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Electromagnetic Fields May Act Directly on DNA

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Abstract A wide variety of environmental stimuli induce the expression of stress response genes, including high temperatures, hypoxia, heavy metal ions, and amino acid analogs. Stress genes are also induced by low frequency magnetic fields. The cellular response to magnetic fields is activated by unusually weak stimuli, and involves pathways only partially associated with heat shock stress. Since magnetic fields interact with moving charges, as we have shown in enzymes, it is possible that magnetic fields stimulate the stress response by interacting directly with moving electrons in DNA. In this paper, we review several lines of evidence that support this hypothesis. J. Cell. Biochem. 75:369–374, 1999.

Key words: DNA; electromagnetic fields; stress genes; initiation of transcription

Stress proteins are synthesized in response to a variety of physical and chemical stressors. This response is ubiquitous, evolutionarily conserved and found in essentially all eukaryotic and prokaryotic organisms [Lindquist and Craig, 1988]. Stress proteins have important protective roles; they act as "chaperones" for transporting cellular proteins to their destinations and help affected proteins refold and retain their conformation [Welch and Brown, 1996].

The initial response to thermal stress is characterized by the movement of heat shock factor (HSF) monomers from the cytoplasm to the nucleus, where they trimerize and bind to specific nucleotide sequences in the promoter, the heat shock elements (HSEs) [Morimoto, 1993]. It is currently believed that activation of the stress response occurs when extracellular signals affect receptors in the plasma membrane. Subsequently specific signal transduction cascades involved in regulating cell proliferation, differentiation, and metabolism are initiated. Each cascade passes its message to the cell nucleus via protein kinases that propagate and amplify the signal, with different molecules activating different pathways. The final step in activation of gene expression requires the binding of specific transcription factors to specific nucleotide sites in the promoter of the gene. Extracellular signals that affect transcription factor activity also affect steps in this process [Hopkin, 1997].

Induction of HSP70 expression is activated within minutes in cells exposed to low frequency magnetic fields [Lin et al, 1997]. Several early steps in this process are similar to the initial steps following heat shock (Table I). There are, however, significant differences from heat shock in the way cells respond to magnetic fields (Table II). These differences between magnetic field-activated and heat-activated stress response suggest that stress-activated signal transduction pathways may be just one of the mechanisms for extracellular signaling to the nucleus, and that direct reaction with DNA may also occur. Magnetic fields penetrate throughout the cell and their interactions are not limited to the membrane.

Direct Interaction Hypothesis

Direct interaction of magnetic fields with DNA and the more generally accepted signal transduction cascade initiated at the cell membrane can both lead to signal amplification. In the membrane hypothesis, signal transduction is via cascades utilizing phosphokinases. In the direct interaction with DNA, signal transduction is by interaction with large electron flows. Both hypotheses leave unanswered the key problem of how DNA can be destabilized to

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TABLE I. Similarities in the Induction of HSP70 by Magnetic Fields and Heat Shock

- Induction of heat shock puffs in the salivary gland chromosomes of *Drosophila melanogaster* [Goodman et al., 1992];
- Induction of HSP70 gene expression in Dipteran and cultured human cells [Goodman and Henderson, 1988; Goodman et al., 1994], and SSA1 in yeast cells [Weisbrot et al., 1993];
- Activation of heat shock factor (HSF1) and increased heat shock element (HSE)-binding [Lin et al., 1997];
- Myc-transactivation of HSP70 expression transcription following exposure to magnetic fields [Lin et al., 1998a];
- Induction of cytoprotection, similar to acquired thermotolerance in heat shock [Goodman and Blank, 1998; Han et al., 1998].

TABLE II. Differences Between Induction of HSP70 by Magnetic Fields and Heat Shock

- The energy needed to induce hsp70 is 14 orders of magnitude lower than heat shock [Goodman and Blank, 1998];
- Normal cellular protein synthesis is *not* reduced by magnetic fields [Blank et al., 1994];
- The HSP70 promoter region responsive to a magnetic field maps to a different domain [Lin et al., 1999];
- HSE sequences, centered at −192, are responsive to magnetic fields; the HSE in heat shock domain downstream, centered at −100, is unresponsive to magnetic fields [Lin et al., 1998a, 1999];
- Three MYC-binding sites in the HSP70 promoter are required for the induction of HSP70: MYC-A at −230, MYC-B at −160, and MYC-C at −166. MYC-C, acts as a regulator [Lin et al., 1999];
- HSP70 expression by magnetic fields *requires* Myc protein [Lin et al., 1998a];
- Magnetic fields induce increased DNA binding activity of AP-1 (Fos/Jun protein), a transcription factor not involved in the heat shock pathway [Lin et al., 1998b].

initiate transcription. The membrane hypothesis relies on reactions with promoter elements, while the DNA hypothesis suggests that disturbance of electron flows in DNA results in chain bending or kinking that destabilizes the quiescent state and leads to binding of transcription factors.

The significant differences between magnetic field-activated and heat-activated stress response suggest that the conventional stress-activated signal transduction pathways, which originate at the cell membrane, may not necessarily be the only mechanisms for extracellular signaling to the nucleus. Summarized below is a discussion of evidence that supports a direct effect of magnetic fields on DNA.

- Magnetic fields penetrate to all parts of cells, and stimulation of transcription can occur in the absence of intact membranes [Tuinstra et al., 1997].
- Magnetic fields accelerate electron transport reactions in cytochrome oxidase [Blank and Soo, 1998a,b].
- Binding of different transcription factors induced by magnetic fields could be due to direct activation of DNA at several sites [Lin et al., 1998b].
- Different responses of cells to magnetic restimulation with different field strengths suggests each field strength activates a different DNA sequence [Lin et al., 1996; Han et al., 1998].
- Electric currents activate DNA in muscle (i.e., stimulate protein synthesis that depends on the frequency of stimuli) [Eftimie et al., 1991; Pette and Vrbova, 1992; Lomo, 1992].
- Single and double strand breaks in DNA at stronger magnetic fields than those that stimulate transcription [Lai and Singh, 1997] suggest that the weaker fields may also perturb the DNA structure.

Magnetic Fields Interact With Moving Charges

Several mechanisms have been proposed for the initial interaction of magnetic fields with cells [EM Goodman et al., 1995], but the most likely explanation is the mobile charge interaction (MCI) model [Blank, 1995], which proposes that the interaction of a magnetic field with a moving charge leads to a change in its velocity. Interaction of magnetic fields with moving charges has been shown in enzymes [Blank and Soo, 1996, 1998a,b]. Changes in the activities of both Na,K-ATPase and cytochrome oxidase exposed to magnetic fields vary inversely with the basal level of enzyme activity, without regard for how these changes in activity were induced. Na,K-ATPase activity can be varied, for example through changes in ion concentrations, temperature, and the specific inhibitor ouabain. In all cases, the magnetic field effects depend only on the final enzyme activity. Although the particular charge movements affected in Na,K-ATPase have not been com-

pletely characterized [Hilgemann, 1994], the correlation between the inhibitory ability of non-specific cations and their redox potentials [Britten and Blank, 1973] suggests that a redox reaction may be critical.

In cytochrome oxidase, an electron transport enzyme at the end of the mitochondrial redox chain, the rate of cytochrome C oxidation in magnetic fields varies inversely with the rate of electron transport by the enzyme. The magnetic field apparently competes with the intrinsic enzyme activity. When the basal rate is fast, above 5×10^{17} electrons/mg protein/min, the magnetic field has no effect [Blank and Soo, 1998a,b]. Changes in the steady state Na,K-ATPase reaction, and in the rate constants of the equilibrating cytochrome oxidase reaction, both show that the effect of a magnetic field increases with field strength and varies with frequency, with maxima in the range of the turnover numbers.

Additional support for the MCI model derives from studies of changes in Na,K-ATPase in electric fields [Blank, 1995]. Electric fields accelerate the reaction at low enzyme activity, but when the enzyme activity is high, electric fields are inhibitory. The similar frequency dependence for both inhibition and stimulation by electric fields, suggests that the effects are due to interaction with the same charge movement in the enzyme. Field strength thresholds for both enzymes are below 0.5 μT; this is in the threshold range for stimulating transcription ($<$0.8 μT) [Goodman and Blank, 1995, 1998] and cutoff thresholds in epidemiology (0.2–0.3 μT) [Wertheimer, 1997].

Although electron transfer rates are accelerated in weak magnetic fields, the calculated force on a moving electron (at assumed velocities based on the time scale of enzyme reaction rates) is very small. The Lorentz force, F (in newtons), on a moving electron is given by the equation, $F = qvB$, where q = charge (1.6×10^{-19} coulombs), v = velocity (in m/s), and B = magnetic flux density (in tesla). For a 10 μT magnetic field, and assuming that electrons cross nanometer distances in 1 nanosecond or 1m/s, the force is 10^{-24} newtons. This force can produce an acceleration of $10^6 m/s^2$ (10^5 times the acceleration of gravity) on an electron of mass 10^{-30} kg. This magnitude of acceleration is large, but it is comparable to the effect of a very weak electric field of 10^{-5} V/m, and the magnetic force has little effect on electron movement in the presence of such an electric field. There are probably sites in a protein where the electric field is sufficiently small for a weak magnetic field to have an effect. The large electric fields at membrane surfaces do not appear to inhibit ion flow driven by much lower transmembrane potential differences.

In line with field-induced changes observed in enzyme activities, it is suggested that magnetic fields interact with moving electrons in the stacked bases of DNA [Blank and Goodman, 1997]. Magnetic field-induced increases in transcript levels have been demonstrated [Goodman and Blank, 1998; Goodman et al., 1994], despite the low calculated forces that are predicted to be "insufficient" to affect a DNA chain [Adair, 1998]. The reported velocities of electron movement in DNA [Dandliker et al., 1997] are comparable to those assumed in the above calculation of the Lorentz force. The forces induced by magnetic fields may be large enough to affect processes that can change the rate of movement of electrons significantly, and thereby initiate changes in the DNA. Different DNA sequences have different conductivities [Meggers et al., 1998] and specific nucleotide sequences may function as "antennae" for these low frequency magnetic fields, leading to changes in DNA conformation in response to fields acting upon the moving charges.

Studies of Magnetic Field Activation of the Stress Response

The diversity of conditions that elicit the stress response raises questions concerning how different stimuli are transduced to the nucleus to stimulate upregulation of the stress genes. This question is especially intriguing in the case of very low energy magnetic fields. Lis and Wu [1993] suggest that the different environmental stressors induce the stress response by activating stress-specific transcription factors that recognize separate promoter elements. Figure 1, which presents a summary of pathways involved in the response to low frequency magnetic field- stimulated stress, shows that several different pathways are involved.

Magnetic fields induce stress through HSF binding and induction of hsp70 synthesis. However, unlike heat shock, the transactivation of HSP70 by magnetic fields requires c-*Myc* protein-binding to nCTCTn sequences in the HSP70 promoter [Lin et al., 1998a] and HSF binding at an HSE centered at −192 [Lin et al.,

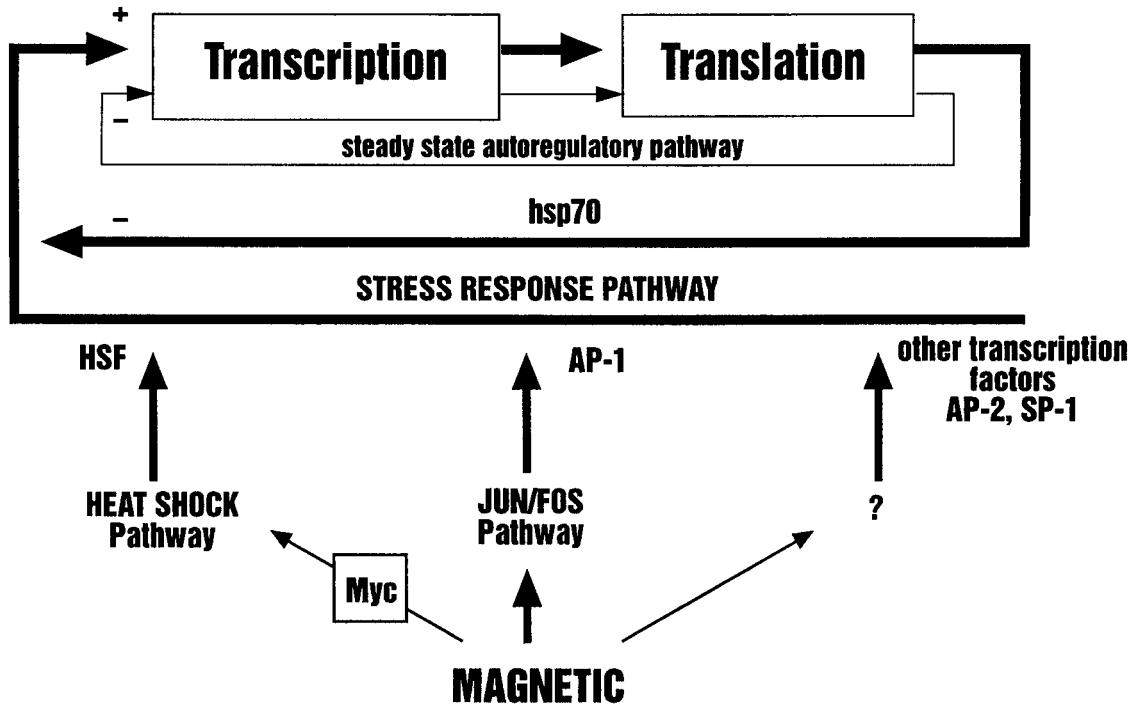

Fig. 1. Circuit diagram illustrating pathways activated by magnetic fields and heat shock. Concentrations of cellular proteins are maintained at a steady state level by a feedback process (light line below the two boxes). Stress proteins (e.g., hsp70) are induced in response to a potentially harmful change in the cell's environment (bold face lines at the bottom of the diagram). Magnetic field induction of HSP70 gene expression acts through an independent signal transduction mechanism that involves the activation of both AP-1 [Lin et al., 1998b] and HSF [Lin et al., 1997] and requires binding of Myc protein to nCTCTn sequences [Lin et al., 1998a] in the HSP70 promoter. Magnetic fields may also act through other transcription factors (indicated by question mark near the unlabeled pathway) [Lin et al., 1998b].

1999], which is at a different site from the heat shock-induced HSF binding downstream. Magnetic fields also induce increased binding-activity of transcription factors *not* affected by heat shock, e.g., increased AP-1 binding involved in the Jun/Fos pathway [Lin et al., 1998b].

That membranes are not necessarily required in some instances was demonstrated using an in vitro cell-free translation system; magnetic field-stimulated protein synthesis occurred in the absence of intact membranes [Tuinstra et al., 1997]. In membrane enzymes studies (e.g., Na,K-ATPase and cytochrome oxidase) membranes were present, however the membranes are *not* required for an enzyme response in the presence of magnetic fields. Since magnetic fields interact with charge movements, stimulation of transcription may occur through interaction with moving electrons within DNA.

In our hypothesis, the magnetic field does *not only* interact with the various membrane receptors to activate parallel cascades simultaneously, but the magnetic fields can interact with conducting electrons in the stacked bases of the DNA that then activate specific binding sites on the DNA by altering the conformation of the DNA. How these sites are selected by the magnetic field may depend on how some characteristic of the signal (e.g., frequency, amplitude) affects the interaction with DNA. Meggers et al. [1998] have recently shown that different DNA sequences have different electronic conductivities.

Magnetic field restimulation studies provide some insight into how magnetic fields may react with particular sites on DNA [Lin et al., 1996; Han et al., 1998]. Stress proteins are synthesized to the same level by any temperature as long as it is above the heat shock threshold. Similarly, in the response to heavy metal ions, a higher concentration still induces the same level of stress response proteins. In contrast, cells respond to different magnetic field amplitudes (field strengths) as *different* stimuli. Hsps are elevated each time the amplitude is changed. Further, changing the amplitude higher or lower makes no difference. It is the

change that counts. Unlike heat shock and heavy metal ion responses, specific DNA sequences may respond directly to different magnetic field strengths. That is, different field strengths interact with electrons in the DNA and activate nucleotide sequences that were not previously responsive.

Magnetic Field-Induced DNA Strand Breaks

Low frequency magnetic fields are generally believed to be too weak to cause any significant movements in DNA chains, let alone lead to single and double strand breaks. Yet, single and double strand breaks have been reported after exposure of brain cells to 0.1 mT (60 Hz) for 2 h [Lai and Singh, 1997]. The field strengths that lead to DNA damage are significantly higher than those (0.008–.08 mT for 20 min) that induce stress proteins. It is possible that the DNA strand breaks could be due to the same kind of interaction of magnetic fields with conducting electrons in the DNA. At lower intensity the forces disrupt the DNA structure to activate transcription factor binding, while at higher intensity the forces lead to real breaks.

Electric Currents Induce Protein Synthesis in Muscle

Muscle mass requires electric stimulation, and muscles atrophy in the absence of stimulation by nerves. Exercises at different speeds and levels of force are known to develop specific muscles. Studies on electric stimulation of muscle have shown that electric currents activate DNA to stimulate specific protein synthesis. For example:

- Levels of mRNA's coding for myogenin and myoD, (two proteins that regulate genes for making muscle proteins), remain high in denervated preparations, but electric stimuli (100 Hz trains of 1 sec duration every 100 sec for six days) depress the concentrations, as do normal nerve action potentials. When electric stimulation is stopped, the transcripts coding for these proteins reappear [Eftimie et al., 1991].
- Structural and functional changes occur in response to electric stimulation. When a fast muscle, normally stimulated at about 100 Hz, is stimulated at 10 Hz, the protein composition changes to resemble a slow muscle after several weeks. The isoforms of troponin T characteristic of fast muscle change to those of slow muscle after 82 days of stimulation at the slow rate (10 Hz, 12 h daily) [Pette and Vrbova, 1989].
- Electric stimulation causes fast-to-slow as well as slow-to-fast transitions in rat muscle, changes in contractile properties and protein composition. Fast and slow muscles in rat are characterized by differences in nerve stimulation rates, muscle contraction rates, and ATPases on heavy meromyosin (that determine contraction rate). The nerve stimulation rate appears to control muscle composition and contraction rate [Lomo, 1992].

The changes in proteins are due to the frequency of action potentials and not to secretions from nerve endings, since eliminating the nerves and stimulating with electrodes shows the same effect. The chemical changes associated with ionic fluxes during action potentials in muscle are not as important as the frequency of the signals, since there are different proteins when the slow (200 pulses at 20 Hz every 15 sec) and fast (25 pulses at 150 Hz every 15 sec) stimulation patterns yield almost the same number of action potentials.

The changes in muscle composition due to electric stimulation mean that segments of DNA previously de-activated during differentiation are re-activated, and that the particular segments which control specific proteins appear to depend upon the frequency of the action potentials. The nuclei of striated muscle are very close to the cell membrane, and action potentials cause ionic (eddy) currents in the muscle cytoplasm to pass around nuclei. The repeated currents (depending upon the frequency) activate particular segments of DNA that result in the synthesis of specific proteins. Eddy currents in the muscle cytoplasm (approximately $1A/m^2$) are much higher than the currents (approximately $0.1–1mA/m^2$) used to induce biosynthetic responses in human HL60 leukemic cells [Blank et al., 1992], so it is possible for the eddy currents in muscle cells to stimulate transcription, if they acted by the same mechanism.

CONCLUSION

Significant differences between the magnetic field-activated stress response and other forms of activation suggest that the conventional stress-activated signal transduction pathways may not necessarily be the only mechanisms for

extracellular signaling to the nucleus. As reviewed above, several lines of evidence support a direct effect of magnetic fields on DNA through interaction with conducting electrons in the DNA. Since cells are minimally perturbed during magnetic field activation of the stress response, magnetic field stimulation could provide a unique experimental tool to study the steps involved in cellular activation mechanisms.

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REFERENCES

Adair RK. 1998. Extremely low frequency electromagnetic fields do not interact directly with DNA. Bioelectromagnetics 19:136–137.

Blank M. 1995. Na/K-adenosine-triphosphatase. Adv Chem 250:339–348.

Blank M, Goodman R. 1997. Do electromagnetic fields interact directly with DNA? Bioelectromagnetics 18:111–115.

Blank M, Khorkova O, Goodman R. 1994. Changes in polypeptide distribution stimulated by different levels of electromagnetic and thermal stress. Bioelectrochem Bioenerg 33:109–114.

Blank M, Soo L. 1996. The threshold for Na,K-ATPase stimulation by electromagnetic fields. Bioelectrochem Bioenerg 40:63–65.

Blank M, Soo L. 1998a. Enhancement of cytochrome oxidase activity in 60Hz magnetic fields. Bioelectrochem Bioenerg 45:253–259.

Blank M, Soo L. 1998b. Frequency dependence of cytochrome oxidase activity in magnetic fields. Bioelectrochem Bioenerg 46:139–143.

Blank M, Soo L, Lin H, Henderson AS, Goodman R. 1992. Changes in transcription in HL-60 cells following exposure to alternating currents from electric fields. Bioelectrochem Bioenerg 28:301–309.

Britten JS, Blank M. 1973. Effects of cations on biologically active surfaces—Specific binding sites in the Na,K-ATPase. J Coll Interf Sci 43:564–570.

Dandliker PJ, Holmlin RE, Barton JK. 1997. Oxidative thymine dimer repair in the DNA helix. Science 275:1465–1469.

Eftimie R, Brenner HR, Buonano A. 1991. Myogenin and MyoD join a family of skeletal muscle genes regulated by electrical activity. Proc Nat Acad Sci USA 88:1349–1353.

Goodman EM, Greenebaum B, Marron MT. 1995. Effects of electromagnetic fields on molecules and cells. Int Rev Cytol 158:238–279.

Goodman R, Blank M. 1995. Biosynthetic stress response in cells exposed to electromagnetic fields. Adv Chem 250:423–436.

Goodman R, Blank M. 1998. Magnetic Field Induces Expression of hsp70. Cell Stress Chaperones 3:79–88.

Goodman R, Blank M, Lin H, Khorkova O, Soo L, Weisbrot D, Henderson A. 1994. Increased levels of hsp transcripts are induced when cells are exposed to low frequency electromagnetic fields. Bioelectrochem Bioenerg 33:115–120.

Goodman R, Henderson A. 1988. Exposure of salivary gland cells to low-frequency electromagnetic fields alters polypeptide synthesis. Proc Natl Acad Sci USA 85:3928–3932.

Goodman R, Weisbrot D, Uluc A, Henderson A. 1992. Transcription in Drosophila melanogaster salivary gland cells is altered following exposure to low frequency electromagnetic fields: Analysis of chromosome 3R. Bioelectromagnetics 13:111–118.

Han L, Lin H, Head M, Jin M, Blank M, Goodman R. 1998. Application of magnetic Field-induced Hsp70 for presurgical cytoprotection. J Cell Biochem 71:577–583.

Hilgemann DW. 1994. Channel-like function of the Na,K pump probed at microsecond resolution in giant membrane patches. Science 263:1429–1431.

Hopkin K. 1997. How and where might signal-transduction cascades intersect? J of NIH Res 9:25–27.

Lai H, Singh NP. 1997. Acute exposure to a 60Hz magnetic field increases DNA strand breaks in rat brain cells. Bioelectromagnetics 18:156–165.

Lin H, Blank M, Jin M, Goodman R. 1996. Electromagnetic field stimulation of biosynthesis: Changes in c-myc transcript levels during continuous and intermittent exposures. Bioelectrochem Bioenerg 39:215–220.

Lin H, Opler M, Head M, Blank M, Goodman R. 1997. Electromagnetic field exposure induces rapid, transitory heat shock factor activation in human cells. J Cell Biochem 66:482–488.

Lin H, Head M, Blank M, Han L, Jin M, Goodman R. 1998a. Myc-mediated transactivation of HSP70 expression following exposure to magnetic fields. J Cell Biochem 69: 181–188.

Lin H, Han L, Blank M, Head M, Goodman R. 1998b. Magnetic field activation of protein-DNA binding. J Cell Biochem 70:297–303.

Lindquist S, Craig E. 1988. The heat shock proteins. Annu Rev Genet 22:631–677.

Lis J, Wu C. 1993. Protein traffic on the heat shock promoter: parking, stalling and trucking along. Cell 74:1–4.

Lomo T. 1989. Long term effects of altered activity on skeletal muscle. Biomed Biochim Acta 48:S432–S444.

Meggers E, Michel-Beyerle ME, Giese B. 1998. Sequence dependent long range hole transport in DNA. J Am Chem Soc 120:12950–12955.

Morimoto RI. 1993. Cells in stress: Transcriptional activation of heat shock genes. Science 259:1409–1410.

Pette D, Vrbova G. 1992. Adaptation of mammalian skeletal muscle fibers to chronic electrical stimulation. Rev Physiol Biochem Pharmacol 120:115–202.

Tuinstra R, Greenebaum B, Goodman EM. 1997. Effects of magnetic fields on cell-free transcription in E. Coli and HeLa extracts. Bioelectrochem Bioenerg 43:7–12.

Weisbrot D, Khorkova O, Henderson A, Goodman R. 1993. The effect of low frequency electric and magnetic fields on gene expression in Saccharomyces cerevisiae. Bioelectrochem Bioenerg 31:167–177.

Welch WJ, Brown CR. 1996. Influence of molecular and chemical chaperones on protein folding. Cell Stress Chaperones 1:109–115

Wertheimer N. 1997. Comment on 2mG Intensity Cutpoint. Bioelectromagnetics Newsletter, number 138: 4–5, 8–10, Sept/Oct 1997.