Mini-review

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Magnetic field stress induces expression of *hsp70*

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LOW FREQUENCY MAGNETIC FIELD INTERACTIONS WITH CELLS: AN OVERVIEW

Magnetic fields with frequencies lower than 300 Hz and field strengths less than 1 Gauss (1000 mG) induce a variety of effects in cells and tissues (for reviews see Blank 1995; Goodman et al 1995; Hong 1995). Among these effects are altered transcription and translation, including *hsp70* (Lin et al 1997, 1998) and the immediate early response genes *myc, jun* and *fos* (Philips et al 1992; Lin et al 1996; Jin et al 1997); increased enzyme activities of ornithine decarboxylase, Na, K-ATPase and cytochrome oxidase (Byus et al 1988; Blank 1995; Blank and Soo 1998); changes in melatonin release (Reiter 1995) and alterations in receptor binding (Luben 1995). The increased expression of stress genes in the presence of magnetic fields suggests that cells respond to magnetic fields as an environmental stress.

Research on changes in cells caused by magnetic fields is focused on mechanisms at two levels: the initial physical transduction step and the cellular/biochemical pathways that are stimulated. Several physical mechanisms have been proposed to account for the initial interactions with cells. The Mobile Charge Interaction (MCI) model (Blank 1995) proposes that magnetic fields interact with moving charges in cells and change their velocities, as in the classic interaction of a magnetic field with any moving charge. If the charge flow is associated with a biological function, as in the case of an enzyme, that function will change. Field-induced changes in enzyme activity, proportional to charge flow, have been demonstrated in Na,K-ATPase and cytochrome oxidase reactions (Blank 1995; Blank and Soo 1998).

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Resonance models in which the AC magnetic fields interact with the earth's DC field, and the response is determined by the charge/mass ratio of ions involved in the reaction, have also been proposed as a mechanism of initial interaction. These models include Ion Cyclotron Resonance (Liboff et al 1987) and several variants (Lednev 1991; Blanchard and Blackman 1994). Theoretical objections have been raised to resonance models, however, and a clear connection to a biological process is lacking (Halle 1988; Adair 1992). Free radical reactions, which involve unpaired electrons, are also affected by magnetic fields, but generally at much higher field intensities (over 10 Gauss).

Recent studies using electromagnetic noise fields (a signal containing a random mixture of frequencies 30–300 Hz) have shown that cells must be exposed for at least 10 s to elicit a response (e.g. increase in ornithine decarboxylase activity) (Litovitz et al 1991, 1993a, 1993b, 1994). Superimposing a noise field on a 60 Hz signal eliminates the effect of that signal. To be effective, the energy level of noise must match the energy level of the signal that is causing the change.

Magnetic fields are believed to interact initially with the cell membrane, activating signal transduction pathways that lead to the nucleus and result in stress protein synthesis. In any case, the cell membrane is clearly a site of interaction with magnetic fields. Increases in receptor binding and activation have been shown (Luben 1995), as well as increases in the activities of membrane enzymes (e.g. Na,K-ATPase and cytochrome oxidase). [In the latter case, however, the enzymes need not be in membranes for interaction with magnetic fields (Blank 1995).]

Theoretically, since low frequency magnetic fields penetrate the cell (unlike electric fields), interactions can occur anywhere in a cell. The assumption that magnetic fields stimulate changes in cells by first interacting with cell membranes is based on the belief that they act only indirectly through their induced electric fields, which are

very small because of cell dimensions. The membrane has been proposed as the site of initial interaction because it offers the possibility of signal amplification by established cascade mechanisms. It is necessary, however, to show that the signal transduction pathways lead to the nucleus where biosynthesis is stimulated, a connection not yet established. That membranes are not required for magnetic field interaction is also demonstrated by in vitro studies in cell-free systems that show magnetic field-stimulated protein synthesis in the absence of intact membranes (E. M. Goodman et al 1993; Tuinstra et al 1997).

The initial stress-sensing reactions are not characterized for the many different stimuli that elicit the stress response. This is especially true of magnetic fields, which induce the stress response at remarkably low energies compared to heat. Magnetic fields could interact with nuclear DNA directly since magnetic fields, in contrast to electric fields, can be as effective at the nucleus as at the cell membrane. Extrapolating from recent reports of Barton and her colleagues that showed electron-flow through the stacked bases of DNA (McClellan et al 1990; Dandliker et al 1997), we have proposed that these electrons may respond to magnetic fields, and that specific nucleotide sequences/transcription factors may be targeted (Blank and Goodman 1997).

ELECTROMAGNETIC FIELD EXPOSURE CONDITIONS

As in most studies of extremely low frequency magnetic field effects, the fields are generated by a pair of double-wrapped Helmoltz coils and the cells are placed between them as seen in Figure 1. There are two identical sets of coils, and either one can be set for either active (exposure) or zero field (sham-exposed mode). In the sham-exposed mode, the opposing directions of current flow create a zero field. Cells are exposed to frequencies < 300 Hz in flasks or Petri dishes. Mammalian cell cultures are generally exposed at cell densities of 10^6/ml. The cells are split from a single flask 18 h prior to each experiment (i.e. control and experimental cell samples are derived from the same parental flask), and are plated out at ~3–5 × 10^5/ml, so that by the following morning they are 10^6/ml. In our laboratories 15 ml of cells at 10^6/ml are used for exposures. The Helmholtz coils are shielded from stray AC and DC fields in mu metal containers within the incubator. The sinusoidal field is generated by a function generator connected to a power regulator. Signal parameters are monitored by a calibrated inductive search coil with an oscilloscope (see diagram in Fig. 1). Cells are sham-exposed at the same time in the same incubator, and shielded in an identical mu metal container. (For details of coil construction, see Lin et al 1996; Jin et al 1997.)

To ensure that no heating results from the active coils, temperatures are carefully monitored. We use a thermocouple probe attached to the coils throughout all exposures (sensitivity ± 0.1°C). To compare magnetic field stimulation with thermal stimulation, flasks or Petri dishes containing the cells are wrapped in Parafilm and placed in a mu metal container (*to avoid the magnetic fields generated by heating unit in the waterbath*) and immersed in a water bath at 43°C for mammalian human cell cultures and 37°C for dipteran cultures.

MAGNETIC FIELD-INDUCTION OF THE STRESS RESPONSE

Various lines of evidence show that cells respond to magnetic fields as a stress:

- Transcription autoradiography on Dipteran salivary gland chromosomes identifies nascent RNA chains following short magnetic field exposures; transcription on specific regions of the salivary gland chromosomes of *Sciara coprophila* and *Drosophila melanogaster* is induced (R. Goodman et al 1983, 1992a; 1992b; Weisbrot et al 1993a)
- Magnetic field exposures increase the synthesis of the stress protein Hsp70 in Diptera (R. Goodman and Henderson 1988)
- The induction of stress gene expression by magnetic fields was observed in yeast and cultured human cells (Weisbrot et al 1993b; R. Goodman et al 1994; Blank et al 1994)
- Critical early steps in the magnetic field-induced stress response have been identified, such as activation of heat shock factor (HSF1) and increased heat shock element (HSE)-binding (Lin et al 1997)
- A Myc-binding region on the *hsp70* promoter is required for a magnetic field response (Lin et al 1998)
- Magnetic fields stimulate another characteristic of the stress response, a temporary stress resistance in cells that is strongly reminiscent of acquired thermotolerance in the case of thermal stress. This points to potential therapeutic applications, e.g. for pre-conditioning prior to surgery.

Based on evidence discussed below, magnetic-field induction of *hsp70* gene expression appears to act through an independent signal transduction chain, as well as through the heat stress-sensing pathway.

SIMILARITIES AND DIFFERENCES BETWEEN THERMAL AND MAGNETIC FIELD STRESS

Transcriptional and translational activation

Magnetic fields elicit biological responses at very low field strengths (R. Goodman and Blank 1995); the energy

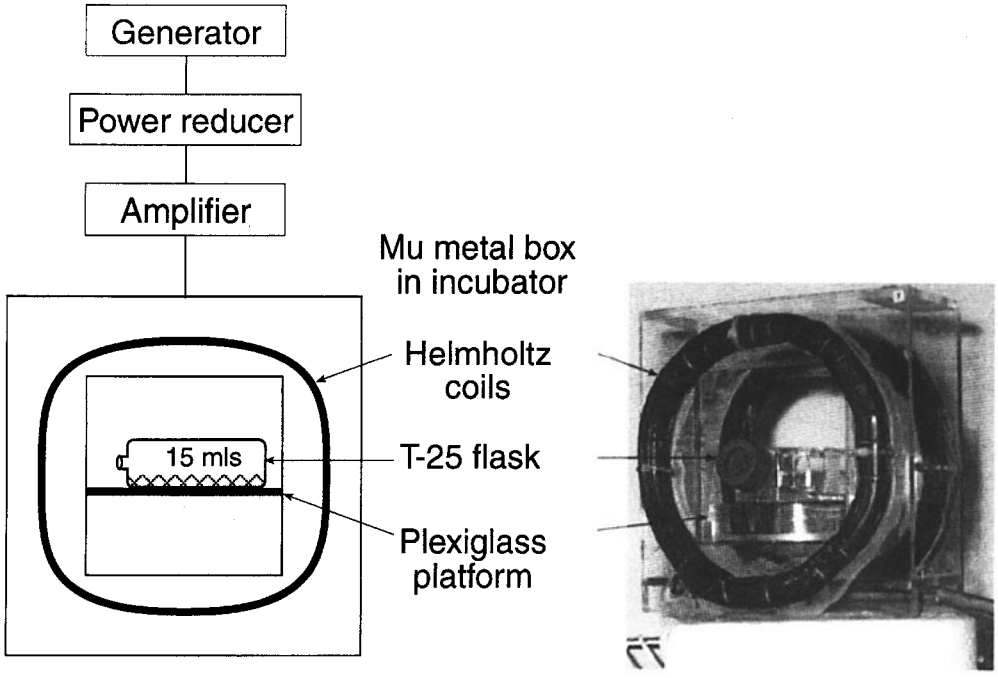

Fig. 1 Magnetic field exposure system diagram and a photograph of Helmoltz coil with T25 flask.

input to the cell is negligible compared to the energy needed to induce heat shock. Nevertheless, magnetic field exposures of 20 min at 80 mG, 60 Hz *at ambient growth temperatures* induces 'heat shock' puffs in *Drosophila* salivary gland chromosomes, including puffs at 93AC and 87AC (R. Goodman et al 1992a; Fig. 2). In contrast to thermal stress, magnetic field-induced stress augments transcriptional activity in constitutively active loci, as well as in chromosome regions that were not detectable as active in control cells (R. Goodman et al 1987, 1992a, 1992b; Weisbrot et al 1993a).

Changes in protein synthesis resulting from magnetic field-induced stress and thermal stress were compared by analyzing two-dimensional (2D) gel electrophoresis of ^{35}S-labeled polypeptides. The changes in molecular weight (MW) and isoelectric point (pI) distribution patterns in magnetic field- and heat shock-induced protein synthetic patterns were surprisingly similar (Blank et al 1994). The similarities between magnetic field- and heat shock-induced protein with respect to their sequence of changes in response to increasing stress, and the return to control levels, suggest a common 'stress response' pathway.

Heat shock factor activation and heat shock element-binding

Other differences and similarities between magnetic field stress and thermal stress became evident with electrophoretic mobility shift assays (EMSAs) to determine whether magnetic fields induce activation of heat shock transcription factor and heat shock element-binding activity (Lin et al 1997). Cells exposed to magnetic fields showed a 3- to 4-fold increase in binding activity (somewhat less than seen in the heat-shocked sample; Fig. 3A). Magnetic field-exposed cells exhibited HSF1 DNA-binding activity (Fig. 3B). There was also a suggestion that HSF2-binding activity occurred in magnetic field-exposed cells, as judged by partial reduction of the HSF/HSE band in a 'wipeout' assay. Thus, stimulation of human promyelocytic HL60 cells by a 60 Hz magnetic field at normal growth temperatures results in heat shock factor 1 activation and heat shock element-binding, a sequence of events that mediates the stress-induced transcription of the stress gene *hsp70* and increased synthesis of the stress response protein Hsp70.

Activation of other transcription factors

Transcriptional initiation is an important stage in the control of gene expression. Changes in cell behavior induced by extracellular signaling molecules or environmental stresses require a complex program of transcriptional events involving transcription factor activation. Magnetic field-responsive transcription factor activation has been identified for AP-1, AP-2 and SP-1 in human estrogen receptor-positive breast carcinoma cells (MCF7, T47D), in normal estrogen-receptor positive breast cells (HTB124) and in human leukemic cells (HL60).

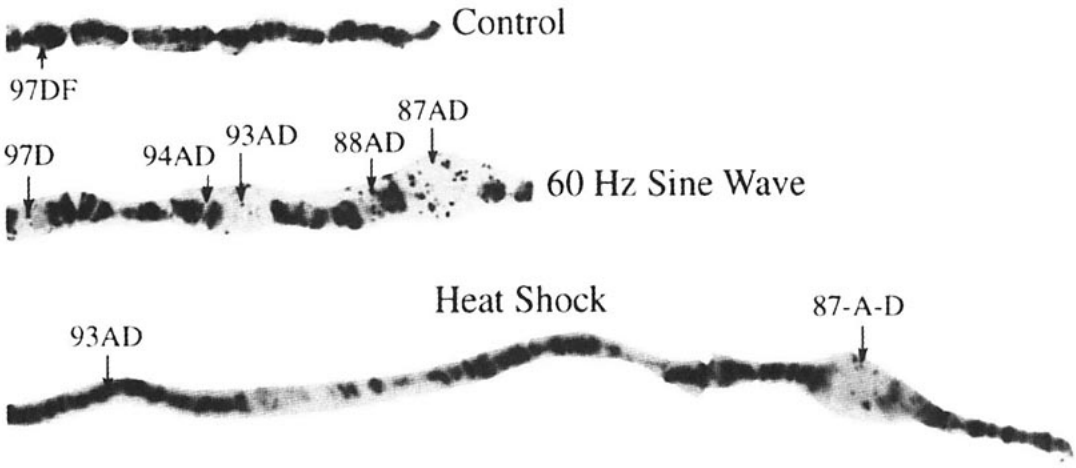

Fig. 2 Transcription autoradiogram of chromosome 3R in *Drosophila melanogaster.* Heat shock puffs at 87AC and 93AC with thermal (37°C) and magnetic (60 Hz sine wave, 25°C) stresses as compared with control 3R. All exposures were 20 min in the presence of 3H-uridine at 25°C. Autoradiographic exposure time was 48 h. In order to expedite identification of specific regions, chromosomes were cut from photomicrographs and portions positioned to achieve linearity. The transcriptively active regions were identified according to published maps (Bridges 1935; *Drosophila melanogaster* Genome Maps 1991). (Experimental and technical details described in R. Goodman et al 1992a.)

The same level of magnetic field stimulation induced different levels of activation, suggesting cell-type specificity in response to magnetic fields (Fig. 4A). An example of the time curve for the induction of AP-1 in HTB124 cells is seen in Figure 4B. Although induction of HSF by magnetic fields is similar to that of thermal stress, activation of AP-1, AP-2 and SP-1 indicates that additional transcription factors, and/or their corresponding DNA sequences in the promoter, respond to magnetic fields.

Magnetic field-responsive region in Hsp70 promoter

The Myc-binding sites in the *hsp70* promoter play a role in modulating the induction of magnetic field-induced *hsp70* expression, unlike thermal stress (Lin et al 1998). The link between magnetic field exposure and Myc protein is to be expected from reports of a trans-regulatory relationship between the c-*myc* gene and the Hsp70 protein. This is seen in nuclear colocalization of Myc protein with Hsp70 in *myc*-overexpressing cells (Koskinen et al 1991), in c-*myc* protein activation of the human *hsp70* promoter (Kingston et al 1984), in the binding of c-*myc* protein complex to two sites in the *hsp70* promoter (Taira et al 1992), and through increased c-*myc* transcript levels in HL60 cells exposed to magnetic fields (Lin et al 1996; Jin et al 1997). Identification of a 900 bp magnetic field-responsive region in the *myc* promoter (Lin et al 1994) confirms this link.

The two Myc protein-binding sites in the *hsp70* promoter required for magnetic field responsiveness are homologous to the Myc protein complex-binding sequence in the c-*myc* gene, CCTCTCA and CCTCTGA, and are located at nucleotide positions −230 and −160 respectively (Taira et al 1992; Fig. 5A). These two regions overlap with the region reported for the regulation of inducible *hsp70* gene expression by Myc protein in the human *hsp70* promoter (Kingston et al 1984). Additionally to the two Myc binding sites are two HSEs, three AP-2, two SP-1 and a serum response element (SRE), but their roles are not essential since selective deletion of the Myc-binding sites eliminates the response to magnetic fields.

Transfectants exposed to magnetic fields had an average 3-fold increase in expression of CAT activity in constructs containing both Myc protein-binding sites (Hsp-MYC A and Hsp-MYC B), as compared with sham-exposed control transfectants (Fig. 5B). Deletion of Hsp-MYC A reduced CAT activity in magnetic field-exposed transfectants to 2.3-fold. Deletion of both Hsp-MYC A and Hsp-MYC B binding sites resulted in no magnetic field induction of CAT activity. *Without co-transfection of* Myc *protein expression vector, none of the hsp70–CAT constructs responded to magnetic fields.* Transfectants with each of the three constructs expressed increased CAT activity when they were heat-shocked at 43°C (*with or without* Myc *protein expression vector*). Unlike thermal induction of *hsp70* gene expression, magnetic field induction of this stress gene appears to be mediated through the binding of Myc protein to the *hsp70* promoter (Lin et al 1998).

Feedback control of the magnetic stress response

The duration of magnetic field exposure determines the magnitude of the response but, because of an apparent

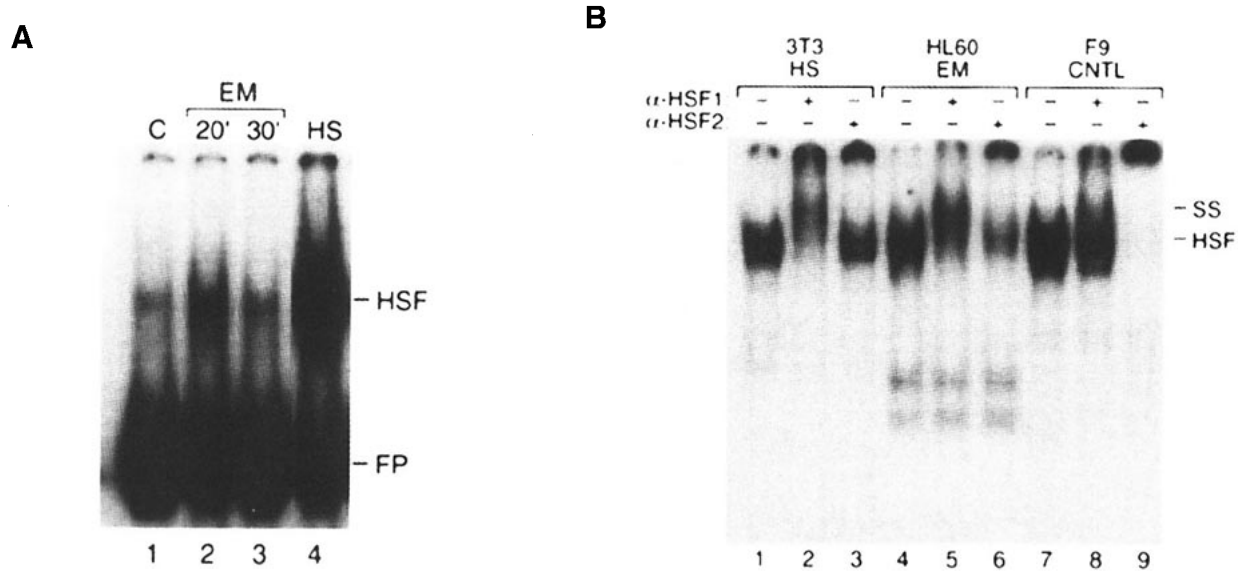

Fig. 3 Electrophoretic mobility shift assays (EMSAs) and supershift assay of magnetic field activation of heat shock factors. (A) HL60 cells were exposed to 60 Hz, 80 mG magnetic fields (EM) for 20 and 30 min at 37°C. Protein lysates for EMSA analyses (Mosser et al 1988; Lin et al 1997) were prepared 20 and 30 min *following* magnetic field stimulation. Cells heat shocked (HS) at 43°C for 20 min were incubated additional 20 min at 37°C before preparation of lysate. Electrophoretic mobility shift assays (EMSAs) used a self-complementary 'ideal' HSE oligonucleotide, 5´-CTA*GAAGC TTC*TA*GAAGC TTC*TAG-3´ containing four inverted nGAAn repeats when annealed (Sarge et al 1993) (provided by Dr R. I. Morimoto, Northwestern University, Evanston, IL, USA). Oligonucleotides labeled with $\gamma[^{32}P]$-ATP were loaded on 4% polyacrylamide gels. Competition assays established sequence specificity of the retarded band and supershift assays using antibodies against HSF1 and HSF2 confirmed the identity of sequence-specific retarded complex (Sarge et al 1993; Head et al 1996; Lin et al 1997). Lane 1 = sham exposed control 20 min, plus 20 min recovery (C). Lane 2 = EM field exposed 20 min, plus 20 min recovery. Lane 3 = EM field exposed 30 min, plus 20 min recovery. Lane 4 = HS 20 min, plus 20 min recovery at 37°C. Lysates prepared of samples exposed to 60 Hz magnetic fields for 20 min, plus a further 20 min recovery, showed a mean increase of 3.4 ± 0.18 in binding activity (lane 2), but somewhat less than that seen in heat shocked sample (lane 4). Control samples were sham-exposed and thus cells are handled identically for both control and experimental samples. Data were entered in QuatroPro for analysis and the results examined with a two-tailed t-test to test the hypothesis that the ratio of the exposed samples over the control samples is equal to unity. Statistical significance using the multifactor analysis of variance program (INSTAT) was also used (for additional details see Lin et al 1997). (B) Binding reactions were performed in presence or absence of monoclonal antibodies against HSF1 and HSF2 (Lin et al 1997). The HSE/HSF retarded band is marked HSF and the HSE/HSF complex supershifted by addition of HSF1 or HSF2 antibody is marked SS. (Antibodies to HSF1 and HSF2 were generously provided by Dr. R.I. Morimoto, Northwestern University, Evanston, IL, USA). Lanes 1, 2 and 3 = protein samples from 3T3 heat shocked cells, positive controls for HSF1 DNA-binding activity. Lanes 4, 5 and 6 = protein samples from HL60 cells exposed to 60 Hz, 80 mG magnetic fields (EM) for 20 min. Lysate was prepared 20 min *following* magnetic field stimulation. Lanes 7, 8 and 9 = protein samples from F9 CNTL cells, positive controls for HSF2 DNA-binding activity.

autoregulatory mechanism (Baler et al 1992; R. Goodman and Blank 1995), the response is not linear. For example, differences in *myc* transcript levels occur in cells exposed for short (20 min) and long (up to 4 h) durations, as well as for repeated short exposures at different intervals (Lin et al 1996). (Similar data have been obtained for Hsp70; manuscript in preparation.) We believe that the stress proteins synthesized in cells continuously 'stressed' by a magnetic field inhibit transcription. In short exposures, on the other hand, stress protein levels are lower and their inhibition of transcription is reduced.

Protein products synthesized in response to magnetic fields *also affect the time required for return to control levels and the level of response to a subsequent stimulus* in cells continuously and intermittently stimulated, providing additional credence for an autoregulatory/feedback control mechanism. These effects of magnetic field stress are similar to *thermotolerance,* in which heat shock at a sub-lethal temperature allows the organism to develop tolerance to a second heat shock at a lethal temperature (Gerner and Schneider 1975; Lindquist and Craig 1988). The interaction of the magnetic field with the thermal pathway is illustrated in the circuit diagram of Figure 6.

We have found that prior magnetic field exposure protects against a lethal thermal stress (manuscript in preparation). Fertilized eggs from *Sciara coprophila* were exposed to a magnetic field. Controls were unexposed fertilized eggs. Both groups of eggs were heat shocked at 37°C (lethal for *Drosophila* and *Sciara*). More than 90% lethality resulted in eggs *not pretreated* with the magnetic field; over 95% of the eggs *pretreated* with the magnetic field survived lethal heat shock and hatched into larvae

A

Cell Lines	HSF	AP-1	AP-2	SP-1
HL60	++	+	+	++
HTB124	+++	+++	++	+
MCF7	+	++	+++	+
T47D	+	++	++	+

B

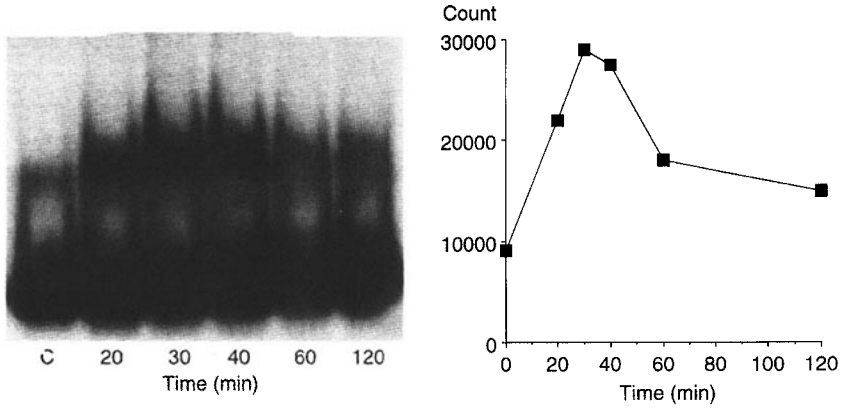

Fig. 4 Transcription factor activation for HSF-1, AP-1, AP-2 and SP-1 in four cell lines. (A) Human leukemic cells (HL60), human breast carcinoma cells (MCF7 and T47D) and 'normal' breast cells (HTB124) were exposed to 80 mG 60 Hz magnetic fields for 20 min followed by 20 min recovery in the absence of the magnetic field at 37°C. Protein lysates for electrophoretic mobility shift assays were prepared as described in Mosser et al (1988) and Lin et al (1997). The Promega Gel Shift Assay System (cat. #E3300; Promega, Madison, WI, USA) was used. Oligonucleotides with consensus sequences for AP-1, SP-1 and AP-2 were supplied with the kit. Oligonucleotides containing consensus sequences for HSF were provided by Dr R.I. Morimoto (Northwestern University, Evanston IL, USA) described in Mosser et al (1988). Oligonucleotides, containing consensus sequences to characterized binding sites, were end-labeled with γ^{32}P-ATP (DuPont/ NEN) and used as protein-specific probes. Protein lysates were run for gel shift assays on 4% non-denaturing acrylamide gels (gels were pre-run for 20 min before loading samples). DNA-binding activation was determined by the intensity of the radiolabeled band as quantified on a PhosphorImager 400A (Molecular Dynamics). The number of pluses (++) is proportional to the magnitude of activation. (B) Time course of AP-1 activation in HTB124 cells, 'normal' human breast cells, exposed to an 80 mG, 60 Hz magnetic field.

(Fig. 7). It appears that magnetic and thermal stresses interact in the stress response, and that cross-protection (between different stress modalities) is possible with magnetic fields.

INDUCTION OF STRESS PROTEINS FOR CYTOPROTECTION IN CLINICAL APPLICATIONS

Stress proteins, by binding to denatured or damaged protein substrates, facilitate refolding and restoration of functional macromolecular complexes during recovery from stress. Evidence that stress proteins play crucial roles in a wide variety of normal cellular processes has made them an object of broad interest to specialists in various fields of medicine. Recent focus has centered on the putative function of stress proteins as cytoprotective factors that limit injury during the development of tolerance to a second, more severe stress. Cardiovascular research has shown the anti-ischemic properties of Hsp70 to have therapeutic relevance; whole-body heat stress reduces infarct size and enhances postischemic contractile function (Marber et al 1993, 1995; reviewed in Plumier and Currie 1996).

Studies show that prior heat stress can limit myocardial injury following subsequent coronary occlusion, suggesting that the abundance of Hsp70 or other stress proteins is an important factor in cell survival during myocardial ischemia (Currie et al 1988; Donnelly et al 1992); that prior heat stress protects the myocardium against ischemia (Currie et al 1988; Yellon et al 1992); that mam-

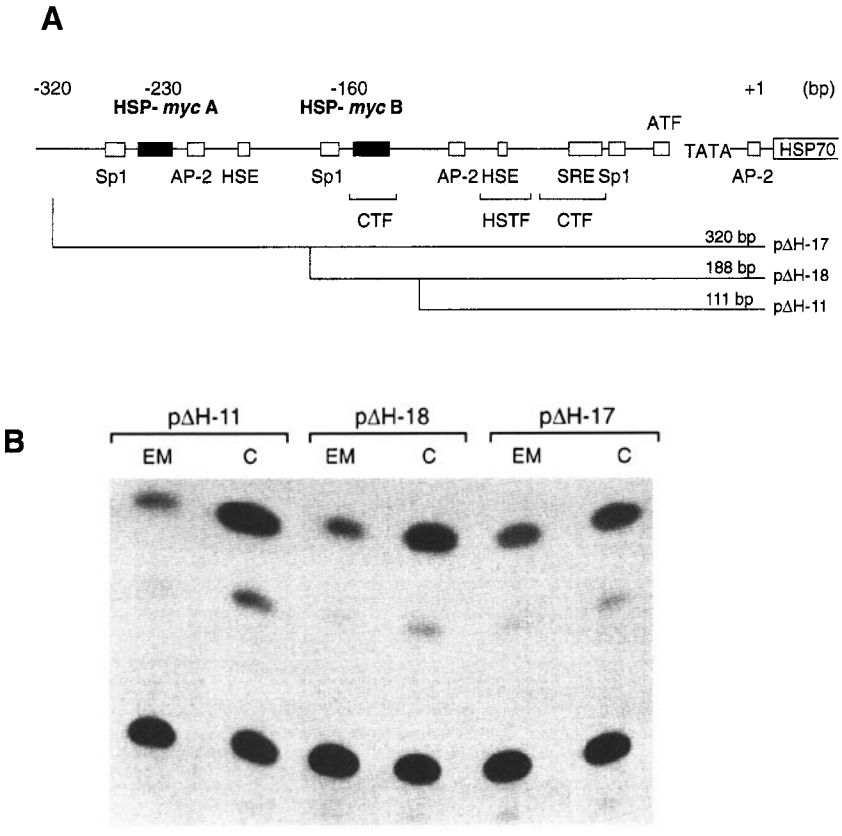

Fig. 5 Portions of DNA upstream of human HSP70 gene linked to CAT gene. (A) Diagram of three constructs of the human *hsp70* promoter. Hsp-MYC A and Hsp-MYC B protein-binding regions are shown as solid boxes. [Open boxes are other transcription factor binding sites.] Deletion constructs were: pΔH-17 *Zem*: Hsp-MYC A and Hsp-MYC B binding sites; pΔH-18 *Zem*: Hsp-MYC A binding site deleted; Hsp-MYC B binding site intact; pΔ H-11 *Zem*: Hsp-MYC A and Hsp-MYC B binding sites deleted. (Deletion constructs described in Kingston et al. 1984 and provided by Dr R. Kingston, Harvard University, Cambridge, MA, USA). (B) Constructs containing portions of DNA upstream of human *hsp70* gene, fused to the bacterial gene encoding chloramphenicol acetyltransferase (CAT), were co-transfected with Myc protein expression vector into HeLa cells as transient transfectants (the protein expression vector was provided by Dr R. Dalla-Favera, Columbia University, New York, NY, USA and is described in Wei et al. 1993). One group of transfectants was exposed to 80 mG (8 μT) 60 Hz magnetic field (at 37°C) for 20 min followed by a 20-min recovery period. The second group of transfectants served as simultaneous sham-exposed controls and the third group of transfectants was heat shocked at 43°C, followed by a 20-min recovery period. Lysates were processed for total protein and prepared for CAT assay using chromatography. CAT activity was calculated as the percentage of chloramphenicol converted to the acetylated form (Lin et al 1998).

malian cells can be protected from cell injury resulting from heat, severe metabolic stress or simulated ischemia by forced expression of *hsp70* (Williams et al 1993; Heads et al 1994; Mestril et al 1994); that synergistic effects in the protection of cells against cytotoxic conditions have been achieved with indomethacin (an anti-inflammatory drug) and elevated temperatures (Lee et al 1995).

Stress proteins, in particular Hsp70, have been implicated in the immune response with respect to infection and neoplasia (Young 1990; Jindal and Young 1991). A recent report by Tamura et al (1997) showed that in immunotherapy of mice with preexisting cancers, using heat shock protein preparations derived from autologous cancer, resulted in retarded progression of the primary cancer. Protection of tumor cells from tumor necrosis factor cytotoxicity by Hsp70 has also been noted (Jaattela and Wissing 1992).

We propose that magnetic fields can be used for stress-conditioning. Magnetic fields offer the advantages of being effective at extremely low energy levels and can be applied under conditions that are convenient for both physician and patient. Magnetic fields have been used since 1972 for healing bone non-unions (Bassett 1995), and the effect probably involves the induction of stress proteins. A strong expression of the stress proteins, Hsp28, Hsp70 and Hsp110 has been demonstrated in growth plate cartilage (Vanmuylder et al 1997) and in articular chondrocytes in response to various physiologically relevant agents (Zafarullah et al 1993).

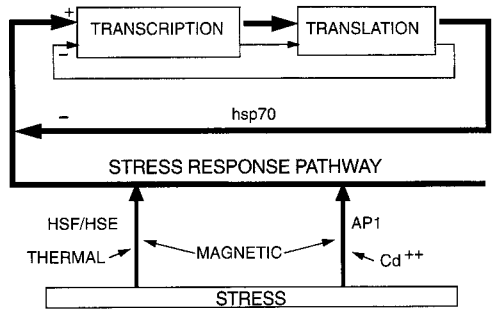

Fig. 6 Circuit diagram of autoregulatory feedback mechanism. Magnetic and thermal stresses (heat shock) feed into a common response pathway (heavy line). Magnetic fields act through the heat shock HSF pathway, but also act through the AP-1 pathway, used by the cadmium metal ion (Cd^{++}). A common negative feedback loop is mediated by Hsp70.

For clinical application it will be necessary to define the optimal magnetic field strength and duration for induction of Hsp70, as well as the duration of elevated Hsp70 levels following exposure to magnetic fields. Because of the ubiquity of stress proteins and ease of induction by magnetic fields, the potential for their use in medical applications is considerable.

UNIQUE ASPECTS OF MAGNETIC FIELD-INDUCED STRESS RESPONSE: A SUMMARY

The evidence reviewed shows that low frequency magnetic fields can be added to the many environmental factors that induce the stress response. There are several unusual aspects of the magnetic field-induced response that should be emphasized, since they appear to be unique:

- The most unusual aspect is that the magnitude of an effective magnetic stimulus is minuscule compared to a thermal stimulus: it evokes responses at an energy density 14 orders of magnitude lower than heat shock (as shown in the table below). Aside from the great sensitivity of cells to this stimulus, it is also likely that magnetic fields affect different cellular components.

Energy	**Input**	**Energy Density** (joules/m^3)
Magnetic	0.8 μT	2.6×10^{-7}
Thermal	+5.5°C	$2.3 \times 10^{+7}$

- Given these energy differences, it is not surprising that magnetic field induction of *hsp70* expression, in the absence of elevated temperature, has unusual features.
- In contrast to thermal stress, magnetic field-induced stress augments transcriptional activity in constitutively active loci as well as in chromosome regions that were not detectable as active in control cells.
- Magnetic fields activate several transcription factors in addition to heat shock factor, e.g. Ap-1, Ap-2 and Sp-1. This suggests that magnetic fields can affect several DNA regions in the promoter.
- Magnetic field activation requires Myc protein. *hsp70* promoter constructs linked to the CAT gene containing both Myc protein binding sites responded to magnetic field stimulation, whereas deletion of one MYC-binding site reduced CAT activity one-third and deletion of both Myc-binding sites resulted in no magnetic field-stimulated activity. All three constructs responded when they were heat shocked

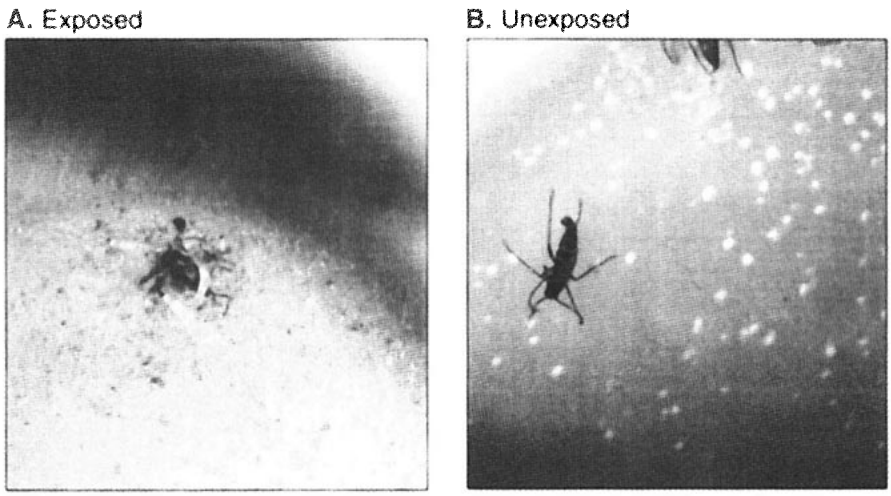

Fig. 7 Magnetic field-induced cross-protection. (A) Fertilized *Sciara coprophila* eggs exposed to 80 mG, 60 Hz for 60 min (at 20°C). (B) Fertilized *Sciara coprophila* eggs unexposed (at 20°C). Eggs from both (A) and (B) samples were heat shocked for 30 min at 37°C and were returned to 20°C incubator. Eggs in (A) hatched into viable larvae (seen as black spots, which are the mouth parts). Eggs in (B) did not survive heat shock, and are dead (white).

at 43°C. Unlike thermal induction of *hsp70* gene expression, magnetic field induction of this stress gene is mediated through the binding of Myc protein to the *hsp70* promoter.

In conclusion, it is apparent that the unusual properties of magnetically-induced stress response have advantages for clinical application, particularly for pre-conditioning to induce cytoprotection.

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