Demarty M, Thellier M, Ripoll C. Plant sensitivity to low intensity 105 GHz electromagnetic radiation. Bioelectromagnetics 2004; 25:403-7.
4. Vian A, Roux D, Girard S, Bonnet P, Paladian F, Davies E, Ledoigt G. Microwave irradiation affects gene expression in plants. Plant Sign Behav 2006; 1:67-70.
5. Roux D, Vian A, Girard S, Bonnet P, Paladian F, Davies E, Ledoigt G. Electromagnetic fields (900 MHz) evoke consistent molecular responses in tomato plants. Physiol Plant 2006; 128:283-8.
6. Vian A, Henry Vian C, Davies E. Rapid and systemic accumulation of chloroplast mRNA binding protein transcripts after flame stimulus in tomato. Plant Physiol 1999; 121:517-24.
7. Stankovic B, Vian A, Henry Vian C, Davies E. Molecular cloning and characterization of a tomato cDNA encoding a systematically wound-inducible bZIP DNA-binding protein. Planta 2000; 212:60-6.
8. Beaubois É, Girard S, Lallechère S, Davies E, Paladian F, Bonnet P, Ledoigt G, Vian A. Intercellular communication in plants: evidence for two rapidly-transmitted systemic signals generated in response to EMF stimulation in tomato. Plant Cell and Environ 2007; 30:834-44.
9. Roux D, Vian A, Girard S, Bonnet P, Paladian F, Davies E, Ledoigt G. High frequency (900 MHz) low amplitude (5 V m^{-1}) electromagnetic: a genuine environmental stimulus that affects transcription, translation, calcium and energy charge in tomato. Planta 2008; 227:883-91.
10. Vian A, Faure C, Girard S, Davies E, Hallé F, Bonnet P, Ledoigt G, Paladian F. Plants do respond to GSM-like radiations. Plant Sign Behav 2007; 2:522-24.
11. Marron M, Goldman E, Greenebaum B, Tipnis P. Effects of sinusoïdal 60 Hz electric and magnetic fields on ATP and oxygen levels in the slime mold, *Physarum polycephalum*. Bioelectromagnetics 1986; 7:307-14.
12. Sanders A, Joines W, Allis J. Effect of continuous wave, pulsed, and sinusoidal-amplitude modulated microwaves on brain energy metabolism. Bioelectromagnetics 1985; 6:89-97.
13. Henry Vian C, Vian A, Dietrich A, Ledoigt G, Desbiez MO. Effect of wounding on nucleotide pools in *Bidens pilosa* L. Biol Plant 1996; 38:191-6.
14. Demidchik V, Nichols C, Oliynyk M, Dark A, Glover BJ, Davies JM. Is ATP a signaling agent in plants? Plant Physiol 2003; 133:456-61.
15. Kim SY, Sigaguru M, Stacey G. Extracellular ATP in plants. Visualization, localization, and analysis of physiological significance in growth and signaling. Plant Physiol 2006; 142:984-92.
16. Novak I. ATP as a signaling molecule: the exocrine focus. News Physiol Sci 2003; 18:12-7.
17. Hanley PJ, Musset B, Renigunta V, Limberg SH, Dalpke AH, Sus R, Heeg KM, Preisig Müller RP, Daut J. Extracellular ATP induces oscillations of intracellular Ca^{2+} and membrane potential and promotes transcription of IL-6 in macrophages. Proc Natl Acad Sci USA 2003; 101:9479-84.

18. Song CJ, Steinebrunner I, Wang X, Stout SC, Roux SJ. Extracellular ATP induces the accumulation of superoxide via NADPH oxidases in Arabidopsis. Plant Physiol 2006; 140:1222-32.
19. Roux S, Steinebrunner I. Extracellular ATP: an unexpected role as a signaler in plants. Trends in Plant Sci 2007; 12:522-27.
20. Mahroura N, Pologea Morarub R, Moisescua MG, Orlowskic S, Levêque P, Mira LM. In vitro increase of the fluid-phase endocytosis induced by pulsed radiofrequency electromagnetic fields: importance of the electric field component. Biochim Biophys Acta 2005; 1668:126-37.
21. Lew RR, Dearnaley JDW. Extracellular nucleotide effects on the electrical properties of growing *Arabidopsis thaliana* root hairs. Plant Sci 2000; 153:1-6.
22. Jeter CR, Tang W, Henaff E, Butterfield T, Roux SJ. Evidence of a novel cell signaling role for extracellular adenosine triphosphates and diphosphates in Arabidopsis. Plant Cell 2004; 16:2652-64.
23. Tang W, Brady SR, Sun Y, Muday GK, Roux SJ. Extracellular ATP inhibits root gravitropism at concentrations that inhibit polar auxin transport. Plant Physiol 2003; 131:147-54.

©2008 Landes Bioscience. Do not distribute.