INFLUENCE OF HF ELECTROMAGNETIC FIELDS ON THE DEVELOPMENT AND THE MOLECULAR BIOLOGY OF THE MOSS *PHYSCOMITRELLA PATENS* AND THE NEMATODE *CAENORHABDITIS ELEGANS*

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Abstract - We propose to investigate the effect of electromagnetic fields on the moss *Physcomitrella patens*, and on the nematode *Caenorhabditis elegans* in the range of 900 MHz – 1 GHz. *Physcomitrella patens* is a plant whose developmental biology and genetics is well known and the nematode *Caenorhabditis elegans* has been completely sequenced and this organism has been and is still the most well known chordate as far as genetics, developmental and behavioural biology are concerned.

1. Introduction

No clear conclusion about possible effects of EMF on health could be put clearly into evidence only by epidemiological studies or in vitro experiments on tissues or cells. The use of well-known complex experimental laboratory organisms is an absolute necessity if we want to make any progress in solving the question of their possible harmful effects or innocuity. We propose therefore to study the effect of EMF on two well-known biological organisms, namely the moss *Physcomitrella patens* and the nematode *Caenorhabditis elegans*.

Physcomitrella patens is a plant whose developmental biology and genetics is well known and which is the object of several private and public programs of functional genomic; it is the only complex pluri-cellular organism showing high frequency homologue recombination of foreign DNA, *P. patens* is therefore susceptible to all reverse genetic approaches that have been applied to baker yeast. The genome of the nematode *Caenorhabditis elegans* has been completely sequenced and this organism has been and is still the most well known chordate as far as genetics, developmental and behavioural biology are concerned.

We propose to study macroscopic aspects of development (such as growth, behaviour, mobility of the worms, growth pattern of plants), as well as relevant molecular aspects such as the integrity of the photosynthetic systems of the plant, the expression of stress related proteins and heat-shock promoters. The appropriate transformed lines are already available for the two chosen organisms. The expression of the "heat shock" protein genes and other stress proteins (oxidative stress) of the nematode *Caenorhabditis elegans*, [1] has been recently investigated; we are planning similar experiments on *P. Patens*. For the analysis of the results morphological studies, an approach including fractal dimensions that has already been tested will be applied.

2. Rationale for the proposed project

Although no clear conclusion about possible effects of EMF on health could be put into evidence by epidemiological or in vitro studies on tissues or cells, the subject, however, is of real importance due to the exponential growth of mobile telephones and base stations. Many countries are defining limits (Ordonnance sur la Protection contre le Rayonnement non Ionisant published by the Office Fédéral pour l'Environnement, la Forêt et le Paysage in Switzerland, controversial discussions in the Italian Parliament) or define preventive recommendations (Recommendation in UK on the limits to the use of mobile phones by children).

This proposal is partially motivated by the recent decision by Food and Drug Administration backed by the CTIA (Cellular Telephone Industry Association) emphasizing the need to work on simple animal, plants and cellular systems [2].

The two biological organisms chosen for this research study are well suited in this respect: *Physcomitrella patens* is a moss whose development is very well known in biology and therefore is the object of several programs of functional genomic (e.g. [3,4]). On the other hand, the *Caenorhabditis elegans* is an extensively studied organism; it has been the object of a recent study on non-thermal effects of microwaves [1] as well as other effects of strong EMF [5].

In a previous research project (1997-2000) financed by the *Électricité Romande*, we have obtained some preliminary results on the effects of low frequency 50 Hz magnetic fields on *Physcomitrella patens* suggesting a certain effect on the moss development, for magnitudes of 1 mT (a value 10 times as large as the ICNIRP and ORNI limits), see [6]. More recent experimental results we have obtained at a lower frequency ($16^{2/3}$ Hz), shows that the moss development appears to be even more negatively affected compared to 50 Hz. This result is in clear contradiction with the exposure limits set in the Federal Ordinance, which at the above-mentioned low frequencies decay as $1/f$.

Although the exact causes for such effects are not established, they seem however to be linked to a modification of some critical internal plant structures as indicated by a negative correlation between the light effects and those of EMF.

Most of the studies dealing with RF radiation consider thermal effects (tissue heating) to be responsible for any biological influence. Exposure limits by international bodies have also been defined according to thermal considerations and expressed in terms of SAR (specific absorption rate). The evaluation of SAR is a very complicated task and can be performed using different approaches

(1) direct measurement of temperature using small probes,
(2) measurement of electric field,
(3) numerical simulations.

The determination of SAR using direct measurements of temperature is subject to important errors, due in particular to heat diffusion [7,8]. The use of field probes [9] and numerical

simulations [10] would result in a more accurate determination of SAR. However, provided the type and the very tiny size of the organisms we have chosen to study in this research, the evaluation of SAR is even a more challenging and difficult task, and none of the above method seems to be well adapted.
The use of proper transgenic constructs in plant or animals should help to clarify the situation and open some new research areas.

3. Goals, expected results

The aim of the present research study is to put into evidence possible non-thermal effects of the EMF on organisms with a reasonable degree of complexity and which can be submitted to strict and reproducible experimental constraints. Such non-thermal effects have been reported but remain still controversial [1]
The parameters that will be studied are of two sorts:

a) morphological parameters such as plant growth pattern, and kinetics aspects of nematode mobility,
b) biochemical effects such as induction of Hs promoters and stress enzymes.

We will try to distinguish between the thermal effects (induction of heat-shock proteins) and the other possible perturbation of the metabolism (general stress effects). The idea is to make correlation analysis between the Hs effects and other possible effects on photosynthesis (kinetics of fluorescence), and other stress responses (developmental parameters). We will look for uncoupling between temperature effects and EMF effects (that is by doing experiments at normal and stress - higher - temperatures). We expect to be able to provide thoroughly quantified and reproducible specific EMF responses, if any. It is clear that we might well reach the term of our research with only a clear thermal response; this will of course not preclude the existence of an unpredictable and unforeseen effect of EMF but this will at least rule out some possibilities. The fact that we will use both a plant and an animal will also increase the significance of our results.

4. Experimental approach

In this study, we propose to use TEM cells, which allow for a good homogeneity of the field. To prevent other RF sources to disturb the experiment, all the TEM cells will be located in a shielded Faraday cage. Two or three plastic Petri dishes will be put inside the working volume of each TEM cell, supplied with a modulated HF sinusoidal source. In order to distinguish between thermal and possible non-thermal effects, we will apply modulated signals with different parameters, but keeping constant the rms value.
Concerning the biological control methods, we plan to use those, which have already been worked out in our study of low frequency magnetic field during the last three years. *P. patens* will be cultivated from spore for up to 4 weeks under the electromagnetic field. Light and temperature conditions are similar to those used already used in previous work [3].
More than 200 plants will be measured for each experimental condition; as variance comparison between samples are of very high value in assessing environmental effects, variance analysis is planned for all experimental setup.
Photosynthesis is expected to be one possible target of EMF on plants. The fast kinetics of photosynthetic fluorescence will be assessed on developed plants (two to three weeks old) by using a “Plant Efficiency Analyzer – PEA” fluorometer
The study of heat shock proteins will be made by isolation of an Hs101 homologue from cDNA and/or gDNA library of *P. patens*. Hs101 has been shown to be essential for thermal resistance in *Arabidopsis*; transgenic plants expressing Hs101 at low level do not survive high temperature stress [11]. By constructing either anti-sense and/or knockout transformant in *P. patens* we hope to be able to reproduce this phenotype. In such case, we would be able to see whether EMF exposure affects transgenic more than wild-type. This should be the case if thermal effects are the main effect of EMF; otherwise we would conclude that EMF is affecting multicellular organisms in a more complex manner.
C. elegans will be synchronized by exposure to low temperature (less than 15°C). After exposition to EMF one day or more, the nematode will be tested for motility in thin agar layers. Animal and animal traces will be monitored by a CCD camera. Speed of the worm [12] and fractal dimensions of the traces will be used for the monitoring of possible EMF effects. We will also measure the overall fertility (egg number) of hermaphrodite worms maintained continuously under EMF.
As far as Hs is concerned we will attempt to reproduce the results published in [1] and to check whether they are correlated with possible effects on mobility parameters.

5. References

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