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RADIATION RESEARCH **164**, 277–285 (2005)
0033-7587/05 $15.00

Extremely Low-Frequency Electromagnetic Fields do not Affect DNA Damage and Gene Expression Profiles of Yeast and Human Lymphocytes

Cristina Luceri,[a,1] Carlotta De Filippo,[a] Lisa Giovannelli,[a] Marta Blangiardo,[b] Duccio Cavalieri,[b] Filippo Aglietti,[b] Monica Pampaloni,[b] Daniele Andreuccetti,[c] Lapo Pieri,[c] Franco Bambi,[d] Annibale Biggeri[b] and Piero Dolara[a]

[a] *Department of Pharmacology and* [b] *Department of Statistics, University of Florence, Florence, Italy;* [c] *Institute for Applied Physics "Nello Carrara", National Research Council, Florence, Italy; and* [d] *Transfusion Unit, Meyer Hospital, Florence, Italy*

Luceri, C., De Filippo, C., Giovannelli, L., Blangiardo, M., Cavalieri, D., Aglietti, F., Pampaloni, M., Andreuccetti, D., Pieri, L., Bambi, F., Biggeri, A. and Dolara, P. Extremely Low-Frequency Electromagnetic Fields do not Affect DNA Damage and Gene Expression Profiles of Yeast and Human Lymphocytes. ***Radiat. Res.*** **164, 277–285 (2005).**

We studied the effects of extremely low-frequency (50 Hz) electromagnetic fields (EMFs) on peripheral human blood lymphocytes and DBY747 *Saccharomyces cerevisiae*. Graded exposure to 50 Hz magnetic flux density was obtained with a Helmholtz coil system set at 1, 10 or 100 μT for 18 h. The effects of EMFs on DNA damage were studied with the single-cell gel electrophoresis assay (comet assay) in lymphocytes. Gene expression profiles of EMF-exposed human and yeast cells were evaluated with DNA microarrays containing 13,971 and 6,212 oligonucleotides, respectively. After exposure to the EMF, we did not observe an increase in the amount of strand breaks or oxidated DNA bases relative to controls or a variation in gene expression profiles. The results suggest that extremely low-frequency EMFs do not induce DNA damage or affect gene expression in these two different eukaryotic cell systems.

INTRODUCTION

Electromagnetic fields have been suggested to have detrimental effects on cell function and to influence chronic pathological processes such as leukemia and cancer, although epidemiological data for exposed humans are conflicting or inconclusive (*1–3*). Experimental evidence linking these putative deleterious effects to plausible biological mechanisms is still lacking. Although the literature on the cellular effects of extremely low-frequency electromagnetic fields (EMFs) is abundant, it is somewhat confusing. Most studies conclude that extremely low-frequency EMFs do not induce DNA damage, point mutations, gross chromosomal alterations or micronucleus formation (*4*). However, some reports show cellular effects of extremely low-frequency EMFs, such as induction of mutations in MeWo cells exposed to high-density (400 mT) magnetic fields (*5*), activation of *FOS, JUN* and *MYC* transcription in T-lymphoblastoid cells exposed for 15–120 min to a 100 μT sinusoidal magnetic field (*6*), and increased expression of the growth-associated protein (GAP43) in human glioma MO54 cells after exposure to 5 mT with a peak at 10 h (*7*). Many studies have been conducted using HL60 human myeloid leukemia cells, which are known to respond to various stressors. In these cells, 50 Hz magnetic fields were able to induce heat-shock proteins (HSP70, A, B, C) at flux densities between 10 and 80 μT (*8*), to increase the expression of two cytokine receptors (TNFRp75 and IL-6Rα) after a 72-h exposure at 0.1 and 0.8 mT (*9*), and to activate CREB (cyclic-AMP responsive element binding protein) binding activity at 0.1 mT in a time-dependent manner (*10*).

Co-exposure of human lymphocytes to benzo(a)pyrene and extremely low-frequency EMFs at 0.8 mT has been reported to cause a cumulative increase in micronuclei and sister chromatid exchanges (*11*). Furthermore, intermittent but not continual exposure to 1 mT for 24 h increases DNA strand breaks in diploid fibroblasts (*12*), as does *in vivo* exposure of rats to 10 μT for 24 h (*13*) and in the brain of mice exposed to 0.5 mT magnetic fields for 14 days (*14*).

Some studies of extremely low-frequency EMFs have focused on variations in cell proliferation and differentiation. For example, increased proliferation in the mammary gland was documented after a 2-week exposure of rodents to extremely low-frequency EMFs at 100 μT (*15*), whereas exposure at 4 μT blocks cell differentiation and at higher intensity (100–1000 μT) stimulates proliferation of erythroleukemia cells (*16*).

Because there are no published systematic studies, we decided to study the variation in overall gene expression after exposure to extremely low-frequency EMFs using DNA microarray technology. The impact of any given exposure on human health is very complex, so gene expres-

[1] Address for correspondence: Department of Pharmacology, University of Florence, Viale Pieraccini 6, 50139, Florence, Italy; e-mail: cristina.luceri@unifi.it.

sion profiling, through the use of microarray technology, can be highly informative and can add to the information obtained with established classical methodologies.

We analyzed the effects of extremely low-frequency EMF exposure on peripheral blood human lymphocytes and on *Saccharomyces cerevisiae*. Human leukocytes, particularly lymphocytes, have been the subject of several studies investigating the effect of EMF exposure and provide easily accessible normal human cells for *in vitro* studies. *S. cerevisiae* is a good model for studying gene expression profiles during exposure to supposedly detrimental environmental stimuli, since it is one of the most intensively studied eukaryotic systems at both the genetic and molecular levels. Gene expression results obtained with microarrays can be interpreted in the context of a well-characterized cellular network (more than 80% of the yeast genes have been assigned to a functional class), and a single microarray containing the entire genome permits exploration of all possible variations of gene expression in this cell system.

We also assessed the genotoxic effects of extremely low-frequency EMFs on human lymphocytes using the microgel electrophoresis method, commonly known as the comet assay, to compare possible variations in gene expression with an established methodology.

MATERIAL AND METHODS

The HC-50 Helmholtz Coil System

The HC-50 exposure system was developed at IFAC-CNR (Institute for Applied Physics ''Nello Carrara'' of the Italian National Research Council, Florence, Italy) and consisted of a Helmholtz pair system comprising two parallel, coaxial, circular coils having a diameter of 40.6 cm. The distance between coils was equal to their radius (20.3 cm). Each coil was made up of 16 turns of 0.5-mm-thick enameled copper wire. The two coils were connected in series so that the same current flowed in both.

The system generated a nominal magnetic flux density (i.e. the magnetic flux density in the ''center'' of the system, which is the midpoint of the segment connecting the centers of the two coils) proportional to the coil current. Therefore, the field strength can be regulated by varying the drive current supplied by an adjustable power unit. The power unit was designed to allow the HC-50 to produce a low-frequency (50 Hz) sinusoidal magnetic flux density, with intensity varying from 1 to more than 150 μT. For example, a nominal magnetic flux density of 100 μT was achieved by applying a coil current of 1.41 A; in this last case, the two coils dissipated a total power of approximately 7 W. The HC-50 system also featured a switch that could reverse the current flow in just one of the two coils. In this way, the coils dissipate the same amount of power (for a given current intensity) but produce a zero nominal magnetic flux density, thus allowing us to check for unwanted temperature effects.

The HC-50 power supply unit was equipped with a solid-state current meter whose liquid crystal display was calibrated to indicate the nominal magnetic flux density intensity in microtesla.

The actual magnetic flux density is a vector function of the position inside the Helmholtz coil system (being exactly equal to the nominal magnetic flux density in the system center only), but calculations and measurements show that intensity and direction errors with respect to the nominal magnetic flux density are less than 1% in a spherical volume centered in the system center and have a radius equal to one-third of the coil radius, approximately 7 cm in the HC-50 (*17*).

The Helmoltz coil system is a very robust device, and its properties are only slightly affected by structural imperfections (*17*). Scrupulous construction and precise calibration of the current meter (made similar to a Keithley 2001 high-performance digital multimeter) guaranteed the overall accuracy, which was within 3% of the 7-cm-radius spherical volume. Although the system accuracy is too high to be assessed with standard magnetic flux density meters (rather, the HC-50 itself can be used as a calibration device for such probes), it was checked and confirmed as far as possible with a professional commercial magnetic flux density meter (EMDEX II by Enertech Consultants); the same instrument was also used to evaluate the background field intensity in the incubators and in the environment where cell exposure took place.

Experiments with Saccharomyces cerevisiae

S. cerevisiae strain DBY747 (*MATa his3-Δ1 leu2-3,112 ura3-52 trp1-289a gal*^{s} *can1 CUP*^{r}) was grown in 1% yeast extract/2% peptone/2% glucose (YPD medium; Sigma, Milan, Italy) at 30°C. The cells exposed to extremely low-frequency EMFs (50 Hz; 1–100 μT) were grown in a water bath inserted in the HC-50 Helmholtz coil system with the water temperature set at 30°C. The motor driving the water bath to the incubator was placed at a distance that avoided any external effects of the extremely low-frequency EMF (more than 2 m). Unexposed cells were grown in a different water bath, at 30°C, located in a laboratory where the measured electromagnetic field was <0.1 μT. The cells were grown for 18 h in the presence or absence of the extremely low-frequency EMF up to a density of 1.6×10^7 cells. Each experiment was performed in duplicate.

Human Lymphocytes

Lymphocytes were isolated from buffy coats of blood from 18 donors (age 20–50 years) from the Transfusion Unit of the Meyer Hospital (Florence, Italy) using the Lymphoprep™ (Axis-Shield, Oslo, Norway) protocol. The experimental plan was approved by the local Ethical Committee, and informed consent was obtained from all donors.

Cells were counted in a Neubauer chamber and analyzed for vitality with the Trypan Blue exclusion method. Lymphocytes were subsequently suspended in RPMI 1640 medium (Invitrogen, San Giuliano Milanese, Milan, Italy), containing 100,000 U/liter penicillin G (Sigma, Milan, Italy), 100 mg/liter streptomycin sulfate (Sigma), 10% heat-inactivated fetal bovine serum (Invitrogen, San Giuliano Milanese), and L-glutamine (2 m*M*) at a final concentration of 1×10^6 cells/ml.

Using an EMDEX II magnetic flux density meter, we determined a background level of EMF of about 1 μT before switching on the Helmholtz apparatus inside the cell culture incubators (set at 37°C, 5% CO_2 and 95% O_2 with 95% humidity) in the zone where the cells were incubated, as stated above.

Each lymphocyte population was divided into two aliquots. The exposed cell aliquots were incubated at the desired field intensity in a cell incubator containing the Helmholtz coil system, and the unexposed cell aliquots were placed in a second incubator used in parallel (Forma Scientific Inc., Columbus, OH and Heraeus Scientific Instruments, Milan, Italy). The temperature was monitored at the site of the cultures in the incubation medium with a 0.1 precision thermocouple connected to an electronic temperature monitor and was maintained at 37 ± 0.5°C. As an additional control, some experiments were performed by reversing the current in one of the coils in the HC-50 (sham exposure).

To determine whether the 1 μT intensity was able to affect gene expression or to induce DNA damage, we created a system capable of eliminating the background field intensity present in the standard cell incubator when the electric appliances (heating systems and electric valves) were turned on. Thus, for low-exposure experiments, the flasks containing the lymphocytes were maintained at 37°C in air by means of two water baths placed in separate areas of the laboratory where the background level was <0.1 μT. To avoid interference from the electrical systems, the water baths were kept at a distance of about 2 m using plastic tubes for water circulation. The temperature in each water bath was monitored as described previously.

The unexposed aliquot of lymphocytes was maintained at 37°C in a water bath exposed to an electromagnetic field <0.1 μT, and the exposed aliquot was incubated simultaneously in a second water bath positioned between the coils of the Helmholtz coil system set at 1 μT.

Comet Assay

Aliquots of the exposed and unexposed lymphocyte suspension containing about 200,000 cells were centrifuged at 250*g* for 10 min, and the resulting pellets were resuspended in low-melting agarose (Fisher Scientific, St. Louis, MO), layered on microscope slides, and run through the comet assay as described previously (*18*). The comet assay was used to measure both DNA breaks and oxidized bases according to the modification described by Collins *et al.* (*19*). This involves using a repair endonuclease, the bacterial formamidopyrimidine DNA glycosylase (FPG), which recognizes oxidized purines and formamidopyrimidine lesions and introduces further breaks at these sites. Briefly, the slides with the agarose-embedded cells were subjected to a lysis step (1 h incubation at 4°C in 1% *N*-lauroyl-sarcosine, 2.5 *M* NaCl, 100 m*M* Na_2EDTA, 1% Triton X-100, 10% dimethylsulfoxide, pH 10.0). After the lysis step, slides were washed three times in enzyme buffer [40 m*M* Hepes-KOH, pH 8.0, 100 m*M* KCl, 0.5 m*M* EDTA, 0.2 mg/ml BSA) and then incubated at 37°C for 60 min with 80 μl of the (1:1000) FPG enzyme for purine oxidation detection, a kind gift of Dr. A. R. Collins, University of Oslo]. Control slides from the same sample were incubated in enzyme buffer without FPG. Each experiment was run in duplicate; thus four slides were run for each subject. All experimental slides were placed in an ice-cold electrophoresis chamber containing alkaline electrophoresis solution (300 m*M* NaOH, 1 m*M* Na_2EDTA, pH 13.0) for 20 min to allow DNA unwinding. The electrophoresis was subsequently conducted for 20 min at 0.8 V/cm and 300 mA. At the end of the electrophoresis, the slides were washed with neutralization buffer (40 m*M* Tris-HCl, pH 7.4), stained with ethidium bromide overnight, and analyzed the following day.

Microscopic analysis was carried out by means of a Labophot-2 microscope (Nikon, Tokyo) provided with epifluorescence and equipped with a rhodamine filter (excitation wavelength 546 nm; barrier 580 nm). The images of 50 randomly chosen nuclei per slide were captured and analyzed using custom-made imaging software coupled with a CCD camera (model C5985, Hamamatsu, Sunayama-Cho, Japan). The system provided a measurement of the percentage of DNA that migrated out of each nucleus (the comet tail). Data expressed as the percentage of DNA in the tail were converted to break frequency by a calibration method using X and γ rays (*20*). The value for DNA damage obtained from slides without enzyme incubation estimated the number of DNA strand breaks, whereas specific oxidative damage on purines (FPG-sensitive sites) was assessed for each subject by subtracting the number of breaks in the cells on buffer-incubated slides from that obtained for cells on the slides incubated with FPG. As a positive control, lymphocytes were exposed *in vitro* to 1 Gy γ rays, a stimulus that is known to induce DNA breakage at a frequency of about 3 breaks/10^{10} Da DNA. The level of DNA damage obtained under these conditions was 4.5 ± 0.32% DNA in the tail. Statistical analysis was carried out by means of one-way ANOVA.

cDNA Microarray Construction

Yeast. A set of clones containing 6212 verified ORFs, representing the entire yeast genome, were obtained from Research Genetics (Huntsville, AL) and amplified to levels required for preparation of DNA microarrays by PCR. Some of the longer ORFs were amplified with the Gibco/BRL Elongase Amplification Kit (Invitrogen, San Giuliano Milanese), using 40 cycles of 1 min at 95°C denaturation, 1 min at 55°C annealing, and 10 min at 68°C elongation. We obtained an amplified product confirmed by agarose gel electrophoresis for 98% of the ORFs. The amplified DNA was precipitated with isopropanol, washed with 70% EtOH, and resuspended in 50 μl of spotting solution (3× SSC). The DNA was spotted on CMT-GAPS amino-silane-coated glass slides (Corning, NY), using the Omnigrid 100 arrayer (GeneMachine, CA).

Human. The human oligonucleotide array was constructed using the Oligo Set™ (Operon Technologies, CA) a commercial collection of 13,971 oligonucleotides, 70 mer, representing well-characterized human genes. The lyophilized oligonucleotides were resuspended in 3× SSC and printed using the Omnigrid 100 arrayer on poly-L-lysine glass slides (Erie Scientific, Portsmouth, NH). After printing, yeast and human microarrays were post-processed on the glass matrix following DeRisi's laboratory procedure (http://derisilab.ucsf.edu/).

RNA Extraction

At the end of incubation, yeast cells were pelleted and washed once in distilled water, and the pellets were flash frozen and stored at −80°C. Total yeast RNA was isolated using a hot-acid-phenol method following DeRisi's laboratory procedure (http://derisilab.ucsf.edu/).

Lymphocytes from six donors were pelleted at the end of the 18-h exposure period, washed once in PBS, suspended in RNAlater (Qiagen, Milan, Italy), and frozen at −80°C. Total RNA was extracted using the RNeasy Maxi kit (Qiagen) and concentrated by precipitation in 96% ethanol. The RNA was quantified spectrophotometrically, and the quality of each sample was checked by electrophoresis on a 1% agarose gel. RNAs were then stored at −80°C. We created pools for each intensity value by mixing equal quantities of RNA; each pool was composed of six RNA samples.

Preparation of Fluorescently Labeled DNA and Microarray Hybridizations

We used the labeling method described by DeRisi (http://derisilab.ucsf.edu/). Briefly, the reactive amine derivative of dUTP, 5-(3-aminoallyl)-2′-deoxyuridine 5-triphosphate (Sigma) was incorporated by Superscript II reverse transcriptase (Invitrogen, San Giuliano Milanese) and 25 μg of total RNA using oligo dT (Invitrogen, San Giuliano Milanese) and random examers (Roche, Monza, Milan, Italy). The ratio between aa-dUTP and dTTP was 2 aa-dUTPmolecules to 3 dTTP molecules. After synthesis of cDNA, RNA was hydrolyzed by addition of sodium hydroxide and EDTA to a final concentration of 100 m*M* and 10 m*M*, respectively, and incubated at 65°C for 10 min. The hydrolysis reaction was neutralized with 1 *M* Hepes. After removing free nucleotides by purification, the amino-allyl labeled samples were coupled to succinimidyl ester of Cy3 and Cy5 (Amersham, Milan, Italy). The exposed samples were labeled with Cy5 and the corresponding samples that were not exposed to the electromagnetic field were labeled with Cy3. The two labeled probes were purified again, mixed and then applied to the microarray. The incubation was performed at 63°C for 14–18 h.

Data Acquisition, Normalization and Analysis

Fluorescent DNA bound to the microarray was detected with a GenePix 4000 microarray scanner (Axon Instruments, Foster City, CA) using the Genepix 4000 software package to locate spots in the microarray.

Since the process of microarray fabrication is subject to much variability and can contain a large amount of experimental noise, we used visual and analytical controls as described by Simon *et al.* (*21*) to detect spots of low quality that might produce potentially erroneous information. There were 4114 (66.2%) yeast and 7181 (51.4%) human cell genes with ''good'' quality signals in all the slides used in the experiments.

Therefore, we analyzed only the variation in expression of the genes that passed the quality controls described above. Since the different pins used in depositing the microarray spots are a possible source of variability, we carried out a local pin tip-dependent normalization (lowess) described in Yang *et al.* (*22*) using the SMA library implemented in R (www.R-project.org).

Each slide was then analyzed following Newton's approach (*23*). Figures 1 and 2 show the variation in expression of each gene and the statistical contour lines at different probability values for each array in human lymphocytes and yeast. For each slide, data were presented as a

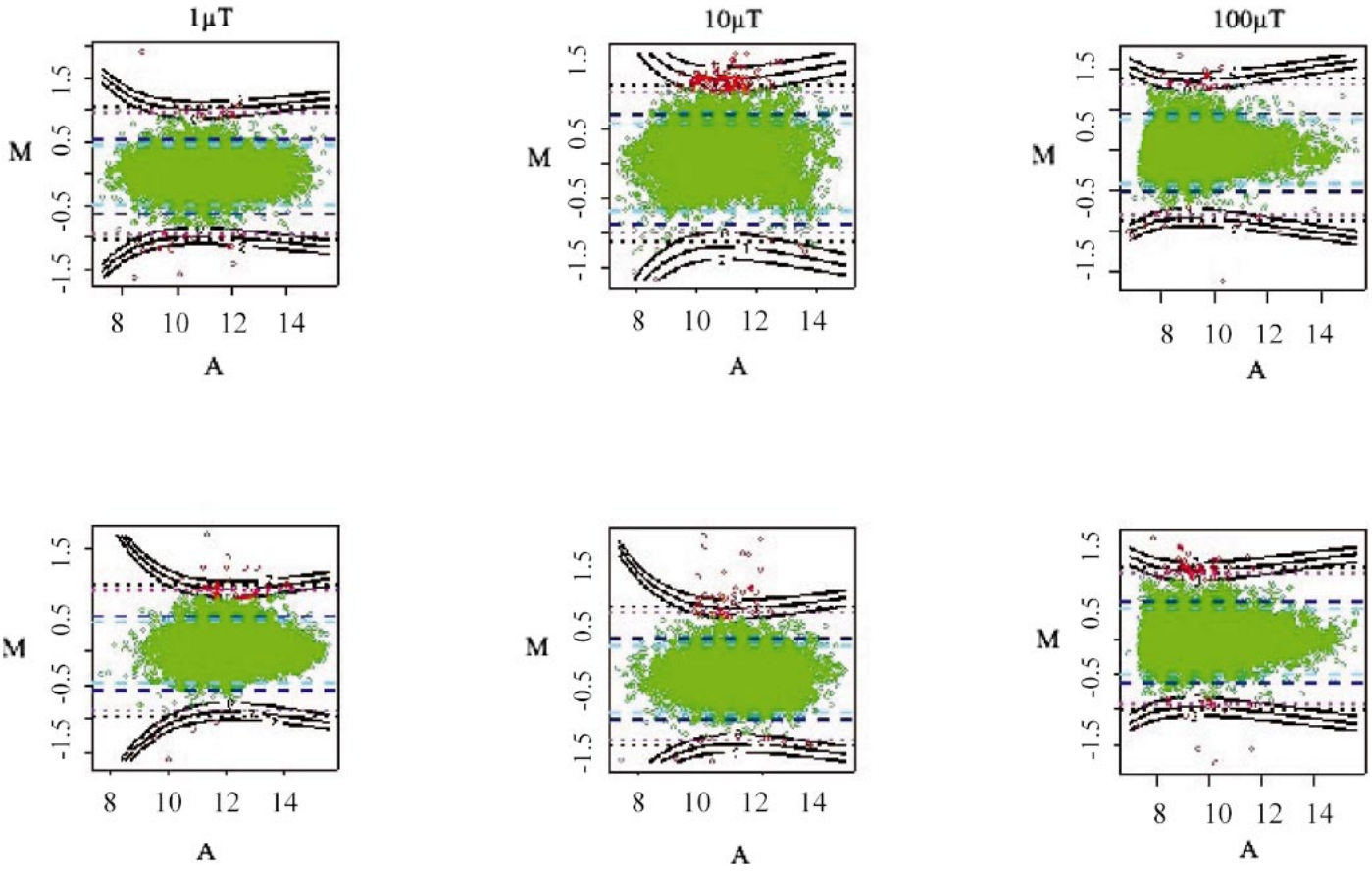

FIG. 1. Distribution of spot fluorescence intensities in human lymphocytes exposed to 1, 10 and 100 μT extremely low-frequency EMFs compared to unexposed cells. The two rows represent the duplicate arrays for the three doses tested. R = red fluorescence (Cy5) and G = green fluorescence (Cy3). *X* axis: values of A = [log(R*G)]; *Y* axis: values of M = $[\log(R/G)]^{0.5}$. In each individual plot the three continuous lines in the upper and lower part correspond to *P* values of 0.5, 0.1 and 0.01. Red spots outside the last line represent up- or down-regulated genes; green spots and red spots inside the lines represent genes with no significant expression variations.

plot of M as a function of A, where M = $\log_2$ (R/G) and A = ($\log_2$ R+$\log_2$ G)/2. In this type of plot, genes that are up- or down-regulated in the exposed cells compared to controls are represented in red; in each plot, the three continuous lines in the upper and lower part of the graph represent change odds of 1:1, 1:10 and 1:100 (corresponding to *P* values of 0.5, 0.1 and 0.01, respectively).

We used the statistical analysis of microarray (SAM) according to Tusher *et al.* (*24*) for yeast data, which were obtained with multiple replicates of the same cells (six arrays and three doses). We consider a *P* value of 0.05 and chose the smallest threshold (Δ) so that the false discovery rate is smaller than 0.05 (*25*).

Data for human cells were obtained with six arrays and three doses; for each dose, we had two replicates on different arrays. In this case we were not able to conduct a dose–response statistical analysis using the SAM method, since different lymphocytes from different donors were used for each exposure. Therefore, we used an empirical Bayesian method as proposed by Lönnstedt and Speed (*26*) and fixed the *P* values at 0.05. A gene with a log odds ratio between posterior probability to be expressed and not expressed >2.9 was considered significant.

RESULTS

DNA Damage

Preliminary experiments were run to ascertain whether the basal levels of DNA damage were influenced by the different experimental conditions (incubation in a water bath in air or in a cell incubator with 5% CO_2 and 95% O_2 atmosphere at 95% humidity). For this purpose, lymphocytes were cultured in parallel in a water bath and in a cell incubator for up to 5 days. The effects of exposure to 50 Hz extremely low-frequency EMFs are shown in Table 1. The last two columns report measures obtained in a water bath at 0.1 and 1 μT. As explained in the Material and Methods section, we used such a device since the background extremely low-frequency EMF level was higher than 0.1 μT in the cell incubator. The levels of basal DNA breaks measured at 1 μT under these conditions were slightly but significantly higher ($P < 0.05$) compared to the cells kept in a cell incubator at the same exposure, although cell viability was greater than 99% in both conditions. Instead, the level of DNA oxidized bases under 1 μT exposure in the cells kept in the water bath was lower than in cells kept in the incubator ($P < 0.05$).

Considering all exposure levels in each incubation system, no difference in DNA breaks or in DNA base oxidation (FPG-sensitive sites) was induced by exposure to increasing levels of extremely low-frequency EMFs.

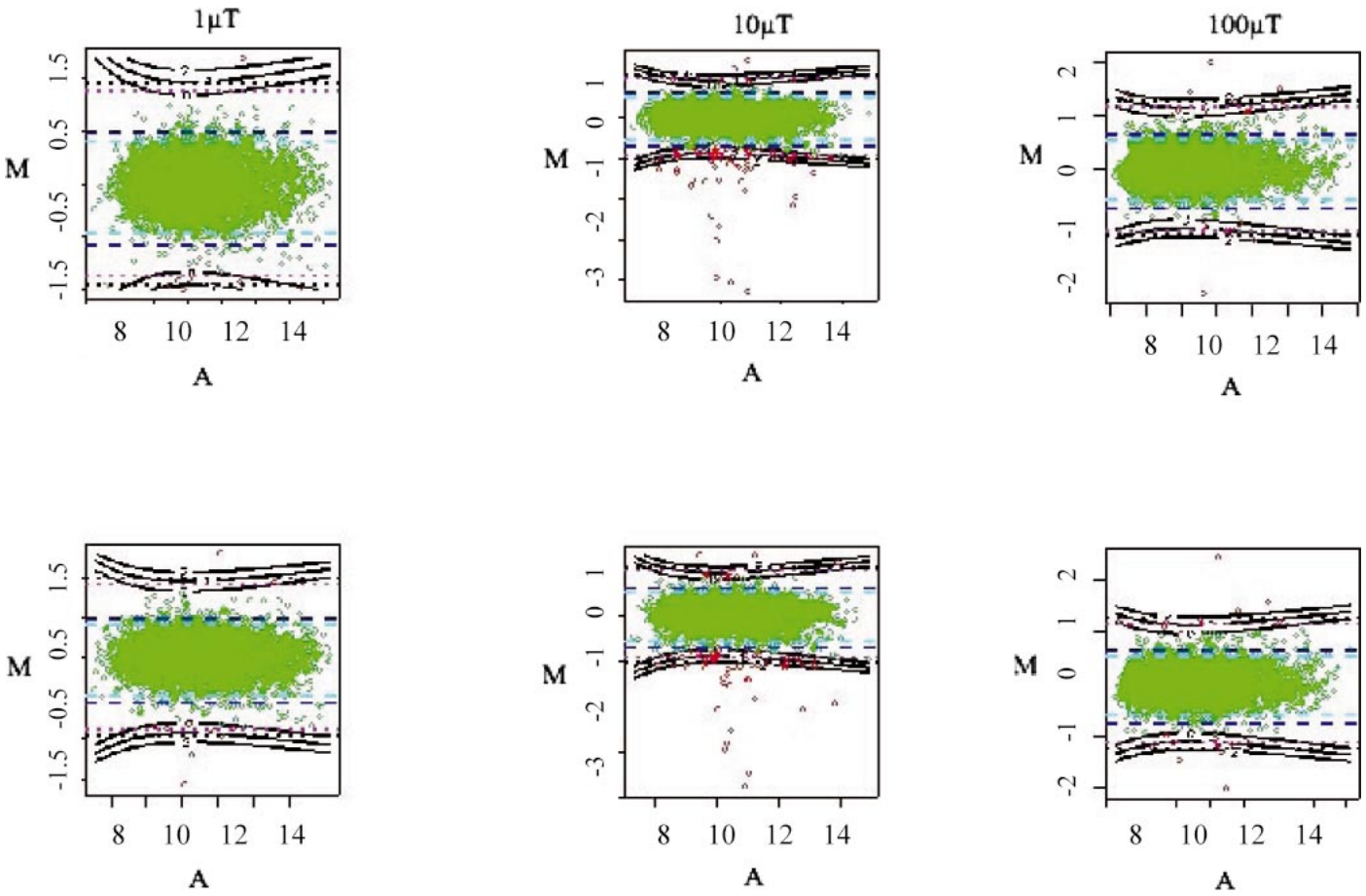

FIG. 2. Distribution of spot fluorescence intensities in *Saccharomyces cerevisiae* cells exposed to 1, 10 and 100 μT extremely low-frequency EMFs compared to unexposed cells. The two rows represent the duplicate arrays for the three doses tested. Red spots outside the last line represent up- or down-regulated genes; green spots and red spots inside the lines represent genes with no significant expression variations.

Gene Expression

We normalized the data using the Lowess normalization approach for analysis of gene expression data, as explained in detail in the Material and Methods. The data are reported as a plot of A as a function of M, in which gene expressions that are not varied by the treatment (in this case extremely low-frequency EMFs) are clustered as dots in the middle of each plot (Figs. 1 and 2). The genes that are significantly up- or down-regulated are shown as red dots and lie outside the third contour lines ($P < 0.01$).

Initially, the analysis of each individual array for each level of extremely low-frequency EMF exposure showed a limited number of genes with a statistically significantly altered expression, as is apparent from the six experimental plots in Figs. 1 (human lymphocytes) and 2 (yeast).

To obtain a summary result for each intensity level, we calculated the replicate average for each intensity level; by doing so, in human lymphocytes we observed just one down-regulated gene at 100 μT exposure; at 10 μT we observed one down-regulated gene and two up-regulated

TABLE 1
Effect of Exposure to Low-Frequency Magnetic Fields on DNA Damage in Cultured Human Lymphocytes

	Culture incubator			Water bath	
	1 μT	10 μT	100 μT	0.1 μT	1 μT
DNA breaks	1.29 ± 0.11	0.86 ± 0.06	1.41 ± 0.09	3.19 ± 0.14	2.76 ± 0.11
FPG-sensitive sites	3.23 ± 0.45	2.22 ± 0.53	1.94 ± 0.27	2.20 ± 0.34	0.27 ± 0.02

Notes. Cultured human lymphocytes were exposed for 18 h to 50 Hz magnetic fields at intensities of 0.1 μT ($n = 6$), 1 μT ($n = 12$), 10 μT ($n = 6$) and 100 μT ($n = 6$) inside a cell culture incubator or in a water bath. The levels of DNA damage are expressed as mean frequencies ± SE of breaks or FPG-sensitive sites/10^{10} Da DNA.

TABLE 2
Genes Apparently Up- or Down-regulated in Human Lymphocytes and *S. cerevisiae* Cells Exposed to Extremely Low-Frequency EMFs

1 μT		10 μT		100 μT	
Human lymphocytes		NM_003988 (PAX2) Transcription factor	2.57	AC00.973 (ZNF208) Zinc finger protein 208	2.56
		AB002359 (PFAS) Phosphoribosylformylglycinamidine synthase	2.03		
		AL031313 Pseudogene	−2.37		
Saccharomyces cerevisiae					
YHR139C (SPS100) Involved in spore development; sporulation-specific wallmaturation protein	2.36	YHR139C (SPS100) Involved in spore development	2.55	YHR139C (SPS100) Involved in spore development	2.5
YMR088C Permease of basic amino acids in the vacuolar membrane	−2.16	YOR200W Molecular function unknown	−2.52	YDR463W (STP1) Transcription factor	2.39
		YML124C (TUB3) Alpha–tubulin	−2.64		
		YLR081W (GAL2) Galactose permease, required for utilization of galactose; also able to transport glucose	−2.72		
		YGR229C (SMI1) Protein involved in (1,3)-beta-glucan synthesis	−2.88		
		YNL112W (DBP2) Essential ATP-dependent RNA helicase	−2.89		
		YOR192C Transporter activity	−3.58		
		YLR301W Molecular function unknown	−3.90		
		YPL252C (YAH1) Iron-sulfur protein of the mitochondrial matrix, involved in heme and biosynthesis	−4.34		
		YFL014W (HSP12) Plasma membrane induced by heat shock, oxidative stress, osmostress	−4.81		
		YFL059W (SNZ3) Member of a stationary phase-induced gene family	−6.80		
		YOR175C Molecular function unknown	−7.70		
		YER013W (PRP22) RNA-dependent ATPase/ATP-dependent RNA helicase	−11.66		
		YER020W (SSP101) Signaling role in response to nutrients	−14.00		
		YAR061W Pseudogene	−16.72		

Notes. For each human gene we report the Genebank accession number, the gene description, and the variation in expression (fold change) compared to the unexposed cells. The ORF IDs for yeast genes are report.

genes. No genes were found to be up- or down-regulated at 1 μT exposure (Table 2).

Individual arrays also showed a small number of significantly altered genes in yeast cells (Fig. 2). Further statistical analysis of these data based on replicates showed 2, 15 and 2 genes as differentially expressed (mainly down-regulated) after exposure to 100, 10 and 1μT, respectively (Table 2). It is interesting to note that the *SPS100* gene,

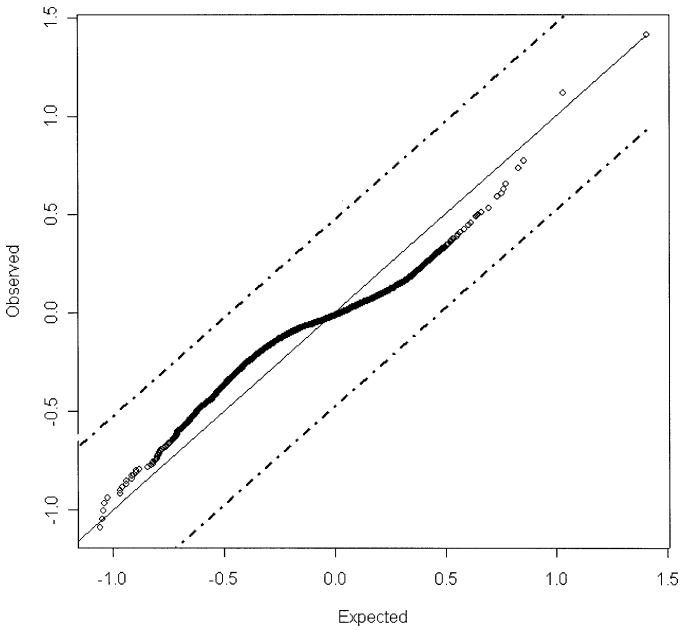

FIG. 3. SAM plot of yeast microarray data (observed relative difference compared to expected relative difference). The solid line is the 45° line (when observed is equal to expected), while the two parallel broken lines indicate the Δ distance from the solid one. We consider a $\Delta = 0.516$. All the points fall inside the bands; therefore, their differences are not statistically significant.

which is involved in sporulation, was consistently up-regulated after exposure to extremely low-frequency EMF at all three intensities analyzed. However, sporulation is a complex phenomenon that requires the variation of whole families of genes to be activated, and the variation on a single gene is difficult to interpret in this context (*27*).

Figure 3 shows the distribution of gene expression variations in yeast for the 4114 genes analyzed with the SAM method for all the exposures tested. With a *P* value that sets the false discovery rate at 5%, no genes were significantly down- or up-regulated in exposed yeast cells compared to controls after an 18-h exposure to extremely low-frequency EMFs, between 1 and 100 μT. Therefore, the variations in a few genes described before could be due to experimental noise effects.

Figure 4 shows the log posterior odds of the 7181 genes that passed the human array quality control. We did not find any gene that was significantly modified by extremely low-frequency EMF exposure at the 5% threshold (equivalent to 2.9 cutoff).

These results indicate that 50 Hz EMFs (1–100 μT) did not affect gene expression profiles in two different eukary-

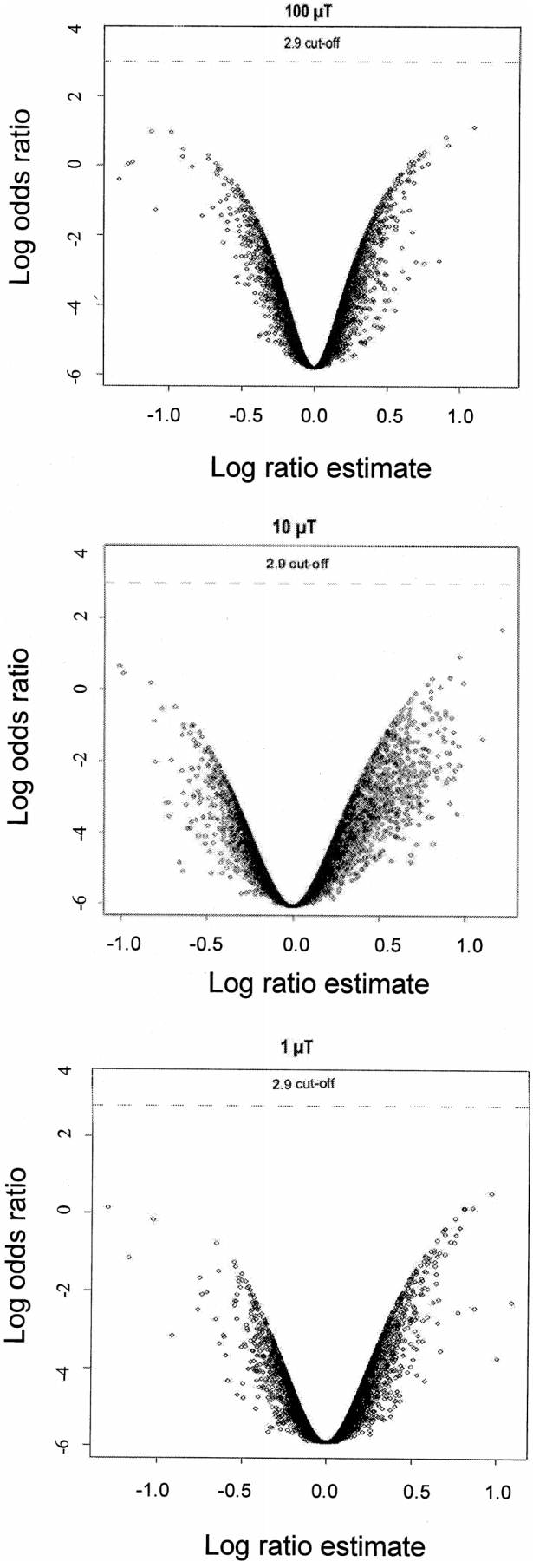

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FIG. 4. Log odds ratio as a function of log ratio estimate for human microarray data. A log odds ratio higher than 2.9 indicates a probability of genes to be differentially expressed higher than 0.95. At all three intensity values, no genes were above the 2.9 cutoff level.

otic cell types (human lymphocytes and *Saccharomyces cerevisiae*).

DISCUSSION

In industrialized countries, humans and other living organisms are inevitably exposed to varying levels of extremely low-frequency EMFs, the effects of which have been the subject of intense debate in the involved communities as well as epidemiological and basic research.

Studies at a cellular level are instrumental in revealing the cellular and molecular mechanisms underlying the possible biological effects and health implications of non-ionizing radiation, such as extremely low-frequency magnetic fields.

We studied the effect of extremely low-frequency EMFs at a frequency commonly encountered in households and offices (50 Hz) using human lymphocytes and *Saccharomyces cerevisiae* as well-characterized eukaryotic cell systems at intensities ranging between 1 and 100 μT, which encompass most human exposures. We used *S. cerevisiae* because it is a useful and informative model for investigating the biochemistry and molecular biology of DNA repair and cell cycle regulation in eukaryotes, since considerable information is available on gene function and regulation in this system [as discussed in detail by Resnick and Cox (*28*), among others]. Yeast has evolved to survive environmental fluctuations by rapidly adapting its internal biochemical machinery to meet challenging environmental variations; one aspect of this adaptation is its remarkable capacity to readjust gene expression to biochemical needs required for growth in each environment. Moreover, gene annotations and regulations in yeast are much more complete than in mammalian cells, and information obtained in yeast can be used to understand the much more complex adaptation mechanisms in the genome of mammalian cells, including humans cells. Human lymphocytes were also used since they are easily accessible and are resistant to experimental manipulation and amenable to *in vitro* study; last but not least, lymphocytes are related to the cells that are a possible target of detrimental effects of extremely low-frequency EMFs after *in vivo* exposure.

To reduce individual variability, for each field exposure, analysis was performed using pools of RNA extracted from lymphocytes from six different subjects, divided into two aliquots, one of which was used as an exposed sample and the other as an unexposed (control) sample.

Global analysis of the yeast and human genome using DNA microarray demonstrated that 18 h of exposure to extremely low-frequency EMFs with intensities ranging from 1 μT to 100 μT did not modify the gene expression profiles. Moreover, no DNA alteration, in terms of either strand breaks or base oxidation, was observed with the comet assay after different extremely low-frequency EMF exposures in human lymphocytes. Differences in the level of lymphocyte DNA breaks and oxidized bases measured at 1 μT exposure were found between the two conditions tested, i.e. in a cell incubator and in a water bath. These are probably due to differences in CO_2 and humidity that can affect cell metabolism and in turn the level of DNA damage.

Our results are in agreement with those of previous studies indicating that extremely low-frequency EMFs (50 Hz, up to 300 mT for 24 h) had no effect on expression of genes related to heat-shock response, DNA repair, respiration, protein synthesis and the cell cycle of *S. cerevisiae* (29). Similarly, other authors observed no effect of extremely low-frequency EMFs (60 Hz at 2 mT for 24 h) on the expression pattern of *MYC, JUN* and *FOS* in HL60 cells (human Caucasian promyelocytic leukemia) (*30*). Recently, Coulton *et al.* (*31*) also reported that extremely low-frequency EMFs in the range of 0–100 μT, either alone or concomitant with mild heating, had no detectable effect on the expression of genes encoding HSP27, HSP70A or HSP70B in human leukocytes. In contrast, some previous studies have found that extremely low-frequency EMFs can increase the binding of transcriptional activators to their related sequences (*32*).

The sensitivity of DNA microarray to detect small changes in gene expression profiles has been demonstrated by Gasch *et al.* (*33*), who detected significant changes in expression between exposure to mild heat shock (from 29°C to 33°C) and high heat shock (from 25°C to 37°C). Recently Debey *et al.* (*34*) found that gene expression profiles can be greatly affected by subtle alterations in sample preparation procedures, supporting the claim that this technology is sensitive enough to register the slightest environmental change. Side analyses performed in our laboratory (data not shown) demonstrated that, when the cells were exposed to 100 μT EMFs with no temperature control, the incubation medium temperature rose from 37°C to 38.5°C, inducing a significant increase in DNA damage. A similar increase from 30°C to 31.5°C in the incubation of *S. cerevisiae* was able to up-regulate heat-shock proteins and many stress-related genes. These results show that these methods can detect the effect of relatively small environmental variables.

In conclusion, the fact that we observed no significant variation in gene expression or DNA damage in cells exposed to EMFs, indicates that EMFs at this frequency and these intensities do not seem to affect cell physiology.

Concerns about the potential health effects of extremely low-frequency EMFs are due in part to the suggested relationships between certain neoplasms, especially childhood leukemia, and exposure to such fields. Such associations are difficult to analyze due to the lack of a known intermediate biological target that can be used to correlate exposure to extremely low-frequency EMFs with specific alterations in cellular processes. Our results suggest that extremely low-frequency EMFs at the intensities currently encountered for nonprofessional human exposures have no impact on DNA integrity and gene expression profiles in

eukaryotic cells. The fact that no effect was observed in two different cell systems supports the biological relevance of our findings.

ACKNOWLEDGMENTS

This work was supported by MAP (Italian Ministry for Productive Activities) in the framework of Public Interest Energy Research project named ''Ricerca di Sistema'' (MAP Decree of 26 January 2000 and following modifications) through a grant from CESI, Milan, Italy.

Received: December 13, 2004; accepted: May 10, 2005

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