SECTION 16

Plausible Genetic and Metabolic Mechanisms for the Bioeffects of Very Weak ELF Magnetic Fields on Living Tissues

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Prepared for the BioInitiative Working Group

December 2012

I. INTRODUCTION

A. The "kT Problem"

The biological effects of weak extremely-low frequency (ELF) magnetic fields (MFs) have long been a subject of controversy, with many expressing skepticism as to their very existence: ELF-MFs have lacked a credible mechanism of interaction between MFs and living material.

A prominent conceptual objection has been the "kT problem" (Binhi, 2007). This "problem" can be summarized by the very large ratio between the energy available from a quantum of ELF radiation (2.47×10^{-13} eV) and the thresholds for ionization of atoms (4.34 eV for potassium), chemical activation (~ 0.7 eV), or even the 0.156 eV able to transfer protons across gA channels (Chernyshev, 2002).

What these numbers show is that ELF MFs are certainly not able to have effects through these particular mechanisms, but a detailed theoretical analysis (Binhi, 2007) does not preclude that ELF-MF effects could occur in other ways. MFs can alter the shape of the orbitals of particles without substantially altering their energies, possibly leading to very low thresholds for MF biological effects. Rather than a pure energy problem, as stated above, the true "problem" is to determine if biological structures exist that can be disturbed by very low-amplitude ELF MFs.

II. KEY SCIENTIFIC EVIDENCE

B. Magnetic Sensors

Modern electronics provides interesting examples, such as the MOSFET, where tiny signals can control large energies: a voltage applied to a gate with nominally zero current allows control of substantial drain currents. Biological systems have their own sources of energy, and the MF need only contribute a perturbing influence.

In the context of ELF MF effects, it is useful to examine the transducers of MF-measuring instruments. Induction coils have long been the item of choice for many such instruments, but they suffer from a lack of analogy with possible biological equivalents, in that they gather signal from

substantial surfaces (the coil core), and then concentrate the action of the magnetic flux variations gathered over that considerable area at a single point, the contact of the winding.

Hall-effect probes are closer to the mark, in that they detect the potential difference created by a MF on a current flowing in a semi-conductor. Here, the MF acts to deflect a current flow that is powered by an extraneous source. This device dissociates the energy available from the MF itself from the energy it controls.

Another electronic device even closer to the biological transducer we seek is the Spin Tunnel Junction (Micromagnetics, 2012). Such a junction is made of two ferromagnetic metal layers separated by an insulating barrier of a few nanometers (Fig. 6). If a small voltage is applied across the junction, electrons will tunnel through the barrier, according to the ambient MF. The device's MF sensitivity is based on spin-coherent tunneling: the probability of an electron tunneling across the barrier is dependent on its spin, because an electron of a given spin must tunnel to an unfilled state of the same spin. Even the simplest free-electron descriptions of Spin Polarization and Tunneling MagnetoResistance confirm that junction characteristics are determined not only by the ferromagnetic layers, but depend as well on the properties of the barrier (Tsymbal, 2003). Solid-state Spin Tunnel Junctions can detect MFs as low as 0.26 nT at 60-Hz. What these solid-state devices demonstrate is that very small MFs can have effects within the bulk of materials, and that changes in the properties of insulating materials can affect electron tunneling.

C. Magnetic Fields and Incubators

MF experiments with living cells are immediately faced with a practical problem. Cell culture incubators have within them relatively large MFs, due to their relatively weak attenuation of environmental MFs, and to the necessity of implementing controlled heating, humidity and CO_2 concentration conditions. The first control simulates body temperature, the second avoids osmotic imbalance through evaporation, and the third stabilizes pH values within cell culture media. Table 1 was compiled in a survey of 46 incubators used in research (Su, 2012), and showed that average MFs in water-jacketed CO_2 incubators range from 0.9 to 13 μT.

The reaction of many investigators to this situation has been to compensate for the high backgrounds by using even larger MFs in their experiments. According to the conventional dose-responses expected in Toxicology, the effect of an agent can be detected even in the presence of a background exposure, since the biological response is expected to rise smoothly with dose. Many

investigators must also have felt that more robust data would be obtained using larger exposures, and that background MFs in incubators could be tolerated.

Table 1. Summary MF Table of 46 Surveyed Incubators (in μT).

Brand	Model	Type	Mean	Min	Max	Max Background
New Brunswick	G-25	Shaker	**0.39**	0.2	0.81	2.06*
Chicago Surgical Ele.	N.A.	General	**0.61**	0.25	1.21	3.32*
Forma Scientific	3956	General	**0.76**	0.2	2.64	0.22
Fisher Sci.	Isotemp	General	**0.76**	0.05	1.85	0.32
Fisher Sci.	637D	General	**0.84**	0.22	2.49	0.23
Forma Scientific	3157	CO_2 W	**0.91**	0.11	2.66	1.77*
Thermo Electron	N.A.	Shaker	**0.98**	0.57	1.58	5.86*
Nuaire	US auto flow	CO_2 W	**0.99**	0.4	2.28	1.34*
Thermo Forma	3310	CO_2 W	**1.04**	0.32	3.75	0.68*
Innova New Brunswick	4200	Shaker	**1.17**	0.31	2.97	0.4
Fisher Isotemp	281	General	**1.86**	1.2	2.22	0.47
Baxter	WJ501	CO_2 W	**1.87**	0.77	5.27	1.6*
Sanyo	N.A.	CO_2	**2.77**	0.85	6.72	0.3
New Brunswick	G-25	Shaker	**2.79**	0.42	16.13	0.31
Sanyo O_2/ CO_2	MCO-18M	CO_2	**2.8**	1.48	4.14	0.81*
Sanyo	MCO_19AIC	CO_2	**2.94**	1.63	5.17	3.31*
Sanyo	MCO-20AIC	CO_2	**3.12**	1.22	6.64	6.68*
Hera Cell	240	CO_2	**3.28**	2.36	4.62	1.48*
Baxter	Tempcon	General	**3.36**	0.61	7.43	1*
Innova New Brunswick	4000	Shaker	**3.47**	1.27	9.53	0.36
Hera Cell	N.A.	CO_2	**3.65**	2.68	4.49	0.26*
Thermo Scientific	370	CO_2	**3.84**	1.9	7.01	0.64*
New Brunswick	C25	Shaker	**3.88**	0.33	17.74	0.96*
Thermo Electron	3110	CO_2 W	**3.91**	1.19	8.56	0.92*
Nuaire	Nu4750	CO_2 W	**3.95**	0.77	10.38	0.64*
Thermo Scientific	370	CO_2	**3.99**	2.03	6.25	0.96*
Forma Scientific	3130	CO_2 W	**4.67**	1.53	11.14	1.37*
Forma Scientific	3110	CO_2 W	**5.44**	1.77	12.59	2.42*
Fisher Sci.	546	CO_2 W	**6.58**	2.36	16.88	0.38
Forma Scientific	N.A.(Old)	CO_2	**6.71**	2.32	16.83	1.36*
Thermo Electron	3130	CO_2 W	**6.79**	1.73	16.97	18.9***
Thermo Electron	3110	CO_2	**7.55**	1.83	18.28	3.92*
Revco	N.A.(Old)	CO_2	**7.67**	3.57	17.76	1.27*
Napco	3550	CO_2	**7.8**	3.52	13.42	2.84*
Thermo Electron	Napco 3550	CO2	**7.83**	3.81	12.13	1.63*
Fisher Sci.	Isotemp 546	CO_2 W	**9.61**	2.34	37.58	0.76*
Thermo Forma	3110	CO_2 W	**9.73**	2.73	24.14	0.47*
N.A.	N.A.	General	**10.46**	3.57	19.51	0.2

Thermo Forma	3110	CO_2 W	**11.89**	3.3	30.41	0.49*
Gallenkamp	N.A.	General	**11.96**	3.06	37.17	2.3*
Fisher Sci.	610	CO_2	**12.3**	5.15	35.52	1.59*
Forma Scientific	3158	CO_2 W	**13.08**	2.62	50.64	1.61*
Labline	3527	Shaker	**14.04**	3.62	42.74	11.87**
WWR international	2005	General	**15.48**	4.92	47.37	1.28
Forma Scientific	546	CO_2	**16.5**	2.61	74.47	3.45*
Sanyo	MIR152	CO_2	**26.98**	5.67	120	0.34*

Type "CO_2 W" means CO_2 incubator with water jacket. "Max Background" refers to measurements outside the incubators. * measured at 50 cm or halfway between the incubator and other electric equipment. ** 5 cm to another incubator. *** 10 cm to a power outlet panel. For more details, refer to Dong and Héroux, 2012.

D. Magnetic Shielding

If it is desired to eliminate the background MFs of incubators to low levels, shielding must be implemented within the incubators. We achieved this in our own experiments using structural steel cylinders 6.3 mm in thickness. As shown in Fig. 1, culture vessels are centered in concentric rectangular structural steel pipes 5.1 x 7.6 x 20 cm, 7.6 x 10.2 x 20 cm and 15.2 x 24.5 x 36 cm. This configuration reduces 60-Hz MFs by a factor of 144, providing "unexposed" cells with a MF environment of 3 nT, slightly below the measurement floor (5 nT at 60-Hz) of our Narda EFA-300 MF instrument (Li, 2012a). The shielding weighs about 20 kg, and is subject to corrosion, if used in the incubator for long periods of time. Fig. 2 shows the change along the axis of the shielding in the triaxially integrated MF. Static MFs within the shields are slightly lower than 50 µT, as structural steel is de-magnetized during production, but of random direction.

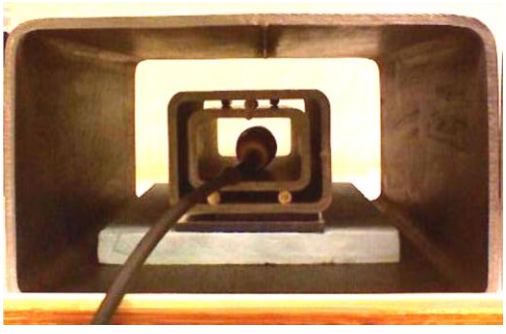

Fig. 1. The three layers of magnetic shielding. The Narda EFA-300's MF probe is in place of the culture vessel. MF coils for exposure are below, but not in contact with the two smaller shields, insulated from the outer shield by a layer of rigid foam.

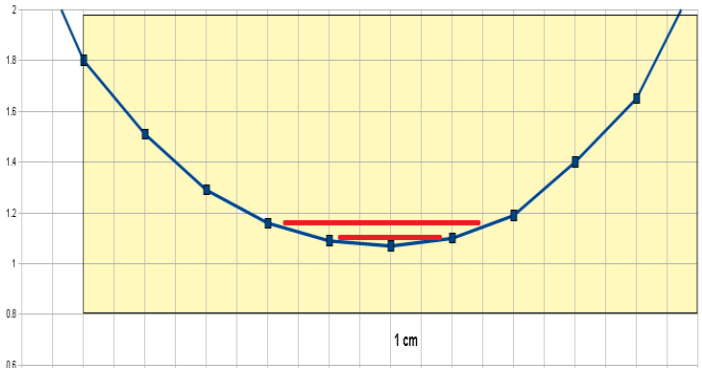

Fig. 2. MF density (μT) generated by an exposure coil vs longitudinal distance inside a magnetic shield pair. The two red lines show the extent of T-25 and T-12 culture vessels, and the yellow rectangle is the smaller shield outline.

E. Experiments on Cells

We conducted experiments on 5 cancer cell lines, with the objective of bringing high precision to our *in vitro* determinations. This objective was reached using automated data acquisition and real-time computer vision, which allowed automated recognition of cells, apobodies and decay particles in cell cultures (Héroux, 2004). In order to reduce deviations related to changing cell culture media, our work used a single synthetic medium (rather than Fetal Bovine Serum) for all 5 cancer models investigated (Li, 2012b).

We first focused our work on changes in the behavior of our cell models under various levels of oxygen. Somewhat surprisingly, all 5 models survived even under anoxic (0 % oxygen) conditions, confirming the exceptional flexibility of cancers cells, able to thrive under anoxia, presumably by finding glycolysis-based sources of cellular energy even in the absence of oxygen.
Low oxygen conditions are actually quite representative of the normal environment of many cells in the body, and are certainly a better *in vitro* representation of the environment of tumor cells, which grow in oxygen and nutrient-restricted environments.

Withdrawal of oxygen suppresses metabolism, as a major combustible of mitochondrial ATP synthesis, oxygen, is eliminated. Metabolism can also be suppressed by a number of chemicals such as oligomycin, imatinib and melatonin-vitamin C, which we collectively designated as “metabolic restrictors”.

F. Karyotype Contraction

When grown under *anoxia* (as opposed to *atmoxia* which is 21 % oxygen, and the commonly used cell culture condition) our 5 cancer cell models lost 6 to 8 chromosomes from their normal

number (Table 3). Further, in the presence of strong doses of antioxidant metabolic restrictors, the cell lines quickly reverted to almost normal chromosome numbers (47 – 49). The anoxic cells showed increases in proliferation rate, and the acquisition of a stable, stem phenotype.

Using our 5 hyperploid (54 – 69 chromosomes) cancer cell models, we found that our cells adjusted their chromosome numbers up or down, to match their micro-environment, through rapid mechanisms of endo-reduplication (unscheduled, extra-mitotic chromosome duplication) or chromosome loss. We called this reversible loss of chromosomes under suppressed metabolism "Karyotype Contraction" (KC).

Anoxic K562 displays a very stable karyotype, with 75 % of the cells having either 61 or 62 chromosomes. With the knowledge that metabolic changes would change these chromosome counts, we then set out to investigate the effects of ELF MFs on this model, while we carefully controlled MFs using the shielding techniques described above. We were then using KC as a metabolic scale.

Starting from cell cultures maintained in a pre-industrial environment (less than 4 nT 60-Hz MF), our 5 cancer cell lines were exposed to constant ELF-MFs within the range of 0.025 to 5 μT, and the cells were examined for karyotype changes after 6 days.

As shown in Table 2, all cancer cells lines lost chromosomes from MF exposures, with a mostly flat dose-response. It seemed that the number of chromosomes lost was more specifically connected to the particular cell type than to the MF level, although the two erythro-leukemia cell types both showed a dose-response between 25 and 400 nT.

Surprisingly, constant MF exposures for three weeks allowed a rising return to the baseline, unperturbed karyotypes. From this point, small MF increases or decreases (10 %) were then again capable of inducing karyotype contractions (Li, 2012a).

Table 2. Karyotype Contraction (mean number of chromosomes lost over 6 days)

Magnetic Field (nT)	Anoxic K562 Erythroleukemia	Atmoxic HEL Erythroleukemia	Atmoxic NCI-H460 Lung cancer	Anoxic MCF-7 Breast cancer	Atmoxic COLO-320DM Colon cancer
25	2.21				
50	4.92	10.22	7.52	11	5.36
100	8.18	11.55			
200	11.04				

400	10.4	12.79	7.55	10.64	5.85
700	9.52				
1000	7.69			10.68	
1500	9.94				
5000	12.1	13.03	7.46	10.95	5.78

Table 3. Karyotype Contraction (mean number of chromosomes lost over 6 days)

Cell	Atmoxic Modal Chromosome Number	Anoxic KC	Anoxic to MF Saturation KC	Atmoxic to MF Saturation KC	Atmoxic to Anti-Oxidant Suppression KC*
K-562 Erythroleukemia	69	7	10.12		21.34
HEL Erythroleukemia	66	7		12.91	18
MCF-7 Breast cancer	82	8	10.82		18
NCI-H460 Lung cancer	57	6		7.51	10
COLO-320DM Colon cancer	54	6		5.66	7.7
Average	*65.6*	*6.8*	*10.47*	*8.69*	*15.01*
Condition	+ O_2	- O_2	- O_2 + MFs	O_2 + MFs	O_2 + Oxidative Inhibition

The conclusion from these observations was that MFs act as a metabolic inhibitor, even at very low levels commonly encountered in the normal environment.

G. ATP Synthase

Supplementary tests carried out by comparing MF-exposed cell cultures to cultures exposed to various metabolic suppressors showed that the MF-exposed cultures were remarkably similar to those exposed to oligomycin A, a specific inhibitor of the Fo segment of the enzyme ATP Synthase (ATPS).

But how could MFs as low as 25 nT alter the activity of ATPS? ATPS has the structure of a motor-generator than normally produces ATP using the energy of a flow of protons through a turbine-like structure, Fo. MFs apparently impaired the flow of protons through ATPS Fo.

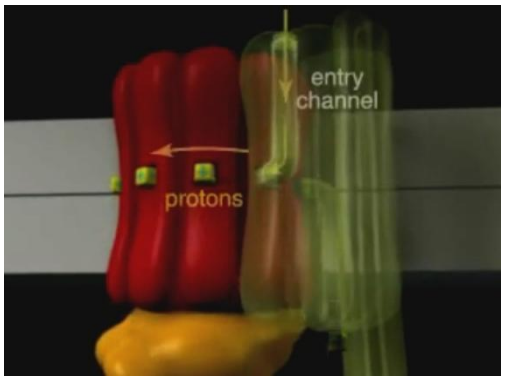

Fig. 3. The structure of ATPS Fo: entry and exit channels for the movement of protons (Yoshida, Tokyo Institute of Technology).

Russian physicists (Semikhina 1981; Semikhina 1988) have reported that very low levels of ELF MFs (25 nT) can alter the structure of water, and that the effects of the altered water structure would be particularly important under high concentrations of protons and water molecules. An interesting aspect of these changes in water structure is that the transition between states takes several hours.

As it turns out, the entry and exit channels of ATPS Fo (Fig. 3) are hydrophilic channels, which means that they are expected to be filled with water molecules, and the intermembrane potential of mitochondria maintains a large electric field (180 kV/cm) which concentrates protons within them. These locations seem ideal to embody the low level effects documented by Semikhina and Kiselev.

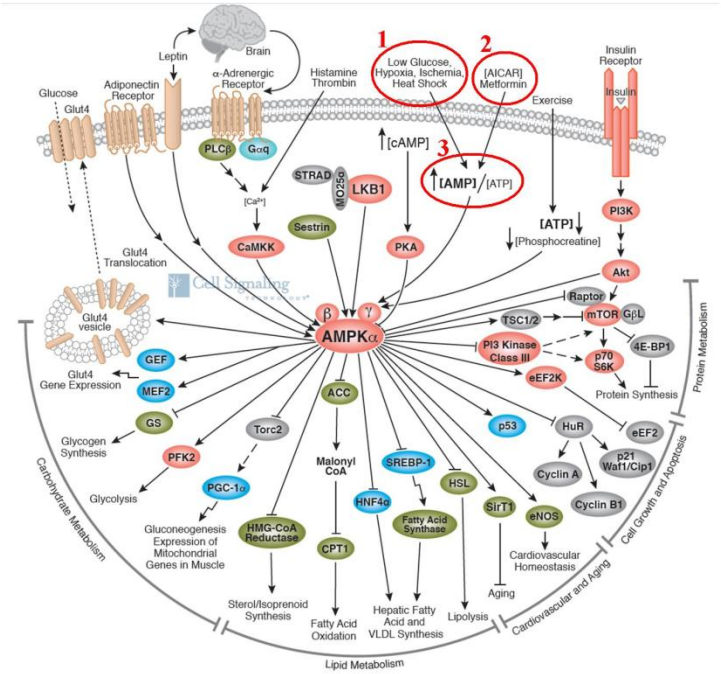

Fig. 4. The many regulatory pathways of AMPK, with the hypoxic (1), metformin (2) and ATPS suppression sites (3) labeled (http://www.cellsignal.com/).

H. AMPK

If the mechanism was indeed as we thought, then MFs would alter the production of ATP in cells. If this happened, another important intracellular enzyme, AMP-activated protein kinase (AMPK), would

immediately be activated, as AMPK is extremely sensitive to changes in the level of ATP. We tested this hypothesis by two supplementary assays involving metformin and resistin. As expected, MF effects were amplified by metformin, an AMPK stimulator, and attenuated by resistin, an AMPK inhibitor (Li, 2012a).

Our data therefore suggests that the karyotype contractions caused by MFs stem from interference with mitochondria's ATP synthase (ATPS), compensated by the action of AMPK. The involvement of AMPK also conveniently explains the slow restoration of karyotypes to their original level after 3 weeks, as AMPK is not only fast-acting to restore ATP levels, but slow-acting through its numerous metabolic and genetic regulation pathways (Fig. 4). It may also explain the unusual observation where increases or decreases in MF exposures can both produce KCs (Li, 2012a).

I. In the Channels

Some enzymes operate faster than predicted by classical thermodynamics, and their increased speed can be explained by tunneling of protons or electrons through activation barriers (Garcia-Viloca, 2004; Olsson, 2004). Quantum tunneling for protons over 6 nm through bridging by water molecules has been observed in tryptamine oxidation by aromatic amine dehydrogenase, for example, and tunneling in enzymatic reactions is now widely accepted in biological models (Masgrau, 2006).

It is of interest to examine how protons may flow through ATPS Fo channels. The protons trickle through a thin pipe of water molecules, propelled by an electric field of about 180 kV/cm. Adiabatic tunneling should be more efficient than non-adiabatic coupling, implying that disturbances along the channel could result in loss of channel transparency. Proton-coupled electron transfer underpins many biological reactions, and may occur as unidirectional or bidirectional, and synchronous or asynchronous, transfer of protons and electrons (Reece, 2009).

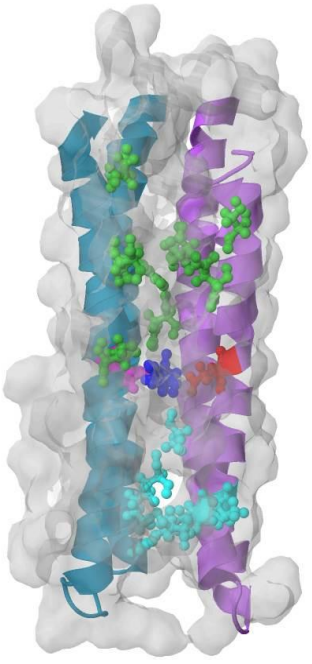

Fig. 5. The ATPS Fo proton hydrophilic channel. Hydrophilic side chains and residues are in green and blue. (from Sasada R, Marcey D. ATP Synthase, 2010. http://www.callutheran.edu/BioDev/omm/jmolxx/atp_synthase/atp_synthase.html#fig1).

It is probable that both electrons and protons tunnel through the channel, making theoretical analysis more complex, especially as electrons meet with different protons along a chain. Since protons are much heavier than electrons (x1836), their wavelength is 43 times shorter (inverse square root), and electrons may transfer over longer distances (Moser, 1992; Gray, 1996). Thus, electron transfer can span fractions of nano-meters, while proton transfer occurs mostly within a hydrogen bond (less than 0.197 nm). The hydrogen bond strength (23.3 kJ/mol) is just 5 times the average thermal fluctuation energy. Quantum chemical calculations show that this strength can vary as much as 90 %, depending on the level of cooperativity or anti-cooperativity within water molecule chains, which corresponds to a bond length change of 9 %, or 0.018 nm (Hus, 2012).

This limited reach of proton tunneling and its delicate dependence on water cluster structure may be major factors underlying the sensitivity of ATPS performance to MF-exposed water.

J. Water 'Remanence'

From our observations, particularly the fact that exposed cell culture medium can retain memory of past MF exposures (Li, 2012a), it does not appear that biological effects of MFs, as we detected them, are based on a direct interaction with electrons or protons, but rather, as suggested by Semikhina and Kiselev, on an interaction between MF and the structure of water, which in turn influences electron and proton tunneling. The exact structure of the water molecule arrays responsible is not known, but may be connected with long-lived hydrogen bond structures which confer particular proton transparency to ATPS Fo water channels. This structure seems vulnerable to interference by MFs over a wide range of intensities and possibly frequencies (Kiselev, 1988). Perturbations to the structure of O-H bond vibrations has even been spectroscopically detected as slow (hours) transitions in water exposed to sunlight radiation (Yokono, 2009).

This would not be the first instance of subtle changes in hydrogen bonds resulting in large influences in biology. A contemporary example relates to the selective uptake of phosphorus rather than arsenic by bacteria. The discrimination by a factor of 4,500 in phosphorus vs arsenic is based on a 4 %

distortion in a unique low-barrier hydrogen bond (Elias, 2012).

III. DISCUSSION

There are similarities as well as differences between semi-conductor tunneling and ATPS tunneling. Both involve oxygen; tunneling distances, as well as the voltages applied (Fig. 6) are similar. But in semiconductor tunneling, only electrons are mobile, while protons move within ATPS. In the semiconductor, magnetic sensing is mainly through shifts in the populations of electrons with a given spin, determined by the electrodes. In ATPS, the transparency of the water channel seems determined by long-term MF exposures.

Perhaps least understood is how cells can metabolically compensate for various MF exposures over time, as shown by the restoration of their chromosome numbers after three week exposures (Li, 2012a). Anoxia leads to permanent KCs, but other KCs from MFs or other anti-oxidants are transient. Most anti-oxidant and MF KCs are larger than the atmoxic to anoxic transition KCs, possibly because some oxygen is still available to cell metabolism, even under anoxic conditions. Anoxia and MFs together are effective metabolic suppressors.

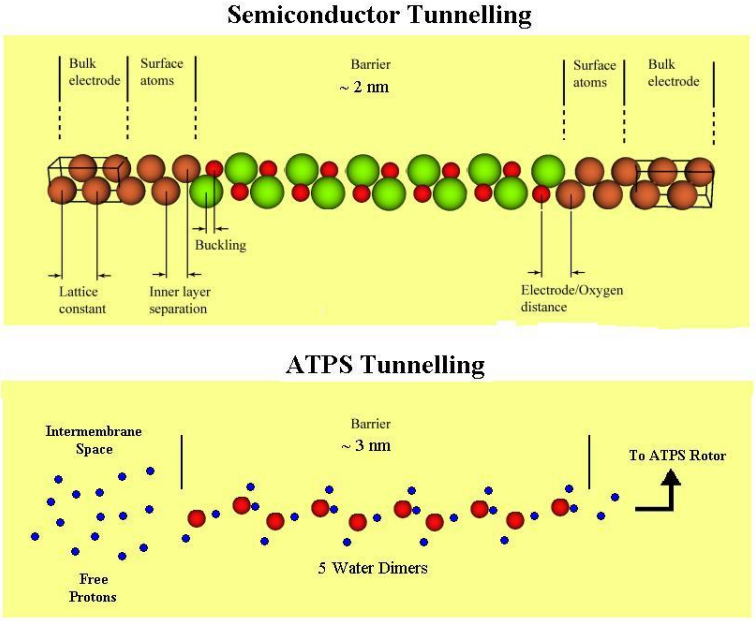

Fig. 6 Tunneling in magnetic sensors and in ATPS water channels.

IV. CONCLUSIONS

The particularities of hydrogen bond structures in water can justify the subtle changes detected in water structure under MF exposures. Under specific circumstances, such water changes may influence the flux of protons in ATPS channels, thus inducing some biological effects of MFs. These interactions seem to involve very small energies, and also seem to require hours to establish themselves, thus bypassing the celebrated "kT problem". These results may be environmentally important, in view of the central roles played in human physiology by ATPS and AMPK, particularly in their links to diabetes, cancer and longevity (Li, 2012a). The wide range of MF amplitudes and frequencies that can potentially disturb ATPS make this effect a global health issue. Although society seems to compile diseases with more enthusiasm that longevity (Li, 2012a), it should be remembered that MF exposures may have both undesirable and desirable effects on health.

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