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Genetics

Exposure of salivary gland cells to low-frequency electromagnetic fields alters polypeptide synthesis

(translational patterns/dipteran salivary gland cells)

REBA GOODMAN*† AND ANN S. HENDERSON‡

*Department of Pathology, Columbia University Health Sciences, New York, NY 10032; and ‡Department of Biological Sciences, Hunter College, The Graduate Program of the City University of New York, New York, NY 10021

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ABSTRACT **This study demonstrates that exposure of cells to extremely low-frequency electromagnetic fields can cause measurable changes in protein synthesis. *Sciara coprophila* salivary gland cells were exposed to five low-frequency (1.5–72 Hz) electromagnetic signals: three signals (1.5, 15, and 72 Hz) produced pulsed asymmetric electromagnetic fields and two signals (60 and 72 Hz) were sinusoidal. Subsequent analyses of two-dimensional gels showed that cell exposure to either type of low-frequency electromagnetic field resulted in both qualitative and quantitative changes in patterns of protein synthesis. Thus, signals producing diverse waveform characteristics induced previously undetectable polypeptides, some of which were signal specific and augmented or suppressed other polypeptides as compared with nonexposed cells. The pattern of polypeptide synthesis differed from that seen with heat shock: only five polypeptides in cells exposed to electromagnetic signals overlap those polypeptides exposed to heat shock, and the suppression of protein synthesis characteristic of heat shock does not occur.**

Diverse effects on cells and organisms have been attributed to exposure to extremely low-frequency (elf) electromagnetic fields. Some examples from experimental exposures include alterations in biopolymers, membranes, ion flux, enzymatic activity, DNA synthesis, and neurotransmitter release (for review, see refs. 1–3). In medical settings, elf electromagnetic fields are used to treat ununited fractures, avascular necrosis, pseudarthroses of bone, and experimentally induced osteoporosis (4, 5). A negative implication (from epidemiological studies) of the cellular response to electromagnetic fields is that such exposure may contribute to or cause environmental diseases. Little is known about cellular responses at the molecular level despite documented biological and clinical effects attributed to elf electromagnetic fields.

The present research was designed to test whether protein synthesis is altered by exposure of cells to elf electromagnetic fields. The response of the endoreduplicated chromosomes of *Sciara* salivary gland cells to elf electromagnetic fields was measured using two-dimensional gel electrophoresis. Measurable changes were monitored by the appearance and/or disappearance of polypeptides or, alternatively, quantitative changes in the existing protein profile.

Our previous studies examined the effect of elf electromagnetic field exposures on transcription in the salivary gland cells of *Sciara* (1, 2, 6, 7). Analyses of transcription autoradiograms showed that previously undetected transcription occurs after short exposures of these cells to several types of electromagnetic signals. The pattern of transcription at interband regions of salivary gland chromosomes was compatible with the hypothesis that mRNA species are affected. Increased synthesis of mRNA was confirmed with analysis of radioactive uridine uptake into molecular size classes expected of mRNA species (6, 7); mRNA with sedimentation coefficients of 6–10S and 20–25S was augmented or induced relative to mRNA from control cells. Most new or augmented mRNA was polyadenylylated, based on binding to oligo(dT)-cellulose columns under hybridization conditions.

In this study, a more sensitive assay of determining alterations in the pattern of polypeptide synthesis in exposed cells was used. The results showed that exposure of salivary gland cells to elf electromagnetic signals with diverse waveform characteristics and shapes caused the appearance of previously undetected polypeptides, some of which were signal specific and altered synthesis in other polypeptides from that seen in control cells. Five polypeptides associated with exposure to elf electromagnetic fields were those seen under heat-shock conditions.

MATERIALS AND METHODS

Experimental groups. The experimental samples included cells (*i*) maintained at normal growth temperature of 20°C and isolated from known electromagnetic field sources (control), (*ii*) exposed to heat shock at 37.5°C, and (*iii*) exposed to the PT-15, E-33, SP-17, and SW signals (60 and 72 Hz) (see below).

Characteristics of Electromagnetic Fields. Three different types of quasi-rectangular, asymmetric pulsed elf electromagnetic fields were used: PT-15 (pulse train at 15 Hz), E-33 (pulse train at 1.5 Hz), and SP-17 (single pulse at 72 Hz). These were compared with two sinusoidal waves (SW) modulated to 60 and 72 Hz. The waveform characteristics for each signal is given in Table 1.

Signal Generation. The generator for producing asymmetric signals was designed by Electro-Biology (Fairfield, NJ). Sine waves were produced by a Radio Shack amplifier (model 32-20-26-A, MPA-80 Realistic) and the generator. Helmholtz-aiding coils also constructed at Electro-Biology were used to deliver the signals; coils were constructed of copper wire bundles ≈1 cm in diameter wound around a square plastic form with a 10-cm opening between the sides. There was a 7.5-cm radius from the center of the form. Local geomagnetic field at the sample location was 46 μT, with an inclination of 76°N. Signals were monitored on a Tektronix (Beaverton, OR) model 2465 (300 MHz) oscilloscope with a calibrated search coil.

Exposure of Cells. Active Helmholtz-aiding coils were positioned in a vertical position so that the magnetic field was generated in a horizontal plane. The coils were placed in a tissue culture incubator at 20°C; no extraneous magnetic

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Abbreviations: elf, extremely low-frequency; SP, single pulse; PT, pulse train; SW sinusoidal waveform.

†To whom reprint requests should be addressed.

Table 1. Physical characteristics of electromagnetic signals*

Signal	Rate, Hz	Positive induced amplitude, mV	Positive duration, μsec	Burst width, msec	Negative space, μsec	Negative spike, μsec	Peak magnetic field, mT	Electric field, V/m
SP-17	72	15	380			4500	3.5	9×10^{-3}
PT-15	15	14.5	200	5	28	24	1.9	9×10^{-3}
E-33	1.5	2.5	250	30	10	4	0.38	1.5×10^{-3}
SW	72	0.8					1.1	5×10^{-4}
SW	60	0.8					1.5	5×10^{-4}

*A more extensive list of characteristics for these signals is given in ref. 6.

fields or other electrical noise above 60-Hz background was detectable at this site. Control cultures were placed in an identical incubator with a sham apparatus in another room. Signal generators were positioned outside the incubator area at room temperature. The cells in 15- × 60-mm Petri dishes were positioned within the plastic form in an area of the coils previously shown to have maximum magnetic field strength. Exposure times were 15, 30 and 45 min for all signals. The 45-min timepoint was used for two-dimensional electrophoresis to ensure adequate incorporation of the isotope for analysis. No thermal changes (detection sensitivity of 0.1°C) were measurable during exposure periods.

Preparation of Cells. Late fourth instar *Sciara coprophila* larvae (females) were used for these experiments. This larval stage can be recognized by the larval "eye spot" index, which is specific for development stage (8). Fifty pairs of salivary glands (1.8×10^4 cells) attached to the larval bodies were placed in 1 ml of Schneider's *Drosophila* media (SDM) minus methionine (GIBCO) and either (*i*) positioned and exposed to a selected electromagnetic field, (*ii*) isolated from the field under sham exposure conditions (controls), or (*iii*) heat shocked at 37°C. The salivary glands were then dissected free of the larval body in ice-cold 0.05 M Tris buffer, pH 7.6. In some experiments, analysis of polypeptide synthesis used one-dimensional gel electrophoresis (9); salivary glands were placed in SDM (minus methionine) containing [^{35}S]methionine at 50 μCi/ml (New England Nuclear; specific activity, 1184 Ci/mM; 1 Ci = 37 GBq). For two-dimensional gel electrophoresis, [^{35}S]methionine at ≈100 μCi/ml was added to each dish. Sample preparation was done in the cold in <1 min to avoid possible degradation or modification of proteins.

Total Protein Determinations. Total protein in salivary gland cells was determined for all groups and times of exposure; total protein was approximately the same in all samples except those from heat-shocked cells. Determination was both by direct measurement of protein (Bio-Rad protein assay kit) and by tracings of stained polypeptide bands on 9% and 12.5% one-dimensional gels (data not shown). The linear uptake of radioactive methionine was ascertained from the trichloroacetic acid precipitation of protein.

Analysis of Polypeptides. For analyses of polypeptides using two-dimensional gel electrophoresis, an equal number of salivary gland cells were suspended in Garrel's buffer, and details of sample preparation were as described (10). All procedures and analyses of two-dimensional gel electrophoresis were done by Protein Databases (Huntington Station, NY) (10, 11). Three sets of samples from cells exposed to five different electromagnetic signals and to heat shock, and three sets of samples from unexposed control cells were analyzed. All samples were coded and run in duplicate. The samples were loaded at 300,000 dpm per gel onto isoelectric focusing gels (pH 3–10 ampholytes) in the first dimension and onto 10.0% and 12.5% acrylamide gels in the second dimension. Exposures used fluorography. All gel analyses relied upon computer assistance for location and determination of molecular weight and ppm; spots are initially quantitated in disintegrations per min and converted to ppm when films are normalized for comparison. A computerized system scanned a section of a two-dimensional fluorogram, detected spots, and integrated spot densities. The results of all sets of experiments using 12.5% gels were standardized with respect to the experimental variables of location, molecular weight, and molecular concentration. The coefficient of variability (% error) was equal to or less than 15% in each case.

Exposures were digitized and submitted to PDQUEST analysis for matching of sets (10). This combined all samples from separate experiments and compared them with one standard (SP-17). In further analyses correlation plots were constructed using tables of matched data in which several samples were simultaneously matched. Correlation of spot analysis provided further data. The relative quantity of each spot was expressed in ppm to correct for normal variations in loading. Using this system, we were able to detect as little as 0.001% of the radiolabeled sample.

Scatter plots provided a tool for examining the reproducibility of spot detection and the integration of densities. The matched data from duplicate scans were plotted as a histogram of ratios and expressed as a scatter plot in which each protein was plotted according to its intensity in the first scan (*x* axis) versus its intensity in the second scan (*y* axis). This type of plot displays both deviations from a ratio of 1 and the intensity of each spot.

RESULTS

Polypeptide patterns in cells exposed to electromagnetic fields were different, both quantitatively and qualitatively, for each type of signal and different from those seen in controls or in heat-shocked cells.

A total of 340 polypeptides was resolved in experimental and control groups. The largest number of polypeptides (96% of total) was seen in cells exposed to the asymmetric single-pulse SP-17 signal, as compared with 73% in control cells (Fig. 1). There were distinct differences in polypeptide

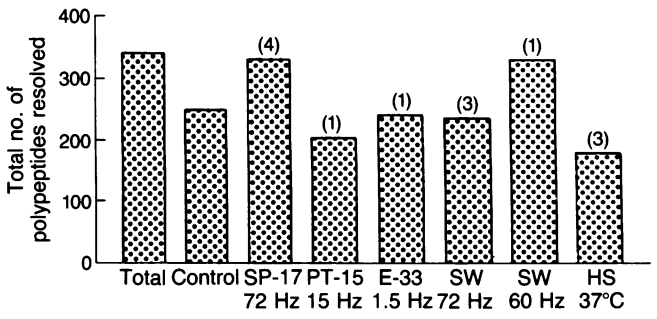

FIG. 1. Distribution of polypeptides among unexposed cells and cells exposed to each of the electromagnetic signals and heat shock (HS). Numbers in parentheses indicate the number of signal-specific polypeptides seen; 340 polypeptides (total) were resolved in experimental and control groups.

profiles in cells exposed to electromagnetic fields as compared with those in nonexposed control cells (see Fig. 2). Relative to controls, polypeptides were increased, suppressed, or absent in exposed cells. In addition, new (undetected in controls) polypeptides were seen in cells exposed to the electromagnetic signals.

Fifty-three molecular weight categories were selected for comparison of polypeptides between unexposed cells and cells exposed to electromagnetic signals or heat shock on the basis of ppm values that exceeded 200. Fig. 3 shows the ratio of exposed cells to unexposed cells. The general pattern is distinctly different for each group. Further, there were both new and deleted polypeptides in the experimental groups as compared with the controls (Fig. 4). Among all polypeptides resolved, some were signal specific, i.e., not present in any other experimental group or the control. Two signal-specific polypeptides were found among the 53 M_r groups in cells exposed to the SP-17 and SW-72 signals. Table 2 summarizes the major differences seen in control, experimental, and heat-shocked cells.

Polypeptide synthesis in heat-shocked cells was suppressed as expected. Five heat-shock polypeptides (polypeptide overlaps with heat-shock polypeptides), not detectable in control preparations, were present in cells that were exposed to electromagnetic signals (Fig. 4). These included a polypeptide with M_r of 63,800 in cells exposed to any of the five signals.

The polypeptide pattern produced in *Sciara* with heat shock differed somewhat from that seen in *Drosophila*. At least seven polypeptides ($M_r \times 10^{-3}$ = 82, 70, 68, 27, 26, 23, and 22) have been reported in one-dimensional gels of *Drosophila* salivary gland cells exposed to heat shock. This pattern in *Drosophila* cells was repeated using our experimental conditions. In *Sciara* cells, the distinction between control and heat-shocked samples was less pronounced in the 82,000 region as compared with the same region in the *Drosophila* polypeptide pattern; the M_r 70,000 band was present in the *Sciara* pattern but was obscured by multiple bands in that region. Other bands were noted in the M_r region of 30,000–40,000 that were not seen in polypeptides from *Drosophila* cells.

Polypeptides were commonly grouped to determine which polypeptides were consistently present in combinations derived from the five signals—i.e., polypeptides with identical characteristics of M_r and isoelectric point that were common to two, three, four, or five of the signal types. This analysis was made to determine whether any feature of the electromagnetic signals correlated with the presence or absence of polypeptides common to the groupings. When consistently low ppm values are omitted, 144 combinations of polypeptides overlapped two or more groups. The most frequent combinations were polypeptides common to exposure to the SP-17 (72 Hz) and SW (72 Hz) signals. Twenty polypeptides were found to be common to SP-17 (72 Hz) and SW (72 Hz) only, a correlation suggesting that the induction of at least some polypeptides is frequency dependent. Other combinations also occurred at high frequency. For example, 13 polypeptides were present in cells exposed to the SP-17 (72 Hz) and SW (60 Hz) signals, and 8 polypeptides were common to cells exposed to the SP-17 (72 Hz) and E-33 (1.5 Hz) signals.

DISCUSSION

The results show distinct and specific changes in protein synthesis patterns in response to short exposures of cells of elf electromagnetic fields. Some changes were signal specific.

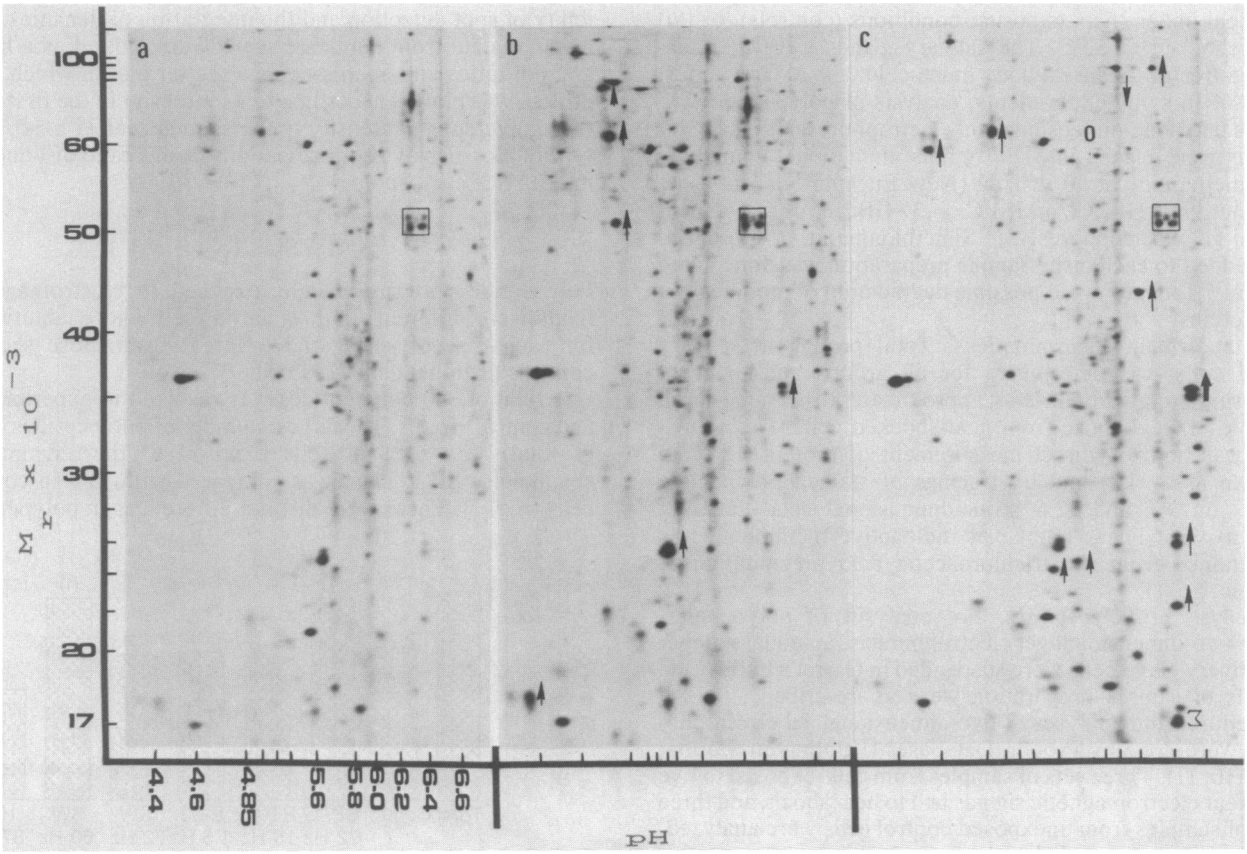

FIG. 2. Comparison of autoradiographic patterns following two-dimensional gel electrophoresis (12.5%) of *Sciara* salivary gland ^{35}S-labeled polypeptides; part of each autoradiograph is shown. Isoelectric points for the gels were 3.5–10.0. The polypeptide pattern characteristic of unexposed control cells (*a*), from cells exposed to the SP (72 Hz) signal (*b*), and from cells exposed to the SW-72 signal (*c*) is shown. Polypeptides characteristic of heat-shock proteins are boxed. Some of the polypeptides that were increased (↑) or decreased (↓) relative to control samples are marked by arrows at right of spot. Polypeptides not previously seen in control groups are designated by ℨ.

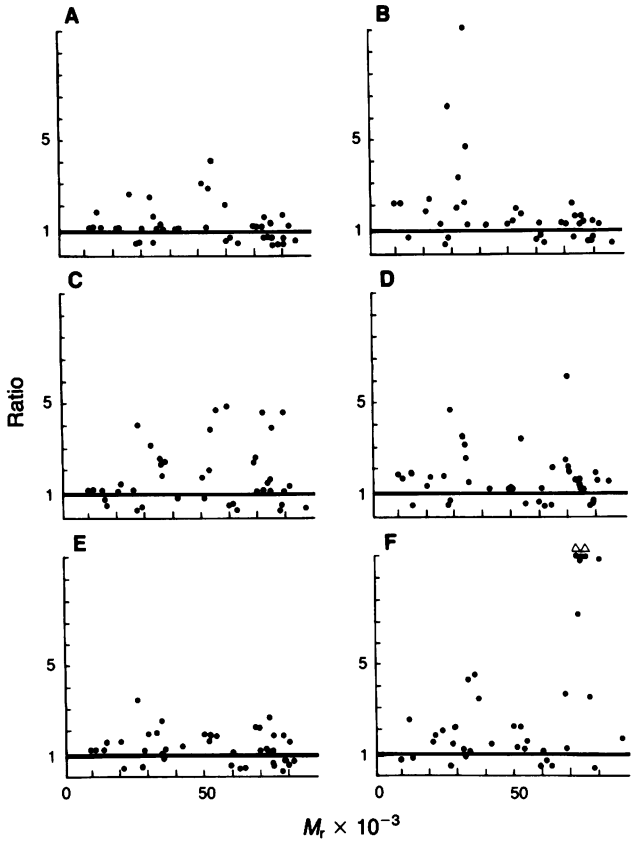

FIG. 3. Relative quantitative distribution of 53 polypeptides that exceeded 200 ppm in any group. The ratio is expressed as ppm for each experimental group relative to control ppm. Letter code represents the type of exposure: (*A*) SP-17 signal; (*B*) SW-72 signal; (*C*) SW-60 signal; (*D*) E-33 signal; (*E*) PT-15 signal; (*F*) heat shock. Triangles on the graph of heat-shock polypeptides indicate values in the M_r 70,000–73,000 range, reflecting distribution around the M_r 70,000 heat-shock protein. Ratios are as follows: M_r 70,000, 6.3; M_r 71,900, 46; M_r 72,100, 17; M_r 72,800, 12.5; and M_r 73,200, 13.

A few polypeptides seen after cell exposure to elf electromagnetic fields overlap those seen after cell exposure to thermal shock. However, the overall pattern, number of polypeptides resolved, and degree of augmentation were distinctly different. Several other factors argue against a heat-shock response in cells exposed to electromagnetic fields, including the fact that no temperature changes in the medium surrounding the cells exposed to the signals was detected. Another possible cause of the effects attributed to electromagnetic fields could be undetected sources of noise,

Table 2. Comparison of 53 major M_r groups in exposed cells with control cells

	SP-17 72 Hz	PT-15 15 Hz	E-33 1.5 Hz	SW 72 Hz	SW 60 Hz	HS 37°C
Highly augmented	0	0	5	3	6	8
Augmented	12	18	19	15	15	14
Suppressed	14	12	10	11	12	9
Equal to control	16	10	5	9	8	7
Absent	5	9	11	11	9	8*
New—not detected in control	5	4	3	3	3	5
New—not present in any other group	1	0	0	1	0	2

Comparison of the distribution of 53 major M_r classes. All comparisons are relative to control values. Highly augmented indicates a value four times or greater that seen in controls. HS, heat shock.

*Two of the eight polypeptides were also missing in controls.

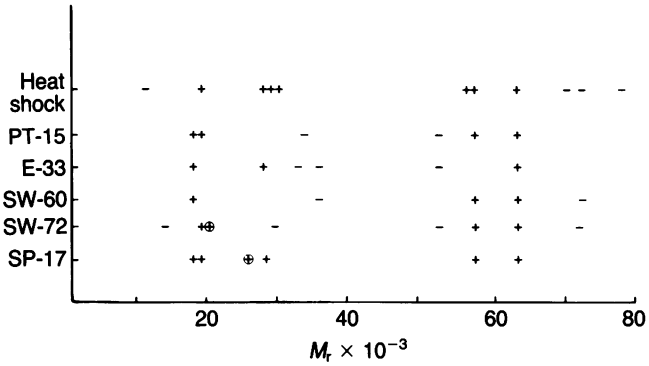

FIG. 4. Molecular weight distribution of previously undetected and deleted polypeptides among 53 major molecular weight groups. A + indicates a new (not detected in controls) polypeptide. Polypeptides seen in controls but not in the experimental group indicated are shown by −. A circle around the + value indicates a signal-specific polypeptide.

such as microwaves, resulting in localized heat shock to the cells, but this is theoretically unlikely under the conditions of the present experiments (for discussion see ref. 12). Finally, other experiments in our laboratory (unpublished data) using heat shock have shown that transcriptional changes are not detectable until the temperature is at least 10°C above the normal growth temperature of *Sciara* (20°C). Therefore, the cellular response to electromagnetic fields in *Sciara* cells probably did not result from a partial heat-shock effect, but we cannot rule out possible synergistic action.

Renewed interest in electromagnetic fields in the 1- to 100-Hz range has occurred within the last few years, partly because of increased environmental exposures to such fields, but also because of reports of experimentally induced cellular changes seen in such fields, including some with relevance to human cancers (13, 14). The exact mechanism(s) whereby such changes occur has not been delineated and is now being investigated by many laboratories. A major problem in this research is the number of variables, among which are characteristics of the signals—e.g., symmetry or asymmetry of the waveshape—and variations within the field, which depend on cell position within the coils and rate of change of coil current. The rate of change of the current depends on many factors, including amplitude, shape of the voltage pulse, and resistance and inductance of the coil (15).

Within a larger framework is the question of whether it is the time-varying-induced electric field, the magnetic field, or a synergistic action that elicits cellular response (see refs. 15 and 16). When current in Helmholtz coils is varied with time, an induced electric field and an induced magnetic field are present. McLeod *et al.* (17) and Parkinson (15) calculated that if induced electric field is the critical factor, then the effects should depend on cell location within the coil geometry, but similar arguments can be made for magnetic fields (16).

In this study, we looked at both symmetrical and asymmetrical signals with frequency ranges from 1.5 to 72 Hz, magnetic field components from 0.38 to 3.5 mT, and electric fields from 1.5×10^{-3} to 5×10^{-4} V/m. A response by the cell, albeit different for each signal, occurred in the form of translational alterations irrespective of signal type. The response was independent of the position of cells within the coil because this was standardized for all experiments. There is some evidence from these studies that suggest some effects seen could be frequency related but this correlation cannot yet be validated.

Theoretically, cell exposure to electromagnetic fields could result in many types of interactions and changes within the cell. The range of reported effects in cells or tissues exposed to elf electromagnetic fields is large (17, 18). Many

proposed models relate electromagnetic fields to cellular activity, including selective gene activation via the nuclear membrane (19) and other specific biological response systems (20). We deliberately chose to study basic molecular features on the hypothesis that any measurable effect within the cell will be caused by or reflected in the transcriptional or translational patterns. All organisms so far examined respond to various kinds of stress (21), and a change in transcription or translation is the most direct measure of stressed cell state. The ensuing result is activation of a limited number of specific genes that were either previously silent or not detectable. We assume that the translational changes seen after exposure to electromagnetic fields is a phenomenon of stress response, albeit different from that seen previously and with some specificity related to electromagnetic field type. How and at what basic level electromagnetic fields influence cells remains to be answered.

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