Effects of Static Magnetic Field on Mutagenesis in *in vitro*

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**Abstract.** Effects of static magnetic field up to 13 T were estimated in *Escherichia coli* and *Saccharomyces cerevisiae*. We observed that exposure to a 5 T static magnetic field resulted in a slight but significant increase in gene recombination frequency while frequency of reverse point mutation was not altered in *S. cerevisiae*. This mutagenic effect showed a dose response relationship as J-shape. To investigate an involvement of reactive oxygen species in possible mutagenic effect of static magnetic field, SOD deficient *E. coli* QC774 was used in thymine synthesis-based mutation assay. The result shows that static magnetic field up to 13 T did not indicate mutagenicity. Thus, it is suggested that frequency of point mutation does not changed under static magnetic field regardless of its susceptibility to superoxide. These results suggest that strong static magnetic field would have small but detectable mutagenic potential. Although mechanism of the mutagenic effect of static magnetic field has not been elucidated yet, the extent of this effect is estimated extremely small by comparison with other mutagens such as ultraviolet irradiation.

1. **Introduction**

The chance for exposure to various magnetic and electromagnetic fields in general public and working environment are increasing. In recent years, the biological effects by exposure to magnetic fields, especially in power frequency and radio frequency were extensively investigated along with growing of social concern. However, there are not enough studies to evaluate the biological effects of static magnetic field while opportunities for exposure to strong magnetic field such as MRI (up to 3 Tesla at present) are increasing. It was reported that exposure to strong static magnetic field caused small increase of somatic recombination in wing spot test of *Drosophila melanogaster* [1], increase mutation frequency in forward mutation assay in SOD (superoxide dismutase) deficient *Escherichia coli* [2] and enhancement of mutagenicity of DNA reactive mutagen in reverse mutation assay in *E. coli* [3]. However, the mechanism of the mutagenic effect has not been determined yet. On the other hand, no mutagenic effect was observed in bacterial mutation assay (Ames’ test) using *Salmonella typhimurium* and *E. coli* [3]. Therefore, additional research evidences will be necessary to understand the mutagenic effects of magnetic fields.

In this study, mutagenicity of strong static magnetic fields were investigated using budding yeast *Saccharomyces cerevisiae* as a eukaryote model and SOD-deficient *Escherichia coli* as a ROS (reactive oxygen species) sensitive model organism.
2. Materials and Methods

2.1 Exposure systems

Two types of superconducting magnet were used for exposure to static magnetic fields. One of the magnets is a superconducting magnet (JS-500 (Toshiba)) and generates homogeneous up to 5 T horizontal magnetic field with 5% fluctuation within 10 cm from the centre of the bore (Magnet 1; Fig. 1 (a)). This magnet was placed in a temperature-controlled room and temperature of the exposure space was maintained at 30 ± 1°C. The other magnet is also a superconducting magnet (JMTD-10C13E-NC (JASTEC)) and generates up to 13 T of vertical magnetic field (Magnet 2; Fig. 1 (b)). Temperature in bore was kept at 37°C by running hot water in silicon tube, which covered around internal surface of the bore of the Magnet 2.

![Figure 1. Exposure systems of static magnetic fields; (a) Magnet 1 (5 T), (b) Magnet 2 (5, 10, 13 T).](image)

2.2 S. cerevisiae mutation assay

*S. cerevisiae* XD83 (*MATa/MATα, lys1-1/lys1-1, arg4-4/arg4-17, RAD*) was obtained from American Type Culture Collection (Virginia, USA) and was used as the tester strain in this study. For magnetic field exposure, the Magnet 1 was used in this experiment. *S. cerevisiae* XD83 was precultured to late log phase (approximately 10⁸ cells/ml) in YPD medium.

In mutation assay, 0.1 ml of cell suspension was mixed with molten soft agar (0.6% Bacto-agar, 0.5% NaCl) and poured on to low lysine synthetic complete plate for detecting point mutation frequency on *lys1-1*. 0.1 ml of 1/100 diluted cell suspension was mixed with molten soft agar and poured on to low arginine synthetic complete plate for detecting gene conversion frequency on *ARG4* allele (between *arg4-4* and *arg4-17*).

At least 6 plates were made for each test condition and randomly divided into two groups. One group was exposed to a static magnetic field for 4 days at 30 ± 1°C as exposed group. The other group was incubated in a conventional incubator as unexposed control. Number of colonies on each plate was scored as revertant and the mutation frequency was calculated.

2.1. E. coli forward mutation assay

Two strains, *E. coli* QC774 cells (Φ*(sodA-lacZ)*49 Φ*(sodB-kan)*1-Δ2 Cm’ Km’) [4], which is defective in SOD gene, *sodA* and *sodB*, and its wild-type (GC4468) were used in this experiment. The SOD is an enzyme which eliminates superoxide from the cells by generation of hydrogen peroxide and oxygen from superoxide and hydrogen ion. The superoxide ion is a kind of the free radicals and causes damage to cellular materials such as DNA.

For magnetic field exposure, the Magnet 2 was used in this experiment. These cells were pre-incubated for 4 hr in Luria-Bertani (LB) medium and the pre-incubated culture was added to fresh LB medium. The plastic tube with suspended culture and fresh LB medium was put into the bore of superconducting magnet for exposure to 0, 5, 10 or 13 T. After 24 hr exposure, cells were poured onto glucose minimum medium plates containing trimethoprim and thymine to select Thy’ (thymine
and gene conversion would be more sensitive to MFs than mutagenesis of point mutation with lack of fields. Moreover, these results suggest that mutagenesis on chromosomal level such as recombination between MF exposed and unexposed control cells in 5, 10 and 13 T, respectively in both S. cerevisiae strains (Fig. 3). This suggests that SOD pathway could not relate to the mutagenic effect of magnetic fields. When cells were exposed to a 1 T static magnetic field, there was no difference in the frequency of reverse mutation in both ARG4 and lys1-1. These results suggest that exposure to a strong static magnetic field shows weak mutagenicity and its threshold would be above 2 T in this tester strain. In addition, the dose response relationship between magnetic field density and mutagenic effect showed J-shape (Fig. 2). In our previous study, extent of mutagenic effect of static magnetic field was increased linearly up to 2 T and saturated in wing spot test of Drosophila melanogaster [1] and co-mutagenicity of Ames test [3]. In case of these assays, tester strains have partial deficiency of DNA repair ability and therefore these strains are sensitive to various mutagens. On the other hand, S. cerevisiae strain used in this study is proficient for DNA repair and its J-shape dose response relationship between magnetic field density and mutagenic effect was different from DNA repair deficient Drosophila melanogaster and E. coli. These different responses would be reasonable if the exposure to strong static magnetic field caused an increase of DNA lesion frequency. Obviously, although a strong magnetic field even at 5T does not have enough energy to modify the covalent bond of DNA directly, indirect effects such as increase of oxidative damage by exposure to a strong static magnetic field that was reported by Watanabe et al [6] would relate to the cause of increase in the mutation frequency.

3. Results and Discussion
When S. cerevisiae XD83 cells were exposed to a 5 T static magnetic field, reverse mutation frequency in ARG4 locus slightly increased (approx. 1.4 times higher than control) while reverse point mutation frequency on lys1-1 was not altered. This result suggests that magnetic field exposure caused chromosomal recombination or gene conversion but not point mutation. This mutagenic effect disappeared on exposure to a 2 T static magnetic field and was even lower than the control on exposure to a 1 T static magnetic field. When cells were exposed to a 0.5 T static magnetic field, there was no difference in the frequency of reverse mutation in both ARG4 and lys1-1. These results suggest that exposure to a strong static magnetic field shows weak mutagenicity and its threshold would be above 2 T in this tester strain. In addition, the dose response relationship between magnetic field density and mutagenic effect showed J-shape (Fig. 2). In our previous study, extent of mutagenic effect of static magnetic field was increased linearly up to 2 T and saturated in wing spot test of Drosophila melanogaster [1] and co-mutagenicity of Ames test [3]. In case of these assays, tester strains have partial deficiency of DNA repair ability and therefore these strains are sensitive to various mutagens. On the other hand, S. cerevisiae strain used in this study is proficient for DNA repair and its J-shape dose response relationship between magnetic field density and mutagenic effect was different from DNA repair deficient Drosophila melanogaster and E. coli. These different responses would be reasonable if the exposure to strong static magnetic field caused an increase of DNA lesion frequency. Obviously, although a strong magnetic field even at 5T does not have enough energy to modify the covalent bond of DNA directly, indirect effects such as increase of oxidative damage by exposure to a strong static magnetic field that was reported by Watanabe et al [6] would relate to the cause of increase in the mutation frequency.

On the other hand, no statistically significant difference on thy forward mutation was observed between MF exposed and unexposed control cells in 5, 10 and 13 T, respectively in both E. coli tester strains (Fig. 3). This suggests that SOD pathway could not relate to the mutagenic effect of magnetic fields. Moreover, these results suggest that mutagenesis on chromosomal level such as recombination and gene conversion would be more sensitive to MFs than mutagenesis of point mutation with lack of SOD pathway. In this study, although we observed the recombinogetic effect of MFs above 2T in S. cerevisiae, the degree of the effects was extremely small. Because, in same experiment, weak UV
light (ca. 1/10 of sunlight) induced both recombination and point mutation approximately 10 times higher than control in *S. cerevisiae*. Thus, our data suggests that mutagenicity of static magnetic field would be negligibly small to assess environmental risks.

### 4. Conclusion

Effects of static magnetic field up to 13 T were estimated in *Escherichia coli* and *Saccharomyces cerevisiae*. For point mutation, we observed no effect for mutation frequency by exposure to up to a 5 T static magnetic field in *S. cerevisiae* and up to a 13 T static magnetic field in SOD-deficient *E. coli*. On the other hand, frequency of recombinogenic mutation was slightly changed by static magnetic field up to 5 T depends on its field density.

These results suggest that strong static magnetic field would have small but detectable mutagenic potential; however, the extent of this effect is estimated extremely small by comparison with other mutagens such as ultraviolet irradiation.

### References

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