

# Electromagnetic Fields Act Similarly in Plants as in Animals: Probable Activation of Calcium Channels via Their Voltage Sensor



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**Abstract:** It has been shown that low intensity microwave/lower frequency electromagnetic fields (EMFs) act in animals via activation of voltage-gated calcium channels (VGCCs) in the plasma membrane, producing excessive intracellular calcium  $[Ca^{2+}]_i$ , with excessive  $[Ca^{2+}]_i$  leading to both pathophysiological and also in some cases therapeutic effects. The pathophysiological effects are produced largely through excessive  $[Ca^{2+}]_i$  signaling including excessive nitric oxide (NO), superoxide, peroxynitrite, free radical formation and consequent oxidative stress. The activation of the VGCCs is thought to be produced via EMF impact on the VGCC voltage sensor, with the physical properties of that voltage sensor predicting that it is extraordinarily sensitive to these EMFs. It is shown here that the action of EMFs in terrestrial, multicellular (embryophyte) plants is probably similar to the action in animals in most but not all respects, with calcium channel activation in the plasma membrane leading to excessive  $[Ca^{2+}]_i$ , leading in turn to most if not all of the biological effects. A number of studies in plants are briefly reviewed which are consistent with and supportive of such a mechanism. Plant channels most plausibly to be involved, are the so-called two pore channels (TPCs), which have a voltage sensor similar to those found in the animal VGCCs.



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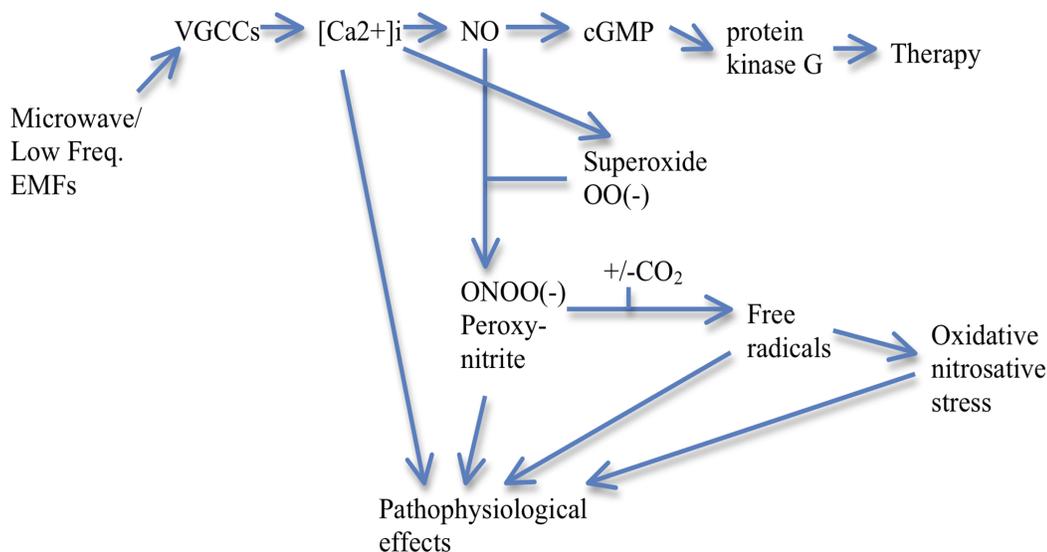
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## INTRODUCTION

Microwave and lower frequency EMFs have been found in animals, to work via activation of voltage-gated calcium channels (VGCCs) [1-4]. Various EMF effects were found to be blocked or greatly lowered by calcium channel blockers [1-3]. Five different calcium channel blockers have been used in these studies, which differ from one another in their structures and sites of action, but are all thought to have high specificity as calcium channel blockers [1-3]. These and other data [1-4],

argue for the centrality of VGCC activation in producing effects of EMFs in animal cells. VGCC activation is thought to act primarily via increased intracellular calcium  $[Ca^{2+}]_i$ , and a wide variety of effects of EMFs in animals are thought to be produced as a consequence of increased  $[Ca^{2+}]_i$  in animal cells [1-4] as diagrammed in Fig. 1. The therapeutic effects of EMFs, such as stimulation of bone growth, are thought to be produced by elevated nitric oxide (NO) and NO signaling [1,5,6]. The pathophysiological effects of EMFs (Fig. 1) are thought to be produced both via excessive  $[Ca^{2+}]_i$  signaling (Fig. 1, center) and also via excessive NO, superoxide, peroxynitrite, free radicals and oxidative stress (Fig. 1, lower right). It will be argued below that pathophysiological EMF

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**Fig. (1).** How Increased Intracellular  $\text{Ca}^{2+}$  Produces Both Pathophysiological and Therapeutic Responses in Animals following EMF Exposure.  $[\text{Ca}^{2+}]_i$ , Intracellular calcium levels; NO, nitric oxide; cGMP = 3',5'- guanosine monophosphate; Protein kinase G = cGMP dependent protein kinase; Peroxynitrite (ONOO(-)); Oxidative Stress, an imbalance between free radicals and other oxidants and antioxidants. Figure taken with permission from Ref. [2].

effects in plants are probably produced as a consequence of very similar mechanisms.

One of the puzzles of the action low intensity microwave frequency EMFs has been how can such very low intensities produce substantial biological effects? The current safety guidelines/standards are based on arguments that there cannot be such effects and only heating effects need be considered. However those arguments have been contradicted by a literature made up of thousands of primary literature citations showing that there are many such non-thermal effects [4], going all the way back to the 1950's and 1960's. Nevertheless, it has been puzzling for many years how such low intensity EMFs can produce biological effects.

Pilla [7] showed that low intensity pulsed microwave EMFs can produce an almost instantaneous increase in  $\text{Ca}^{2+}$ -calmodulin-dependent NO production, all occurring in less than 5 seconds. These observations strongly suggest that low intensity EMFs act directly to activate the VGCCs, because the almost instantaneous increases in both  $[\text{Ca}^{2+}]_i$  and NO leave no time for indirect activation mechanisms. It is known that the VGCCs and also other voltage gated ion channels are controlled by a structure called the voltage sensor [8,9], a structure that contains multiple charges (thought to be 20 charges in the VGCCs [9]). Each of these 20 charges are found on alpha helices

within the lipid bilayer section of the plasma membrane [8,9]. The voltage sensor opens the ion channel due to the action of changes in the electrical force across the plasma membrane acting directly on these 20 voltage sensor charges [8]. The structure of the VGCC voltage sensor is discussed in more detail in the Discussion section below. It is plausible, therefore, that the electrical forces of these low intensity EMFs act through their electrical effects on the voltage sensor to activate the VGCCs. It is predicted that the forces on the 20 charges in the VGCC voltage sensor are highly amplified because of two important factors [2]. The law of physics called Coulomb's law predicts that forces on charged groups are inversely proportional to the dielectric constant of the medium in which the charges occur. Because the dielectric constant of the aqueous phases in the cell or extracellular medium are about 120 times higher than the dielectric constant of the lipid bilayer [2], this predicts that forces on the each of the 20 charges of the voltage sensor are about 120 times higher than are electrical forces on singly charged groups in the aqueous phases. In addition, Sheppard *et al.* [10], predicted that the electrical forces produced by EMFs across the plasma membrane are amplified about 3000-fold compared with the forces in the aqueous phases because of the high electrical resistance of the plasma membrane. It follows from this, that the forces on the voltage sensor are estimated to be vastly increased as compared with

forces on aqueous phase single charges, where most if not all charged groups occur:

$20(\# \text{ of charges in voltage sensor}) \times 120 \text{ (from the dielectric constant)} \times 3000 \text{ (amplification at the plasma membrane)} = 7.2 \text{ million}$

Because of this, the electrical forces placed on the voltage sensor by these EMFs is calculated to be approximately 7.2 million times higher than are the forces placed on singly charged groups located elsewhere in the cell because these singly charged groups are predominantly in the aqueous phase [2]. It is highly plausible, therefore, that this extraordinary sensitivity of the voltage sensor to such weak electrical effects is the final answer to this long puzzle of how such low intensity EMFs can produce biological effects in many animals, including humans.

While there are much fewer data on low intensity microwave EMF effects in plants, what data we have show important similarities both in terms of the probable target in plants and in terms of the consequences of exposure in plants. It is those similarities and the apparent role in plants of calcium channels controlled by a voltage sensor that are the main foci of this paper.

### **Low Intensity Microwave Frequency EMF Effects in Multicellular Embryophyte Plants**

Perhaps the best place to start with plants, is by considering the Beaubois *et al.* study of tomato plants [11]. They [11] looked at the mechanism of microwave EMF-induced stress responses, following up on earlier studies showing increased transcription of certain genes following EMF exposure [11]. Beaubois *et al.* [11], demonstrated a central role of increased  $[Ca^{2+}]_i$  in both direct effects of EMFs on exposed tomato leaves and also in communication of stress signals from such exposed leaves to other shielded parts of the plant. They studied increased transcription of two genes (LebZIP1 and Pin2), in response to 900 MHz EMF exposure, where transcription of each of these genes had been previously shown to increase in response to stressors that increase  $[Ca^{2+}]_i$ . When the whole tomato plant was exposed to 900 MHz radiation, transcript levels in different leaves all increased at times of 0 to 60 minutes following 10 minute EMF exposure. When only one leaf was exposed (other leaves being shielded) the one unshielded leaf responded quickly with the shielded leaves only responding after a 15

minute delay. When the unshielded leaf was sprayed with the calcium chelator EGTA plus the calcium channel blockers  $LaCl_3$ , transcriptional changes were blocked, clearly showing that calcium transport through a calcium channel in the plasma membrane is essential to both the response to the EMF exposure in the unshielded leaf, but also to the delayed response of the shielded leaves as well. The communication to the shielded leaves was shown to involve both abscisic acid (ABA) production and also jasmonic acid (JA) production by the unshielded leaf, communicating the stress to the other leaves. This was shown both by studying mutant plants unable to produce either ABA or JA and also plants treated with naproxen, as specific inhibitor of ABA production. The shielded leaves produced little or no change in gene expression when either ABA or JA production were prevented. Interestingly, both ABA and JA are known to act, at least in part, via elevated  $[Ca^{2+}]_i$ . As stated by Beaubois *et al.* [11] "The effects of decreasing endogenous calcium levels by using EGTA as a chelating agent along with  $LaCl_3$  as a calcium channel blocker, were quite remarkable. Under calcium-depleted conditions, no bZIP transcript accumulation occurred, in either the shielded (distant) leaf or in the directly exposed leaf. The lack of transcript accumulation in the directly exposed leaf is very good evidence of an important role for calcium in gene expression, both in the local (treated) leaf and in the distant (untreated) one..." We will return later to the question of what calcium channel is likely to be activated by EMF exposure.

Roux *et al.* [12] showed that non-thermal 900 MHz exposures of tomato plants produced consistent increases in expression of three stress related genes at 15 minutes following a 10 minute exposure. One of those transcripts, calmodulin-N6 is a major  $[Ca^{2+}]_i$  receptor, causing them to suggest a linkage to "variations in cytoplasmic ...  $Ca^{2+}$  concentrations."

In a subsequent study, Roux *et al.* [13], showed that a low intensity microwave EMF exposure produced rapid (5 to 15 minutes following exposure) increases in transcript levels of 3 genes, each having roles in stress responses in plants. Each of these transcript increases were prevented by shielding the plant from the EMF and also by using either of two calcium chelators (BAPTA or EGTA) or by the calcium channel blocker  $La^{3+}$ . The chelation and calcium channel blocker studies

clearly show that  $\text{Ca}^{2+}$  influx through the plasma membrane produces the transcript changes and argues, therefore, that EMF exposure is acting via activation of a calcium channel in the plasma membrane.

Ripoll *et al.* [14], reviewed evidence for special roles of calcium in responses to several abiotic stimuli in plants, including EMFs. They included in their review, three earlier studies of the same research group showing that non-thermal EMF exposures acted via increased calcium influxes [15-17]. They argued that calcium has a key role in stimulus sensing, possible storage of information and also final expression of effects in the organism. They further state [14] that “there is common agreement that plants react to such stimuli by an almost immediate elevation of the free calcium in the cell cytosol.” Additionally “it was inferred that this elevation of free cytosolic calcium (is) derived from the uptake of external calcium and/or release from internal  $\text{Ca}^{2+}$  stores.” It should be noted that the above-discussed studies [11,13,16] show that initial predominant effects of EMFs are through activation of plasma membrane calcium channels. Ripoll *et al.* [14] also note that “these calcium changes show enormous variability in their nature (transient, sustained or oscillatory), amplitude, kinetics and spatial distribution.”

It can be seen from the previous three paragraphs, that low intensity microwave EMFs act in terrestrial embryophyte plants via activation of calcium channels in the plasma membrane, producing, in turn, large increases in  $[\text{Ca}^{2+}]_i$ . It has also been shown, that plants respond to stressors including elevated sucrose which produce partial depolarization of the plasma membrane which activate voltage regulated calcium channels [18], again producing large increases in  $[\text{Ca}^{2+}]_i$ . Each of these suggest similarities to mechanisms involved in producing the EMF effects, to those found in animal cells -- mechanisms centered on VGCC activation.

Among the important studies cited by Ripoll *et al.* [14] is the study of Kaplan *et al.* [19] showing that in *Arabidopsis*, there are 230 calcium responsive genes, with 162 being upregulated and 68 being downregulated by  $[\text{Ca}^{2+}]_i$  increases.

It will be argued in the Discussion section, that the most plausible targets of EMFs in plants are

the so-called two pore channels (TPCs) [18], with these channels having many similarities to but also differences from the VGCCs in animals (see below).

### Other Studies Consistent with Calcium Increases Include the Following

Pazur and Rassadina [20] used a transgenic *Arabidopsis thaliana* strain carrying the aequorin gene, a gene producing the calcium-dependent bioluminescent protein aequorin to measure increased  $[\text{Ca}^{2+}]_i$  in response to low level 50 Hz sinusoidal EMF exposure. They found rapid increases in cytoplasmic  $[\text{Ca}^{2+}]_i$  levels following exposure onset. Although these increases may have occurred almost instantaneously following exposure, they were only readily apparent after about 2 minutes, possibly because of efficient concentration of  $\text{Ca}^{2+}$  into the vacuole. This study shows that calcium changes in plants are not limited to microwave frequency exposures but also occur from extremely low (50Hz) exposures. This shows another similarity to animals, where many studies have shown that extremely low frequency field, including 50 Hz exposures, are blocked by calcium channel blockers, demonstrating that 50 Hz EMFs in animal cells act via VGCC activation [1]. Pazur and Rassadina suggest that [20] such exposures can produce important  $\text{Ca}^{2+}$  regulatory (“second messenger”) effects.

Shckorbatov *et al.* [21] showed that low intensity 36.6 GHz microwave exposure produced substantial increases in  $[\text{Ca}^{2+}]_i$  in pea root cells, measured via calcium-dependent fluo-3 fluorescence, following exposure.

A number of plant studies showed that plants respond to low intensity microwave EMF exposures in similar ways to the responses in animals, including both cellular DNA damage (genotoxicity) where plants can often be studied more easily than are animals and also oxidative stress (see Gustavino *et al.* [22] for review). Studies have shown that *Vicia faba* (fava bean) [22], *Vigna radiata* (mung bean) [23] and *Lemna minor* (duckweed) [24,25], respond to low intensity exposures to various microwave EMFs by producing oxidative stress responses, similar to those found in animals following EMF exposures [21], suggesting a possibly similar mechanism. In animals oxidative stress responses are thought to be produced by ex-

cessive peroxy nitrite and free radical formation both produced as downstream consequences of excessive  $[Ca^{2+}]_i$  [1,2,]; see Fig. 1.

In terms of DNA damage (genotoxicity), similar changes in plants including single and double stranded DNA breaks and other DNA changes have been widely found in animals [1,2,27-31], and are also found in plants [32-36] where such markers of DNA double strand breaks including chromosomal breaks and micronucleus formation can often be much more easily studied than in animals. These chromosomal changes have been found to occur following low intensity microwave exposures in *Vicia faba* (fava beans) [32], in *Tradescantia* [33], in *allium* (that is onion) root tips [34,35], in *Zea mays* (corn) [35] and in *Lens culinaris* (lentils) [36]. Similar changes in cellular DNA, are thought to be produced in animals via downstream effects of increased  $[Ca^{2+}]_i$  including free radical formation [1,2,27-31] and may, therefore be produced by similar mechanisms in plants.

## DISCUSSION

In animals, low intensity microwave/lower frequency EMFs have been shown to act via activation of voltage-gated calcium channels (VGCCs). This produces excessive  $[Ca^{2+}]_i$  and the downstream effects of such excessive  $[Ca^{2+}]_i$  are thought to produce a variety of pathophysiological effects (Fig. 1). While microwave frequency exposures are of the most environmental concern because of their ever increasing exposure intensities all over the world, low intensity, extremely low frequency EMFs as well as other EMFs/electrical/magnetic fields can also act via VGCC activation [1]. While most of the plant studies involve microwave EMFs, one study showed that 50 Hz (extremely low frequency) EMFs can act in plants via  $[Ca^{2+}]_i$  increases and subsequent calcium effects [20]. As shown above, plants resemble animals by having microwave and extremely low frequency EMFs acting via excessive  $[Ca^{2+}]_i$  and by having such excessive  $[Ca^{2+}]_i$  produced by activation of calcium channels in the plasma membrane. In animals, pathophysiological effects of such EMF exposures often involve excessive calcium signaling (Fig. 1) and the same thing is also true in plants. In animals, EMF exposures produce oxidative stress and also single strand and double stranded breaks in cellular DNA (with the double stranded breaks often being moni-

tored via formation of micronuclei and occasionally via chromosomal rearrangement; these DNA strand breaks are thought to be produced in animals by free radicals produced as breakdown products of peroxy nitrite (Fig. 1) [2,27-30]. Plants are similar to animals in that low intensity EMF exposures also produce oxidative stress and micronuclei and chromosomal changes. It is argued here that it is plausible that the mechanisms for generating these in plants as downstream effects of excessive  $[Ca^{2+}]_i$  may be similar if not identical to the mechanisms involved animals (Fig. 1).

Another critically important observation with regard to the animal studies, is that the activation of the VGCC voltage sensor apparently finally explains how such low intensity EMFs can produce biological effects [2-4]. The reason that this is so important, is that it has long been argued by industry supporters, that there cannot be a biophysical mechanism by which low intensity EMFs can produce biological effects. They acknowledge that it has long been known that microwave EMFs place forces on charged groups and that microwave ovens cook our food by joggling charged groups in our foods back and forth, heating the food and therefore cooking it. However they argue that low intensity EMFs are too weak to produce biological effects because they claim that the forces on such charged groups produced by such low intensity EMFs are too weak to produce biological changes. It is important to note, therefore, that with 20 (there have been some questions whether the actual charge number may be as low as 16 or as high as 24 but I will stay with the 20 figure used earlier [2,9]) positive charges being found in the voltage sensor of the VGCCs and with these charges being found in the lipid bilayer section of the plasma membrane [8], the force of such low intensity EMFs on the voltage sensor is about 7.2 million times the force on individual charged groups found elsewhere in the cell [2; see Introduction, above]. Because of this, the force on the voltage sensor is about 7.2 million times stronger than expected based on industry calculations and consequently we finally have a plausible explanation of how such weak EMFs can produce biological effects. It must be pointed out here, that others have suggested other possible targets for low intensity EMFs. However, with the VGCCs, we have direct, empirical data from the calcium channel blocker studies that these are the actual targets producing EMF

effects, whereas with other proposed targets, there is no such empirical evidence (an exception to this may be the role of magnetite in migrating birds). It seems clear, therefore that in animal cells, we not only have strong empirical evidence that the VGCCs are targets of EMFs, but we have a theoretical basis that provides strong support for why these are the targets – the extraordinary sensitivity of the VGCC voltage sensor to such low intensity EMFs. One of the questions that we will return to later is why it seems to be the VGCCs that are involved in producing the biological effects in animals, when there are similar voltage sensors in voltage-gated sodium, potassium and chloride channels but we see little evidence that activation of those channels have any substantial roles in producing biological responses to low intensity EMFs? This predominance of  $\text{Ca}^{2+}$  ions over other ions also is apparent in plants.

The evidence discussed above in plants provides a similar argument for a very similar mechanism by which low intensity microwave frequency EMFs produce biological effects in multicellular, embryophyte terrestrial plants. There is, however, a difference in the calcium channels in plants as opposed to the VGCCs in animals and there is also a type of confirming information that we have in animals where there is no comparable information for plants.

Monselise *et al.* [37] suggested using alanine accumulation in plants as a measure of cell stress in response to microwave frequency EMFs. However it is the author's view that using transgenic *Arabidopsis* or other plants containing the aequorin gene can allow much easier monitoring of  $[\text{Ca}^{2+}]_i$  which can be used a marker of EMF action (see Pazur and Rassadina [20]). It is the author's view, that using plants rather than animals or animal cells in culture [2,4] as a measure of biological activity of different EMF exposures has substantial merit, as suggested previously [37], given the fact that plants tolerate well a much wider range of temperature and also function well in air, unlike animal cells in culture. However such plant assays must be compared to those of animal cells in culture to determine whether these responses are sufficiently similar to each other for the plants to serve well as a surrogate measure of biological activity in animal cells. Currently, devices producing microwave EMFs are never tested biologically for safety, before they are put out and expose the un-

suspecting public, a major flaw in the whole regulatory system [4].

The apparent central role of the voltage sensor in responding to low intensity, non-thermal EMFs in animals, raises the question of how these voltage sensor-containing four domain channels evolved, how they relate to each other and what these things say about their mechanism of action. There are a number of reviews that have considered the structure and evolution of voltage sensor controlled channels [38,39]. Single domain polypeptides which presumably act as tetrameric structures, occur in bacteria, animals, plants and fungi (including yeasts) [38,39]. This suggests that the domain structure that is essential for both voltage sensor activity and the opening of an ion channel evolved very early in the evolution of life on earth. The two domain and four domain genes and polypeptides, however, occur only in eukaryotic organisms and are presumed to have evolved through tandem duplication within a gene that originally encoded only a one domain peptide. Somewhat surprisingly, four domain polypeptides (and of course genes) occur in a variety of algae, fungi and animals, but have been completely lost in multicellular terrestrial embryophyte plants, such as the plants considered in this review. Thus the plants considered here, evolved from algae containing such four domain peptides and genes, but these have been completely lost in these plants [38,39].

It is the author's view, that the voltage sensor of these channels may play a unique central role in producing biological responses to low intensity microwave/lower frequency EMFs because the special structure of the voltage sensor, causes it to be uniquely sensitive to these weak EMFs, as discussed above. This view needs to be further tested experimentally, of course.

An additional puzzle that must be considered, is why  $\text{Ca}^{2+}$  fluxes have such an important role in both animals and plants in responding to such EMFs? I think that part of the answer is that  $[\text{Ca}^{2+}]_i$  is so important in calcium signaling and that  $[\text{Ca}^{2+}]_i$  is normally maintained at very low levels in most cell types under most conditions, except when brief signaling is needed. In addition, the electrochemical driving force driving  $\text{Ca}^{2+}$  into the cell is very high – it is composed of both the roughly  $10^4$  higher external than internal  $\text{Ca}^{2+}$  (chemical driving force) plus the much higher

electrical driving force because  $\text{Ca}^{2+}$  is a divalent cation, as compared with the other monovalent ions. It is very important for most organisms and tissues to maintain very low  $[\text{Ca}^{2+}]_i$  over extensive time periods to prevent calcium toxicity.

A study providing important information on a channel that probably has a central role in mediating such  $\text{Ca}^{2+}$  fluxes into plants, identified the AtTPC1 channel in *Arabidopsis thaliana* as the main mediator of  $\text{Ca}^{2+}$  influx through the plasma membrane of leaf cells [18]. In this study, transgenic *Arabidopsis* plants were used expressing the calcium sensor protein aequorin, a protein that binds  $\text{Ca}^{2+}$  to produce an easily measured blue luminescence which serves, then, as a measure of  $\text{Ca}^{2+}$  concentration in the cytoplasm (“cytosol”) of the cell. In this study, the  $\text{Ca}^{2+}$  fluxes were produced by depolarization of the plasma membrane produced in turn by high sucrose levels. In Fig. 4 of Furuichi *et al.* [18], they showed that increased expression of the AtTPC1 gene produced greater sucrose-induced  $\text{Ca}^{2+}$  aequorin luminescence and that greatly lowered AtTPC1 expression produced a ten-fold decrease in such luminescence; both of these observations show that AtTPC1 channel is central to producing the  $[\text{Ca}^{2+}]_i$  cytoplasmic increase. Furuichi *et al.* [18] also showed that the AtSUC1 and 2 sucrose symporters each have roles in producing the depolarization causing the  $[\text{Ca}^{2+}]_i$  increases. There are similar genes occurring in other plants as well as similar depolarization-activated  $\text{Ca}^{2+}$  channels in other plants and these are often collectively referred to as TPC channels [38,39]. The AtTPC1 channel in *Arabidopsis* is universally expressed across various tissues in the plant [18].

What can we say about the structure of the AtTPC1 gene and the protein that it encodes? The overall structure of the protein is [18] “similar to half of the general structure of the  $\alpha$ -subunits of voltage-activated (that is gated)  $\text{Ca}^{2+}$  channels.” The VGCCs have four very similar domains, each containing 6 transmembrane  $\alpha$  helices, with the 4<sup>th</sup> helix out of the 6 each containing 5 positive charges [9]. The 4<sup>th</sup> helices in these 4 domains collectively making up the voltage sensor. However the AtTPC1 protein only contains two domains [18], making up half of the structure for the VGCCs but such proteins are thought to act as dimers in the membrane, such that the two identical subunits may act together to function much like a

single VGCC  $\alpha$ -subunit does in animals. The sequence of the AtTPC1 protein (shown in Fig. 1A of [18]), predicts that the first domain 4<sup>th</sup> helix contains 4 positive charges and the second domain 4<sup>th</sup> helix contains 5 positive charges. This predicts that the voltage sensor of the AtTPC1 protein (acting as a dimer), has a total of 18 positive charges, similar but not identical to the 20 positive charges thought to be in the voltage sensor of the animal VGCCs [9]. There is a 4 domain VGCC-like protein occurring in the yeast *Saccharomyces cerevisiae*, which when knocked out in yeast produces a mutant that grows much more slowly and has less  $\text{Ca}^{2+}$  uptake compared with the wild type. Expression of the AtTPC1 cDNA in the mutant yeast, allows the yeast to grow at almost normal rates and to accumulate  $\text{Ca}^{2+}$  from the medium more normally [18]. Thus apparently, expression of the AtTPC1 cDNA sequence in yeast can allow the plant protein to function almost normally in place the missing VGCC-like protein. While the AtTPC1 protein has not yet been directly tested to determine if it is the main target of these weak EMFs in plants, it seems highly plausible that in plants, as in animals, the unique properties of the voltage sensor causes such  $\text{Ca}^{2+}$  channels to be the main targets of weak EMFs in plants. There is a possibility that cyclic nucleotide gated channels encoded by single domain genes may be a target of EMFs leading to  $\text{Ca}^{2+}$  influx in plant cells; however, these have only weak voltage gating [40] and therefore seem to be unlikely to be effective targets of these EMFs.

In summary, plants resemble animals in their responses to low intensity microwave frequency EMFs as follows:

1. Plants resemble animals in that low intensity microwave EMFs activate one or more plasma membrane calcium channels, allowing calcium influx into the cell, raising  $[\text{Ca}^{2+}]_i$ .
2. Plants resemble animals in that agents which block calcium channels can block responses produced by low intensity EMF exposure.
3. Plants resemble animals in that extremely low frequency EMFs act like microwave EMFs, also by raising  $[\text{Ca}^{2+}]_i$ .
4. Plants resemble animals in that they undergo both oxidative stress and DNA strand breaks, with those strand breaks leading to both for-

mation of micronuclei and to chromosomal rearrangements.

5. Plants resemble animals in that increased  $[Ca^{2+}]_i$  levels following microwave EMF exposure produce many (possibly most) of the biological effects of such EMF exposure.
6. Plants resemble animals in that candidate channels in plants for possibly producing this effect, the TPC channels, contain a voltage sensor that is activated by partial depolarization of the plasma membrane and is predicted to be extremely sensitive to low intensity EMFs because of its structure and physical location in the plasma membrane.

However, the plant channels in #5 above are candidate channels, and have not been clearly shown to have an essential role in producing the  $[Ca^{2+}]_i$  increases following EMF exposure. There is a simple type of experiment which should be done to determine whether this is correct or not. There are mutants of Arabidopsis which are completely deficient in its TPC function (mutants in the AtTPC1 gene). If the channel protein encoded by this gene is essential to producing responses to EMFs, than those mutants should be unresponsive to EMF exposure.

### CONFLICT OF INTEREST

There is no conflict of interest concerning the materials presented in the article.

### ACKNOWLEDGMENTS

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### REFERENCES

- [1] Pall ML. Electromagnetic fields act via activation of voltage-gated calcium channels to produce beneficial or adverse effects. *J Cell Mol Med* 2013;17:958-65
- [2] Pall ML. Scientific evidence contradicts findings and assumptions of Canadian Safety Panel 6: microwaves act through voltage-gated calcium channel activation to induce biological impacts at non-thermal levels, supporting a paradigm shift for microwave/lower frequency electromagnetic field action. *Rev Environ Health* 2015;30:99-116.
- [3] Pall ML. Microwave frequency electromagnetic fields (EMFs) produce widespread neuropsychiatric effects including depression. *J Chem Neuroanatomy*, 2015 Aug 21. pii: S0891-0618(15)00059-9. doi: 10.1016/j.jchemneu.2015.08.001. [Epub ahead of print]
- [4] Pall ML. How to approach the challenge of minimizing non-thermal health effects of microwave radiation from electrical devices. *Int J Innov Res Engineer Managem (IJIREM)* ISSN: 2350-0557, Volume-2, Issue -5, September 2015; 71-6.
- [5] Pilla AA. Nonthermal electromagnetic fields: from first mes- senger to therapeutic applications. *Electromagn Biol Med* 2013;32:123-36.
- [6] Pall ML. Electromagnetic field activation of voltage-gated calcium channels: role in therapeutic effects. *Electromagn Biol Med* 2014;33:251.
- [7] Pilla AA. Electromagnetic fields instantaneously modulate nitric oxide signaling in challenged biological systems. *Biochem Biophys Res Commun* 2012;426:330-3.
- [8] Catterall WA. Ion channel voltage sensors: structure, function, and pathophysiology. *Neuron* 2010; 67:915-28.
- [9] Dolphin AC. Calcium channel auxiliary  $\alpha 2\delta$  and  $\beta$  subunits: trafficking and one step beyond. *Nat Rev Neurosci* 2012;13:542-55.
- [10] Sheppard AR, Swicord ML, Balzano Q. Quantitative evaluations of mechanisms of radiofrequency interactions with biological molecules and processes. *Health Phys* 2008;95:365-96.
- [11] Beaubois E, Girard S, Lallechere S, Davies E, Paladian F, Bonnet P, Ledoigt G, Vian A. Intercellular communication in plants: evidence for two rapidly transmitted signals generated in response to electromagnetic field stimulation in tomato. *Plant Cell Envir* 2007;30:840-4.
- [12] 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. *Physiologia Plantarum* 2006;128:283-8.
- [13] 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.
- [14] Ripoll C, Le Sceller L, Verdus M-C, Norris V, Tafforeau M, Thellier M. 2009 Memorization of abiotic stimuli in plants: A complex role for calcium. In *Plant-Environment Interactions: From Sensory Plant Biology to Active Plant Behavior*. F. Baluska (Ed.), Springer-Verlag Berlin Heidelberg 2009.
- [15] Tafforeau M, Verdus, MC, Norris V, White GJ, Demarty M, Thelier 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.
- [16] Tafforeau M, Verdus MC, Norris V, Ripoll C. Plant sensitivity to low intensity 105 GHz electromagnetic radiation. *Bioelectromagnetics* 2004;25:403-7.
- [17] Tafforeau M, Verdus MC, Norris V, Ripoll C, Thellier M. Memory processes in the response of plants to environmental signals. *Signaling & Behav* 2006;1:9-14.
- [18] Furuichi T, Cunningham KW, Muto S. A putative two pore channel AtTPC1 mediates  $Ca^{2+}$  flux in Arabidopsis leaf cells. *Plant Cell Physiol* 2001;42:900-5.
- [19] Kaplan B, Davydov O, Knight H, Galon Y, Knight MR, Fluhr R, Fromm H. 2006 Rapid transcriptome changes induced by cytosolic  $Ca^{2+}$  transients reveal ABRE-related sequences as  $Ca^{2+}$ -responsive cis elements in Arabidopsis. *Plant Cell* 18:2733-48.
- [20] Pazur A, Rassadina V. Transient effect of weak electromagnetic fields on calcium ion concentration in Arabidopsis thaliana. *BMC Plant Biol* 2009;9:47.

- [21] Shckorbatov YG, Karazin Kharkov VN, Pasiuga OS, Kovalenko IF, Ryabukha SS. 2013 Effect of microwave radiation of low intensity and magnetic fields on Ca<sup>2+</sup> contents in pea root cells. *Microwave and Telecommunication Technology (CriMiCo)*, 2013 23rd International Crimean Conference, pp 1101-2.
- [22] Gustavino B, Carboni G, Petrillio R, Paoluzzi G, Santovetti E, Rizzoni M. Exposure to 915 MHz radiation induces micronuclei in *Vicia faba* root tips. *Mutagenesis* 2015, Oct. 17, pii,ge017, 1-6 [Epub ahead of print].
- [23] Sharma VP, Singh HP, Kohli RK, Batish DR. Mobile phone radiation inhibits *Vigna radiata* root growth by inducing oxidative stress. *Sci Tot Environ* 2009; 21: 5543-7.
- [24] Tkalec M, Malaric K, Pevalek-Kozlina B. 2005 Influence of 400, 900, 1900 MHz electromagnetic fields on *Lemna minor* growth and peroxidase activity. *Bioelectromagnetics* 2005;26;185-93.
- [25] Tkalec M, Malaric K, Pevalek-Kozlina B. Exposure to radiofrequency radiation induces oxidative stress in duckweed *Lemna minor* L. *Sci Total Environ* 2007; 388: 78-89.
- [26] Yakymenko I, Tsybulin O, Sidorik E, Henshel D, Kyrylenko O, Kyrylenko S. Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. *Electromag Biol Med* 2015;19: 1-16.
- [27] Szabo G, Bährle S. Role of nitrosative stress and poly(ADP-ribose) polymerase activation in myocardial reperfusion injury. *Curr Vasc Pharmacol* 2005; 3: 215-20.
- [28] Moon HK, Yang ES, Park JW. Protection of peroxy-nitrite-induced DNA damage by dietary antioxidants. *Arch Pharm Res.* 2006; 29: 213-7.
- [29] Sakihama Y, Maeda M, Hashimoto M, Tahara S, Hashidoko Y. Beetroot betalain inhibits peroxy-nitrite-mediated tyrosine nitration and DNA strand damage. *Free Radic Res* 2012; 46: 93-9.
- [30] Watanabe N, Miura S, Zeki S, Ishii H. Hepatocellular oxidative DNA injury induced by macrophage-derived nitric oxide. *Free Radic Biol Med* 2001;30:1019-28.
- [31] Inoue S, Kawanishi S. Oxidative DNA damage induced by simultaneous generation of nitric oxide and superoxide. *FEBS Lett* 1995;371:86-8.
- [32] Qian X-W, Luo W-H, Zheng O-X. Joint effects of microwave and chromium trioxide on root tip cells of *Vicia faba*. *J Zhejiang Univers SCIENCE B* 2006; 7: 221-7.
- [33] Haider T, Knasmueller S, Kundi M, Haider M. Clastogenic effects of radiofrequency radiations on chromosomes of *Tradescantia*. *Mutat Res* 1994;324;65-8.
- [34] Tkalec M, Malaric K, Pavlica M, Pevalek-Kozlina B, Vidakovic-Cifrek Z. Effects of radiofrequency electromagnetic fields on germination and root meristem of *Allium Cepa* L. *Mutat Res* 2009; 672: 76-81.
- [35] Pesnya DS, Romanovsky AV. Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in *Allium cepa* test. *Mutat Res* 2013;750:27-33.
- [36] Akbal A, Kiran Y, Sahin A, Turgut-Balik D, Balik HH. Effects of electromagnetic waves emitted by mobile phones on germination of root growth and root tip mitotic division of *Lens culinaris*. *Medik Pol J Environ Stud* 2012;21:23-9.
- [37] Monselise EB, Levkovitz A, Gottlieb HE and Kost D. Bioassay for assessing cell stress in the vicinity of radio-frequency irradiating antennas. *J Environ Monit* 2011;13:1890-1896.
- [38] Verret F, Wheeler G, Taylor AR, Farnham G, Brownlee C. 2010 Calcium channels in photosynthetic eukaryotes: implications for evolution of calcium-based signalling. *New Phytol* 2010; 187: 23-43.
- [39] Wheeler GL, Browlee C. Ca<sup>2+</sup> signalling in plants and green algae – changing channels. *Trends Plant Sci* 2008; 13: 506-14.
- [40] Leng Q, Mercier RW, Hua B-G, Fromm H, Berkowitz GA. Electrophysiological analysis of cloned cyclic nucleotide-gated ion channels. *Plant Physiol* 2002; 128: 400-410.