EFFECTS OF ELECTRIC AND MAGNETIC FIELDS ON MUTATION AND GENE EXPRESSION

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ABSTRACT

Mutagenicity of electric and magnetic fields (EMFs) and their effect on gene expression profiles were examined using *Saccharomyces cerevisiae*. Exposure to a 5 T static EMF resulted in a slight increase in gene conversion frequency while exposure to 0.5, 1 T static and up to 40 mT, 50 Hz EMFs did not alter the frequency. In the global gene expression profiles, several genes, but not any of the gene cascades were induced slightly after exposure to a 14 T static EMF while a 5 T static and up to 40 mT, 50 Hz EMFs did not affect the expression profiles.

INTRODUCTION

The chances of being exposed to extremely low frequency (ELF, 0-300 Hz) electric and magnetic fields (EMFs) are increasing due to an increase in power consumption in developed and developing countries. EMFs are generated from various sources such as power lines, electric appliances at homes and offices, electrified transportation systems including urban railway systems and diagnostic devices such as magnetic resonance imaging (MRI). However, we do not yet have convincing scientific evidence that guarantees that exposure to such EMFs is not hazardous to our health. Recently, the scientific working group of the Monographs Program of the International Agency for Research on Cancer (IARC) (http://www.iarc.fr) stated that environmental exposure to ELF EMFs is categorized as possibly carcinogenic to humans (Group 2B) while static EMF could not be classified as carcinogenic to humans (Group 3) because of limited evidence. In such situations, investigation of the biological effects of exposure to various EMFs has a high priority. In fact, the World Health Organization (WHO) stated that there is an urgent need for an accelerated programme to provide scientific consensus and clarification of possible health effects of EMFs and has been conducting an international EMF project since 1996 (http://www.who.int/peh-emf/index.htm).

Although the final goal of our study is to estimate the effect, especially carcinogenicity of weak EMFs that we are actually exposed to, here we decided to explore biological effects of high density EMF since that is a common strategy for safety evaluation of chemical substances and ionizing irradiation. Recently, we reported a slight increase of somatic recombination frequency in *Drosophila melanogaster* [1,2] and also about enhancement of chemically induced point mutation frequency in *Escherichia coli* [3] by exposure to static or ELF EMFs. The aim of this study is to estimate the safety of exposure to such EMFs. Mutagenicity of EMFs was estimated by examining point mutation and gene conversion frequency in budding yeast *Saccharomyces cerevisiae*. Changes in the whole genome expression profiles after exposure to EMFs were also examined.

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MATERIALS AND METHODS

Exposure Systems

A superconducting magnet (SCM, Toshiba JS-500) with a horizontal bore of 20 cm in diameter that generates a homogeneous static magnetic field up to 5 T (T: Tesla=10,000 Gauss) was used. To avoid temperature fluctuation, the SCM was located in a constant temperature room (MCU-3000, Sanyo, Japan) with the exposure space maintained at 24-37 ± 0.5 ^{o}C. Another SCM (type14/50/1, Oxford Horizontal Magnet, U.K.) with a horizontal bore of 5 cm diameter that generates a static magnetic field up to 14 T was used. Temperature in the bore was controlled by water circulating pipe (4.5 cm diameter) which was made using silicon tubes filled with circulating hot water. For exposure to a 50 Hz magnetic field, a pair of water-cooled Helmholtz coils (ISM-12K12-2, IDX Co., Tokyo, Japan) were located in a constant temperature room (MCU-2000, Sanyo Co., Osaka, Japan) and the exposure space was maintained at 24 ± 0.5 to 37 ± 1 ^{o}C during exposure period. The coils were energized to generate horizontal sinusoidal EMFs of 50 Hz, up to 40 mT. Spatial inhomogeneity of flux density in the exposure space was less than 10 %.

Strains

S. cerevisiae XD83 (*Mata/Matá, his4-519/his4-519, leu2-3/leu2-3, lys1-1/lys1-1,+/pet1, arg4-4/+, +/arg4-17, ade2-18/+, thr/+*) was obtained from American Type Culture Collection and used for mutagenicity assay. *S. cerevisiae* 4228 (IAM4206, diploid, wild type) was obtained from IAM Culture Collection (Japan) and used for a experiment in profiling of the global genome expression.

Mutagenicity Assay

S. cerevisiae XD83 was precultured to late log phase with YPD medium. Cells were harvested and washed with 0.1 M phosphate buffer (pH 7.4) and re-suspeded in same volume of phosphate buffer. 0.1 ml of cell suspension was mixed with molten soft agar (0.6 % Bactoagar, 0.5 % NaCl) and poured on to SD plate with amino acids except trace of lysine for detecting point mutation frequency on *lys 1-1*. 0.1 ml of 1/100 diluted cell suspension was mixed with molten soft agar and poured on to SD plate with amino acids except trace of arginine for detecting gene conversion of recombination frequency on *ARG4* allele (between *arg 4-4* and *arg 4-17*). At least 6 plates was made both condition and randomely divided two groups and one group was exposed to a EMF for 5 days at 30 ± 0.5 ^{o}C. The other group was incubated in conventional incubator as control. Number of colony on each plate was scored as revertant and calculated the mutation frequency.

Profiling of the Global Gene Expression

Magnetic Field Exposure

S. cereivisiae 4228 strain was precultured to mid log phase with YPD medium. 50 µl of precultured cells was inoculated into 50 ml of YPD medium (final cell density was 10^4 cells/ml) in 50 ml disposable tubes and sealed tightly. The stationary culture of yeast cells was exposed to EMFs for 24 h at 30 ± 0.5 ^{o}C.

DNA Microarrays

DNA microarrays of yeast open reading frames were obtained from DNA chip research Inc. (Yokohama, Japan). One microarray included 5,876 (out of 6,400 ORFs of *S. cerevisiae*) cDNA spots. RNA isolation, cDNA preparation and hybridization were performed by the protocols which provided by the DNA chip research Inc. with slight modification.

Signal Detection and Analysis

Microarrays were scanned using a scanning laser confocal microscope Scanarray™ 4000Lite (Packard BioChip Technologies, USA) at 5 µm. The data obtained from the scanner were imported into Quantarray™ software package (Packard BioChip Technologies, USA) for quantification and then the data were imported into Excell software package (Microsoft Co., USA) for normalization and analysis. Ratios of gene expression in exposed cells compared to that of control cells were calculated using the normalized gene expression values. The

twofold cutoff value for identifying genes was based on recommendations by the developer of the DNA microarray. Clustering analysis was performed with selected genes related to several cellular processes (TCA cycle, DNA damage response, oxidative stress response, protein synthesis and other stress response which was annotated by the Saccaromyces genome database (http://genome-www.stanford.edu/Saccharomyces/)) using Cluster and TreeView software (Stanford Univ., USA). At least two independent experiments were performed under every experimental condition.

RESULTS AND DISCUSSION

Mutagenicity of EMFs

Exposed to a 5 T static EMF resulted in a slight increase in gene conversion frequency in *ARG4* locus while reverse mutation in *lys1-1* was not altered (Table 1). On the other hand, when cells were exposed to 0.5, 1 T static and up to 40 mT, 50 Hz EMFs, frequency in reverse mutation in both *ARG4* and *lys1* was not altered. The results are shown in Table 1. This suggests that 50 Hz EMFs weaker than 40 mT do not cause point mutation or gene conversion. There are several mutagenicity mechanisms or causes of mutagenesis. In bacteria, point mutation, frame shift or small deletion are major mutations. Besides these types of mutation, large deletion, loss of heterozygote, gene conversion and chromosome recombination occur frequently in eukaryote cells. Therefore, it is important to examine various types of mutation to evaluate genotoxic effects of MFs. For this reason, we used *Saccharomyces cerevisiae* XD83 to detect multiple genetic endpoints (point mutation and gene conversion), as in this strain, we can detect various genetic endpoints simultaneously in the same genetic background and under the same experimental conditions [4]. In our results, it is inferred that EMF exposure caused chromosomal recombination or gene conversion but not point mutation. This result is consistent with our previous study that suggests exposure to a 5 T static EMF cause an increase in chromosomal recombination in fruit fly *Drosophila melanogaster* [1]. On the other hand, weak UV irradiation, which was estimated to be approximately 1/5 to 1/10 that of the average sunlight in Japan, caused significant increase of both *ARG4* and *lys1* reversions (Table 1). In a comparison of the results in the exposure to MFs and the UV irradiation in this study, the extent of mutagenicity of MFs was estimated to be extremely small since weak UV exposure (18 J/m^2) is approximately 20 times more effective than MFs which are at least 10,000 times stronger than those in the environment.

Table 1 Mutagenicity of EMFs in *S. cerevisiae* XD83

Treatment	Ratio of *lys*$^+$ mutants (point mutation)	Ratio of *ARG*$^+$ mutants (gene conversion/recombination)
Control[a]	1.0	1.0
Static, 0.5 T	N.D.[b]	1.0
Static, 1 T	N.D.	0.9
Static, 5 T	1.0	1.4 *
50 Hz, 40 mT	1.0	0.9
UVB, 18 J/m^2	15.1 *	7.7 *
UVB, 36 J/m^2	32.1 *	11.4 *

*Significantly higher than the control group at 1 % level

a) *lys*$^+$ mutatnts was 9.7±2.5/10^7 survivor and *ARG*$^+$ mutants was 2.4±0.7/10^4 survivor in control

b) Not Determined

Effect of EMFs on Gene Expression Profiles

Expression of approximately 90 % of the whole ORFs of yeast genome was examined using DNA microarray. When cells were exposed to a 14 T static EMF, weak inductions of several genes including respiratory processes were repeatedly observed (Table 2). On the other hand, no significant difference was observed by exposure to a 50 Hz, 40 mT EMF. To compare the change in gene expression profiles by various stresses, we investigated the global gene expression profile in DNA damaged cells which was treated with chemical mutagen and microaerobic grown cells which was treated with oxygen. DNA repair genes and oxidative stress response genes were strongly induced by exposure to chemical mutagen, N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG, 30 µg/ml). Oxidative stress response genes as catalase, cytochrome-c oxidase and respiration related genes were strongly induced by transferring cells to aerobic cultivation from microaerobic environment for 3 h. On the

other hand, exposure to EMFs did not cause clear induction of specific gene cascades or operons even in a 14 T strong static EMF. It is inferred that the exposure to static EMFs up to 14 T or to 50 Hz EMFs up to 40 mT does not cause major changes in gene expression profiles in *S. cerevisiae* under the experimental conditions used in this study.

Table 2 Induction of genes by exposure to a 14 Tesla magnetic field

Systematic Gene Name	Induction ratio	Gene Name	Process	Function
YNR002C	7.97	FUN34	unknown	unknown
YLR174W	3.39	IDP2	TCA cycle	isocitrate dehydrogenase
YKL217W	2.78	JEN1	transport	lactate transporter
YDL191W	2.75	RPL35A	protein synthesis	ribosomal protein L35A
YBR241C	2.60		unknown	unknown; similar to sugar permeases
YKL187C	2.58		unknown	unknown; similar to 4-mycarosyl isovaleryl-CoA transferase
YEL024W	2.18	RIP1	respiration	protein
YCL042W	2.11		unknown	unknown
YNL195C	2.10		unknown	unknown
YDL067C	2.06	COX9	oxidative phosphorylation	cytochrome c oxidase assembly
YNL202W	2.06	SPS19	sporulation	peroxisomal 2,4-dienoyl-CoA reductase
YBR050C	2.05	REG2	glucose repression	(putative) Glc7p regulatory subunit
YKL085W	2.03	MDH1	TCA cycle	malate dehydrogenase
YEL012W	2.00	UBC8	protein degradation, ubiquitin-mediated	E2 ub.-conjugating enzyme

CONCLUSION

Experimental results suggest that exposure to a 5 T static EMF has a small effect in increasing chromosomal recombination, while up to 14 T static EMFs did not cause reproducible alteration of any specific gene cascade in genome-wide expression analysis using DNA microarray. In addition, a 50 Hz, 40 mT EMF did not affect either mutation frequency or gene expression profile under experimental condition. These results suggest that the effect of exposure to EMFs in environment would be extremely small.

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