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Magnetic Field Activation of Protein–DNA Binding

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Abstract The mechanisms involved in sensing, signaling, and coordinating changes resulting from magnetic field-induced stress show substantial similarities to those of heat shock, e.g., magnetic field-induced heat shock 70 gene (HSP70) expression involves heat shock factor (HSF) activation and heat shock element binding. However, an additional requirement for transactivation of HSP70 expression by magnetic fields is the binding of Myc protein, indicating that additional elements and/or pathways are involved in the induction of HSP70 expression by magnetic fields. To investigate the possible participation of additional genetic elements in magnetic field-induced HSP70 expression, we examined both magnetic field exposure and heat shock on protein–DNA binding of the transcription factors HSF, AP-1, AP-2, and SP-1 in four human cell lines. The binding sites for these transcription factors are present in the HSP70 promoter. AP-1 binding activity, normally not increased by heat shock, was increased by magnetic fields; heat shock induced an increase only in HSF binding. Although intersecting and converging signaling pathways could account for the multiplicity of elements involved in magnetic field-induced HSP70 transcription, direct interaction of magnetic fields with DNA is also a possible mechanism. Because magnetic fields penetrate the cell, they could well react with conducting electrons present in the stacked bases of the DNA. J. Cell. Biochem. 70:297–303, 1998.

Key words: magnetic fields; heat shock; HSP70 gene expression; protein binding sites; nucleotide sequences

MAGNETIC FIELDS INDUCE HSP70 EXPRESSION

Cellular responses to a wide variety of microenvironmental cues; for example, environmental stressors, hormones, and growth factors are believed to originate at the outer surface of the plasma membrane. The signals are transmitted to the nucleus by signal transduction via protein kinase–phosphatase cascades. Transcription factors, the ultimate targets of many of these cascades, are regulated posttranslationally by phosphorylation at serines/threonines [Hill and Treisman, 1995]. The route of the signal from the cell membrane to a transcription factor can be traced in an increasing number of cases; however, due to intersecting pathways, the specificity of the transcriptional response to different stimuli is often ambiguous [Hopkin, 1997].

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Environmental-frequency magnetic fields induce significant biological changes in a variety of cells and tissues [Goodman et al., 1995; Hong, 1995]. These changes include the induction of the stress response heat shock 70-kD protein (hsp70) [Goodman et al., 1994] and several immediate early response genes, including c-*myc* [Jin et al., 1997]. The induction of heat shock 70 gene (HSP70) expression by magnetic field exposure is mediated through trimerization of heat shock factor (HSF) and binding to heat shock element (HSE) [Lin et al., 1997]. Magnetic field-induced trimerization of HSF and its binding to the HSE consensus sequence, steps involved in sensing, signaling, and coordinating cellular changes, are similar to those described for heat shock [Lis and Wu, 1993; Morimoto, 1993]. An important difference from heat shock, however, is the binding requirement of Myc protein to n**CTCT**n sequences for mediation of HSP70 expression in cells exposed to magnetic fields [Lin et al., 1998]. The Myc binding sites, C**CTCT**CA and C**CTCT**GA, in the HSP70 promoter are homologous to the Myc protein complex-binding sequence in the promoter of the c-*myc* gene [Taira et al., 1992], and they overlap with the region reported for the

regulation of inducible HSP70 expression by Myc protein in the human HSP70 promoter [Kingston et al., 1984].

Although both heat shock- and magnetic field-induced stress lead to the induction of HSP70 expression, they may use steps in several stress-activated signal transduction pathways. Because magnetic fields penetrate to the nucleus without attenuation, an additional mechanism may be direct interaction with the DNA. DNA can conduct electrons through its pi-stacked arrays of heterocyclic base pairs [McClellan et al., 1990]. Vulnerable sites on the DNA (e.g., guanine) can suffer damage due to electron flow even when an oxidizing agent is attached to the DNA at a distance [Dandliker et al., 1997]. Magnetic fields interact with moving charges, thus their interaction with the conducting electrons in the DNA may result in conformational changes. Identification of DNA regions/sequences that change their binding activity in response to magnetic field exposure would provide evidence of magnetic field interaction at the regulatory level of the gene and perhaps structural information about a magnetic field receptor.

MAGNETIC FIELDS INCREASE DNA-BINDING ACTIVITY OF HSF, AP-1, AP-2, AND SP-1

Transcription of the human HSP70 is regulated by a complex array of cis-acting promoter elements that respond to normal cell growth, disease states, and induction following physiological stress, among other conditions [Lis and Wu, 1993; Morimoto, 1993]. Multiple forms of stress-induced transcription, including heat shock and exposure to heavy metal ions, require the mediation of a single heat shock element at −100, which binds the heat shock factor in the HSP70 promoter. The sequences required for heat shock or metal ion responsiveness map to a distal domain between −107 and −68 relative to the transcription initiation site [Williams and Morimoto, 1990]. The sequences required for magnetic field responsiveness map to a domain between −230 and −160, with an HSE at approximately −195 and two Myc binding sites at −230 and −160 [Lin et al., 1998].

Genetic elements inherent in regulatory modes other than heat induction have been identified in chicken, *Xenopus*, mouse, and human HSP70 promoters [reviewed in Williams and Morimoto, 1990] and include AP-1, AP-2, and SP-1. AP-1, a sequence-specific transcriptional activator composed of members of the Jun and Fos families, forms DNA-binding dimers with other members of the AP-1 family and with c-*fos* through leucine zipper formation. AP-1 mediates gene induction by the phorbol ester tumor promotor TPA and is induced by growth factors, cytokines, T-cell activators, neurotransmitters, and ultraviolet irradiation [Karin, 1995]. The binding activation of AP-1 is pertinent because magnetic fields induce increased transcript levels for c-*fos* [Phillips et al., 1992], and an upstream DNA region of 138 base pairs (−363 to −225) in the c-*fos* promoter is responsive to magnetic fields [Rao and Henderson, 1996]. SP-1 is an *O*-glycosylated transcription factor with sequence specificity conferred through three zinc fingers in the DNA-binding domain. AP-2, responsive to retinoic acid, probably functions in morphogenesis and may act independently as both a TPA- and cAMP-inducible element.

We examined the magnetic field-induced activation of DNA binding of AP-1, AP-2, and SP-1 in four human cell lines: MCF7 and T47D, two estrogen receptor-positive breast carcinoma cell lines; HTB124 (ATTC HTB100), a normal breast cell line from a patient with a genetic predisposition to breast cancer; and HL60, human promyelocytic leukemic cells. We showed previously that electromagnetic field exposure induces rapid, transitory HSF activation in human cells and that the HSF involved is HSF1 [Lin et al., 1997]. In the present study, the magnetic field- and heat shock-induced binding activity of HSF served as a positive control for induction of binding activity. Cells at 37°C were exposed to magnetic fields (8 μT, 60 Hz) within Helmholtz coils for 20 min, followed by 20 min without magnetic fields before preparation of protein lysates for electrophoretic mobility shift assays. To ensure transcription factor specificity, competition experiments were performed. Protein lysates from sham-exposed and heat-shocked (43°C) cells served as controls.

The results from electrophoretic mobility shift assays in Table I are presented as the ratio of experimental/control ± standard error of the mean. Four to seven experiments were performed for each data point. DNA-binding activation was determined by the intensity of the radiolabeled band as quantified on a PhosphorImager 400A (Molecular Dynamics, Sunnyvale, CA). Magnetic field-exposed cells showed increased binding activity for HSF in HL60,

TABLE I. Magnetic Field-Induced Activation of HSF, AP-1, AP-2, and SP-1 in HL60, HTB124, MCF7, and T47D Cells

	Factors			
Cell lines	HSF	AP-1	AP-2	SP-1
HL60				
EM*	3.6 ± 0.12	1.3 ± 0.08	1.2 ± 0.02	1.4 ± 0.02
HS**	8.6 ± 0.3	1.0 ± 0.13	0.97 ± 0.12	1.07 ± 0.03
HTB124				
EM	2.68 ± 0.13	2.7 ± 0.16	1.7 ± 0.14	1.1 ± 0.03
HS	9.0 ± 0.021	0.98 ± 0.06	1.07 ± 0.21	1.03 ± 0.15
MCF7				
EM	1.5 ± 0.06	1.8 ± 0.05	2.1 ± 0.06	1.0 ± 0.03
HS	6.9 ± 0.18	0.91 ± 0.12	0.9 ± 0.07	1.08 ± 0.13
T47D				
EM	1.06 ± 0.04	1.6 ± 0.03	1.8 ± 0.04	1.3 ± 0.06
HS	7.5 ± 0.12	0.97 ± 0.18	0.97 ± 0.23	1.0 ± 0.09

The results are presented as the ratio of experimental/control ± SEM (controls are lysates from cells that were sham exposed). Four to seven experiments were performed to determine the binding activity of each transcription factor for each of the four cell lines. DNA-binding activation was determined by the intensity of the radiolabeled band as quantified on a PhosphorImager 400A. *Magnetic field (EM): 8 μT (80 mG), 60 Hz; 20 min of exposure followed by 20 min of no exposure at 37°C. **Heat shock (HS): 43°C, 20 min followed by 20 min at 37°C (oligonucleotides with consensus sequences for AP-1, SP-1, and AP-2; Promega E3300; oligonucleotides containing consensus sequences for the heat shock factor were kindly provided by Dr. R.I. Morimoto, Northwestern University [Mosser et al., 1988]. The MCF7 cells were generously supplied by Dr. Robert Liburdy, Lawrence Berkeley Laboratories).

HTB124, and MCF7 cells but not in T47D cells. Magnetic field-induced binding activity was observed for AP-1 and AP-2 in lysates from all four cell lines, and increased binding activity of SP-1 was observed only in HL60 and T47D cells. The variation in binding activity suggests that the responses of the transcription factors studied may be cell-type dependent.

As expected, heat shock activated HSF in all four cell lines. It did not increase binding activity of AP-1, AP-2, or SP-1. Figure 1A presents an electrophoretic mobility gel shift assay that illustrates the effects of heat shock and magnetic field exposure on DNA-binding activity of HSF and AP-1 in HL60, MCF7, and HTB124 cells. The time course for magnetic field-induced AP-1 activation in HTB124 human breast cells is shown in Figure 1B. AP-1/DNA binding specificity was demonstrated with competition experiments in Figure 1C.

CLUES TO MAGNETIC FIELD INTERACTION MECHANISMS

Energy density is a measure of the energy required for magnetic field-induced stress. Magnetic fields are a small perturbation compared with heat shock. Cellular responses are induced at an energy density 14 orders of magnitude lower than heat shock. Thus, it is not surprising that separate domains in the HSP70 promoter are responsive to magnetic fields and heat shock, including different HSEs and probably different stress-activated signal transduction pathways. Based on the magnetic field-induced binding activation of both HSF and AP-1, changes in cell behavior induced by magnetic fields may well result from a combination of the major stress-responsive transcriptional regulatory pathways. The multiplicity of elements involved in magnetic field-induced HSP70 transcription could result from intersecting and/or converging signaling pathways [Hopkin, 1997], e.g., the stress-activated protein kinase cascade terminating in the phosphorylation and activation of the AP-1 by jun N-terminal kinase and p38 [Karin, 1995] and/or the stress-activated pathway that induces nuclear translocation of the HSF monomer, HSF trimerization, and HSE binding [Lis and Wu, 1993; Head et al., 1996].

It must be considered, however, that the conventional stress-activated signal transduction pathways may not be the only mechanisms used. For example, direct stimulation of DNA by electric currents in muscle has been suggested, based on frequency-dependent protein synthesis resulting from muscle action potentials [reviewed in Blank, 1995]. Also, magnetic

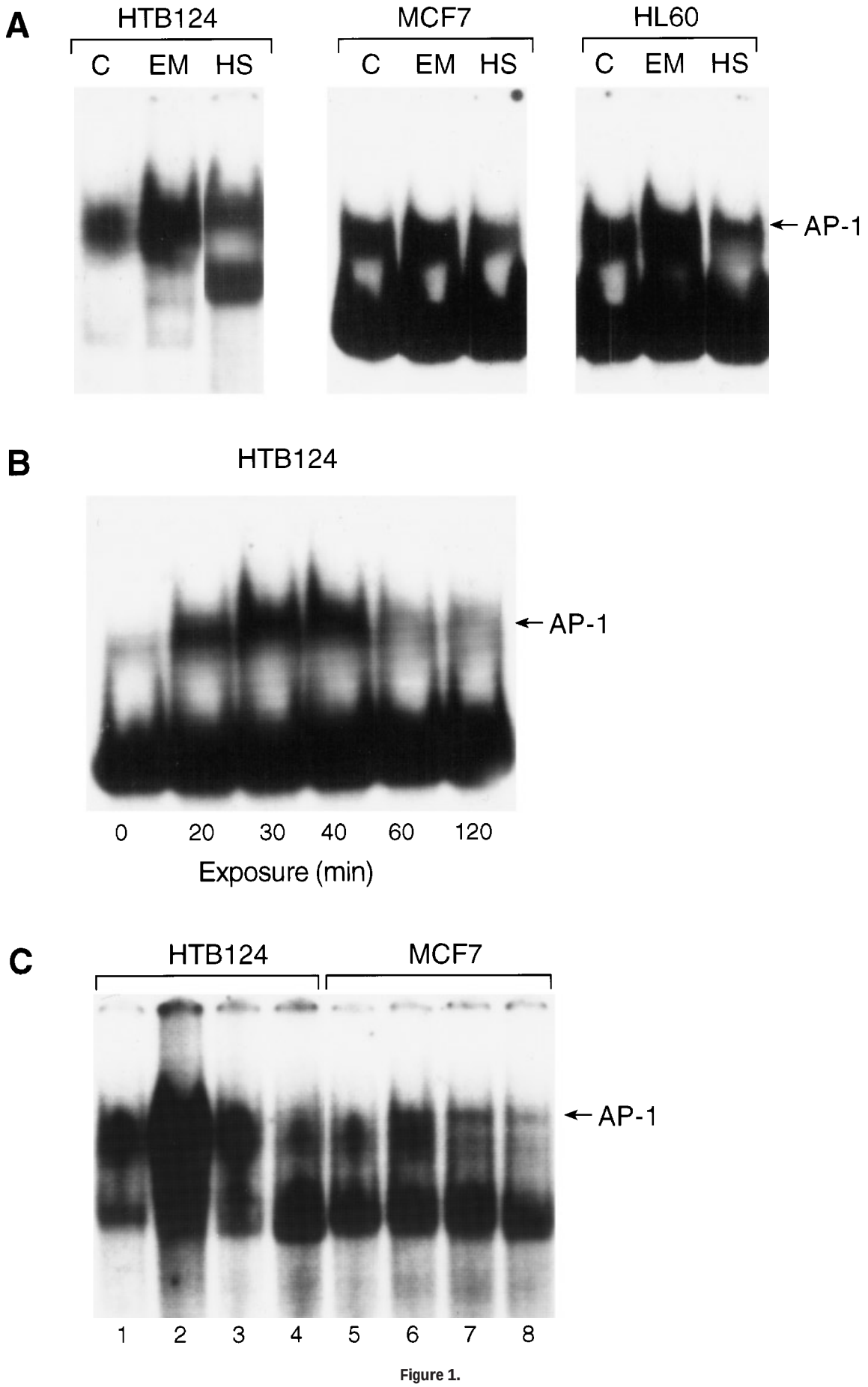
A
HTB124
C EM HS
MCF7
C EM HS
HL60
C EM HS
AP-1
B
HTB124
AP-1
0 20 30 40 60 120
Exposure (min)
C
HTB124
MCF7
AP-1
1 2 3 4 5 6 7 8

Figure 1.

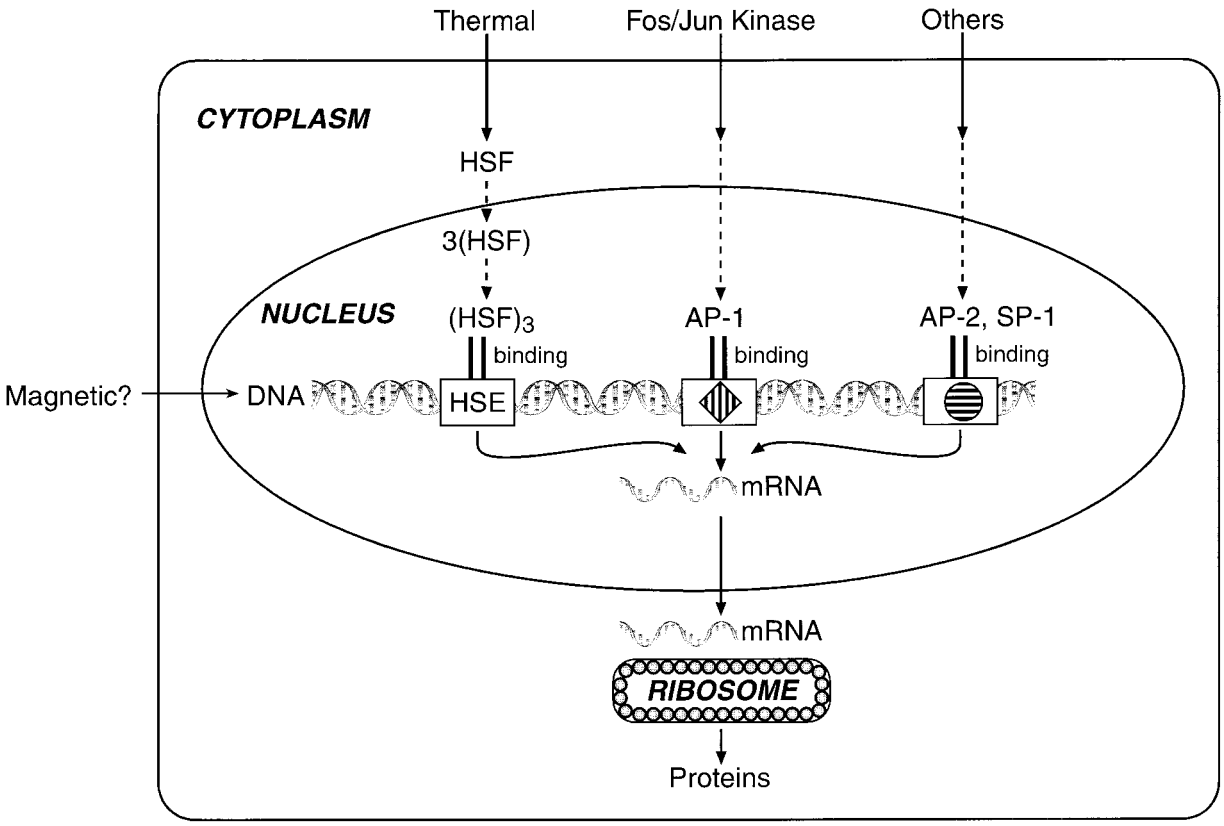

Fig. 2. Model of magnetic signal transduction pathways.

Fig. 1. A: Electrophoretic mobility shift assays showing increased heat shock factor (HSF) and AP-1 DNA binding in protein extracts from HTB124, MCF7, and HL60 cells exposed to magnetic fields. Cells were exposed for 20 min to 8 μT 60 Hz magnetic fields (EM), sham exposed (C), or heat shocked (HS). Lysates were prepared 20 min after magnetic field exposure [Lin et al., 1997]. Cells were heat shocked (HS) at 43°C for 20 min and incubated for 20 min at 37°C, and lysates were prepared. Control cells were sham exposed for 20 min, and lysates prepared after 20 min. Specific oligonucleotide consensus sequence for AP-1 (c-*jun*) was obtained from Promega (E3050). The position of AP-1 is marked. **B:** Time course for magnetic field-induced AP-1 binding activity in HTB124 cells. Cells were exposed for 0, 20, 30, 40, 60, and 120 min to an 8 μT 60 Hz magnetic field, followed in each case by an additional 20 min out of the field prior to preparation of the lysates. The specific oligonucleotide consensus sequence for AP-1 (c-*jun*) was obtained from Promega (E3050). The position of AP-1 is marked. **C:** Electrophoretic mobility shift assays for sequence specificity, AP-1–DNA binding, in competition experiments using protein extracts from HTB124 and MCF7 cells exposed to magnetic fields (8 μT, 60 Hz) for 20 min plus 20 min out of the field. **Lanes 1 and 5:** Sham-exposed control samples. **Lanes 2 and 6:** AP-1 binding activity in magnetic field exposed cells. **Lanes 3 and 7:** Competition experiments; 1:10 dilution with cold AP-1 oligonucleotide. **Lanes 4 and 7:** Competition experiments; 1:100 dilution with cold AP-1 oligonucleotide.

fields have been shown to stimulate transcription in cell-free preparations [Goodman et al., 1993; Tuinstra et al., 1997], implying that interaction with a membrane is not essential. Because magnetic fields can penetrate cells and are not limited to interactions with the membrane, a direct effect of magnetic fields on the DNA has been proposed [Blank and Goodman, 1997] through interaction with the conducting electrons in the stacked bases of the DNA [Dandliker et al., 1997]. The rate of electron flow within the stacked bases of DNA is greater than 10^6 per second, which is a current density of about 5×10^4 amperes/m^2 through the cross-sectional area of the DNA interior (2 nm in diameter). If currents of this magnitude flow in the DNA as part of normal tonic activity, then significant interactions could result from the relatively weak magnetic fields that stimulate transcription. Figure 2 presents a model of possible direct interaction of magnetic fields with upstream DNA and the respective pathways implicated in HSF, AP-1, AP-2, and SP-1 binding. Arguments using Occam's razor would suggest a simpler hypothesis: the direct interac-

tion of magnetic fields with DNA rather than the simultaneous activation of several pathways.

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APPENDIX: MATERIALS AND METHODS

Cells

HL60 (human promyelcytic leukemia) cells and T47D (estrogen positive breast carcinoma) cells were grown in RPMI 1640; MCF7 (estrogen positive breast carcinoma cells) and HTB124 (ATTC100, normal breast cells from patient with family history of breast cancer) were grown in Dulbecco's Minimum Essential Medium. Ten percent fetal bovine serum (Sigma, St. Louis, MO) was added to all cultures. Cells that grew in suspension were exposed to electromagnetic fields at cell densities of 1×10^6 cells/ml in T25 flasks (Falcon Plastics, Lincoln Park, NJ) at 15 ml/flask at 37°C. Cells that grew attached were exposed at subconfluence in Petri dishes. Cells were prepared for each experiment the previous day by aliquoting cells from a single T75 flask into individual T25 flasks or Petri dishes, thereby ensuring that control and experimental samples derived from the same original batch of cells and cell concentrations were identical. The medium was not changed again before exposure of cells to the magnetic field.

Electromagnetic Fields

Magnetic fields [Jin et al., 1997] were generated by double-wrapped Helmholtz coils (164 turns of 19-gauge copper wire around a 13- × 14-cm Plexiglas form; Electric Research Man-

agement, Pittsburgh, PA). Coils were activated prior to placing the cells in the coils to avoid exposing cells to transients. Cells were removed from the field before deactivating the coils. The coils were enclosed within mu-metal containers to shield cells from stray fields and the geomagnetic field (Ammuneal Manufacturing Corp, Philadelphia, PA) within the incubator. The sinusoidal field was generated by a Wavetek function generator (Wavetek model 21, 11 MHz) connected to a power regulator. The function generator and power regulator were situated outside the incubator. Signal parameters were monitored by a calibrated inductive search coil (Electro-Biology, Inc., Parsipanny, NJ) with a Hitachi (V-1065, 100 MHz) oscillscope. Control cells were sham exposed at the same time in the same incubator and shielded in an identical mu-metal container. To ensure that no heating resulted from the coils, temperatures were monitored with a PhysiTemp thermocouple probe (PhysiTemp, Inc. Hackensack, NJ) attached to the coils throughout all exposures (sensitivity ± 0.1°C).

Exposures

Cells were exposed to an 8 μT 60 Hz field for 20 min (at 37°C) and then removed from the field for 20 min before preparation of protein lysates. Dishes or flasks containing cells for experimental or sham exposures were within an area of the coils with a uniform field.

Heat Shock

Cells in Petri dishes were wrapped in Parafilm, placed in a mu-metal box, and immersed in a water bath at 43°C for 20 min, followed by 20 min at 37°C prior to extraction for protein lysate.

Protein Lysates

Lysates were prepared from whole cells [Mosser et al., 1988]. Cells were harvested, washed with cold phosphate buffered saline, centrifuged, and rapidly frozen at −70°C. The frozen pellets were suspended in a buffer containing 20 mM HEPES, pH 7.9, 25% (vol/vol) glycerol, 0.42 M NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 1.0 mM dithiothreoitol and were centrifuged at 14,000 rpm for 20 min. The supernatants were frozen in liquid nitrogen and stored at −70°C. Protein concentrations were determined by using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA).

Gel Mobility Shift Assay

Oligonucleotides with consensus sequences for AP-1, SP-1, and AP-2 were obtained from Promega (Madison, WI; E3300). Oligonucleotides containing consensus sequences for HSF were kindly provided by Dr. R.I. Morimoto (Northwestern University) and are described in Mosser et al. [1988]. Competition experiments used the AP-1-specific oligonucleotide diluted 1:10 and 1:100 with cold AP-1-specific oligonucleotide. These experiments ascertained sequence specificity. Protein lysates from sham-exposed and heat-shocked (43°C) cells served as controls. For supershift assays for HSF, anti-HSF1 was provided by Dr. R.I. Morimoto. Oligonucleotides containing consensus sequences to characterized binding sites were end-labeled with γ^{32}P-ATP and used as protein-specific probes. Protein lysates were run for gel shift assays on 4% nondenaturing 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).