Nutritional conditions and oxygen concentration affect spontaneous occurrence of homologous recombination events but not spontaneous mutagenesis in *Escherichia coli*

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Effects of environmental factors for growth of *Escherichia coli* on spontaneous mutagenesis and homologous recombination events are described. By analyzing rifampicin-resistant (Rif') mutation frequencies in an *E. coli* strain lacking MutM and MutY repair enzymes, which suppress base substitution mutations caused by 8-oxoguanine (7,8 dihydro-8-oxoguanine; 8-oxoG) in DNA, we examined levels of oxidative DNA damage produced in normally growing cells. The level of 8-oxoG DNA damage was about 9- and 63-fold higher in cells grown in M9-glucose and M9-glycerol media, respectively, than in those grown in LB medium. We also found that about 14-fold more 8-oxoG DNA damage was produced in cells grown in about 0.1% oxygen than in those grown in the normal atmosphere. However, Rif' mutation frequency in wild-type cells was unchanged in such different growth conditions, suggesting that the capacity of repair mechanisms is sufficient to suppress mutations caused by 8-oxoG even at very high levels in cells growing in the particular conditions. On the other hand, the frequency of spontaneous homologous recombination events in wild-type *E. coli* cells varied with different growth conditions. When cells were grown in M9-glucose and M9-glycerol media, the spontaneous recombination frequency increased to about 4.3- and 7.3-fold, respectively, higher than that in LB medium. Likewise, the spontaneous recombination frequency was about 3.5-fold higher in cells growing in the hypoxic condition than in cells growing in the atmosphere. When cells were grown in anaerobic conditions, the recombination frequency decreased to half of that in the atmosphere. These data indicated that spontaneous homologous recombination is highly responsive to environmental factors such as nutrition and oxygen concentration.

Key words: 8-oxoguanine, homologous recombination, hypoxia, nutrition, oxidative DNA damage

INTRODUCTION

It has long been postulated that the rate of spontaneous mutation is maintained constant per cell division at a very low level, and, thus, cumulative base changes in the genome are dependent on the number of cell division cycles (Drake et al., 1998). Such a low spontaneous mutation rate is mainly determined by a balance between the rate of emerging pre-mutagenic DNA damage and the efficiency of repair of the DNA damage in normally growing cells (Maki, 2002). Previous studies using *Escherichia coli* showed that DNA replication errors and oxidative DNA lesions are the major types of pre-mutagenic DNA damage spontaneously occurring in normally growing *E. coli* cells and that they are almost completely eliminated by the mismatch repair system and various pathways of base excision repair, respectively (Fujii et al., 1999; Friedberg et al., 2006; Sakai et al., 2006). Mutant strains lacking either of these repair functions show mutator phenotypes having elevated rates of spontaneous muta-
tion, 100- to 10,000-fold higher than that in the wild-type strain (Miller, 1996; Maki, 2002). This indicates that huge amounts of pre-mutagenic DNA damage are produced in normally growing cells. It is also obvious that the DNA repair mechanisms have a strong capacity to reduce the mutation rate to a very low level. Thus, the mutation rate in wild-type cells reflects the capacity of repair functions that eliminate the pre-mutagenic DNA damage. In other words, the DNA repair capacity seems to determine the very low and constant level of spontaneous mutation. However, it is unclear how the effectiveness of DNA repair determines the level of spontaneous mutation. If the low frequency of spontaneous mutation results from a stochastic escape of DNA damage from DNA repair, spontaneous mutation frequency should be double when the level of spontaneous DNA damage is double. In this study, we tried to answer this question.

*Escherichia coli* cells carrying ΔmutS, a deletion mutation in the mutS gene, are totally defective in the mismatch repair function and show high rates of base substitution and single-base frameshift, comparable to the rates generated during *in vitro* DNA replication by DNA polymerase III (Fujii et al., 1999). The site and class distributions of base substitution and single-base frameshift that occurred in the ΔmutS cells were quite different from those in wild-type cells but closely resembled those caused by DNA replication errors in *in vitro*. Therefore, it is widely considered that mutations occurring in ΔmutS cells are caused by replication errors made in the cells, and that the frequency of mutations in ΔmutS cells reflects how frequently replication errors are made in the cells. Base substitutions and single-base frameshifts identified in a target DNA sequence in ΔmutS cells were very rare in the mutation spectrum in the same target sequence in wild-type cells, leading to an estimate that more than 99.9% of replication errors are corrected by the mismatch repair function (Fujii et al., 1999).

Among various kinds of oxidative DNA lesions, 8-oxoguanine (7,8 dihydro-8-oxoguanine, 8-oxoG) is the most mutagenic because it does not block DNA replication and is able to pair with adenine as well as cytosine (Shibutani et al., 1991; Maki and Sekiguchi, 1992). When 8-oxoG is produced in chromosomal DNA, G:C→T:A base substitution is exclusively induced with a probability 1 in 2 via two rounds of DNA replication. Two DNA repair enzymes are involved in suppression of G:C→T:A base substitution caused by 8-oxoG: MutM protein encoded by the *mutM* gene is an 8-oxoG:C-specific 8-oxoG DNA glycosylase, and MutY protein encoded by the *mutY* gene is an 8-oxoG:A- and G:A-specific adenine DNA glycosylase (Michaels et al., 1992). Although cells carrying either ΔmutM or ΔmutY show a weak mutator phenotype, ΔmutM ΔmutY (ΔmutMY) double mutant cells show a strong mutator phenotype, having about a 100-fold higher frequency of rifampicin-sensitive (Rif”) mutation than that in wild-type cells (Nghiem et al., 1988; Michaels et al., 1992; Tajiri et al., 1995; Sakai et al., 2006). Sequencing analyses of base substitutions occurring in wild-type and ΔmutMY cells revealed that G:C→T:A is a very rare type of base substitution in wild-type cells and that the frequency of G:C→T:A increases about 1,000-fold in ΔmutMY cells (Sakai et al., 2006). ΔmutMY cells increase only G:C→T:A base substitution, and no other repair enzyme is involved in suppression of 8-oxoG-induced G:C→T:A base substitution, suggesting that G:C→T:A base substitutions induced in ΔmutMY cells are caused by 8-oxoG spontaneously produced in the cells. This notion is further supported by the observation that the increased frequency of G:C→T:A in ΔmutMY cells was completely suppressed to the wild-type level when the cells were grown under strict anaerobic conditions (Sakai et al., 2006). Therefore, it is conceivable that the frequency of G:C→T:A in ΔmutMY cells reflects how frequently 8-oxoG is produced in chromosomal DNA in normally growing *E. coli* cells. The cellular level of 8-oxoG in DNA can be determined by chemical analysis of chromosomal DNA extracted from cells. However, it is very hard to avoid unfavorable oxidation of DNA during the extraction and purification processes, resulting in a 100× to 1,000× overestimate for the 8-oxoG level in wild-type cells (Tajiri et al., 1995; Alhama et al., 1998). From this, the determination of mutation frequency in ΔmutMY cells is considered to be the most reliable way to estimate the cellular level of 8-oxoG in DNA. Comparing the frequency of G:C→T:A in ΔmutMY cells with that in wild-type cells, it has been estimated that about 99.9% of 8-oxoG residues in DNA are corrected by the MutM-MutY repair function (Sakai et al., 2006).

Spontaneous mutagenesis in *E. coli* has been studied mostly using cells grown aerobically in LB medium at 37 °C. Therefore, it has been unclear whether the spontaneous mutagenesis is affected by environmental factors in growth conditions, such as nutrition, oxygen concentration, pH of growth media, and growth temperature. Hypothesizing that the pre-mutagenic DNA damage could be produced differently in different growth conditions that alter metabolic processes in cells, we started to examine the effects of such environmental factors on spontaneous mutagenesis in wild-type and several mutator mutant strains of *E. coli*. Here, we show evidence that nutrition and oxygen concentration among the growth conditions affect the production of oxidative DNA damage in *E. coli* cells. Using these different growth conditions to vary the cellular level of pre-mutagenic DNA damage, we examined the strength of the DNA repair capacity that eliminates the oxidative DNA damage by analyzing spontaneous mutagenesis in repair-proficient wild-type cells.

Chromosomal rearrangement is another type of genetic alteration that promotes evolution of organisms as well as
cancer development and other genetic diseases. Among various mechanisms involved in the generation of chromosomal rearrangement, homologous recombination ectopically occurring between repetitive sequences dispersed in the genome is the most frequent and causes deletions, duplications, inversions and translocations with a repetitive sequence as their endpoints (Umezu et al., 2002; Friedberg et al., 2006). We developed a genetic system to detect the spontaneous occurrence of a homologous recombination event between 735 bp of repetitive sequences in E. coli (Kanie et al., 2007). In this system, the homologous recombination event is detected as a gene conversion type of allelic recombination between two rpsL genes, one authentic and the other transgenic, separated by 548 kb on the bacterial chromosome. The frequency of allelic recombination between the two rpsL genes increased significantly when cells were irradiated with UV or treated with H2O2 (Kanie et al., 2007), suggesting that DNA damage could induce the homologous recombination events. Therefore, it seems plausible that spontaneous DNA damage is a cause of homologous recombination events in normally growing cells. This prompted us to examine spontaneous events of allelic recombination in cells grown in different growth conditions that alter the cellular level of oxidative DNA damage.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions** Strains used in this study are derivatives of E. coli K12 and listed in Supplementary Table S1. Construction of a ΔmutMY strain (MK7180) and an rpsL hemi-diploid strain (MK9547) is described in Supplementary Materials. LB contained 1% (w/v) Bacto tryptone (Difco, Detroit, MI, USA), 0.5% (w/v) yeast extract (Difco) and 1% (w/v) NaCl. M9 media were prepared as described (Miller, 1992). For M9 media, filter-sterilized 0.2% (w/v) glucose, 0.2% (w/v) succinic acid or 0.2% glycerol (v/v) was added. Agar plates were prepared by adding 1.5% (w/v) of Bacto agar (Difco) to the media. When needed, antibiotics were added to the media as follows: 100 μg/ml ampicillin, 50 μg/ml kanamycin, 25 μg/ml chloramphenicol, 100 μg/ml streptomycin and 100 μg/ml rifampicin. To ensure the anaerobic environment, a commercial enzyme additive, Oxyrase for agar (Oxyrase, Mansfield, OH, USA), was added to LB plates in accordance with the supplier’s manual. LB plates with Oxyrase for agar were prepared one day before use. To perform assays for spontaneous mutation and spontaneous allelic recombination, cells in an overnight culture were appropriately diluted with LB or M9 salt, spread on agar plates to form about 100 colonies per plate, and incubated at 37 °C until the colony diameter became 1.5 mm. For growth in hypoxic and anaerobic conditions, LB and LB+Oxyrase plates, respectively, were placed in a gas-tight plastic bag with oxygen-absorbing materials (Anaeropack with KENKI, Mitsubishi Gas Chemical, Tokyo, Japan) at room temperature one day before plating the cells. During the hypoxic and anaerobic incubations, oxygen was monitored using an oxygen indicator sheet for Anaeropack and a CO2-tolerant O2 monitor, OXY-M (Sugiyama-Gen, Tokyo, Japan).

**Assay for Rif′ and Str′ mutations** Cells grown on agar plates (about 100 colonies/plate, 1.5 mm colony diameter, about 107 cells/colony) were collected in 5 ml LB, and appropriate dilutions were spread on LB plates to determine the number of total cells and on LB plates containing rifampicin or streptomycin to determine the number of Rif′ or streptomycin-resistant (Str′) cells, respectively. If needed, 4.5 ml of the cell suspension was concentrated 10-fold by centrifugation, and all the cells were plated on antibiotic-containing LB plates. Colonies formed on the plates were counted after incubation for 24 h at 37 °C. The mutation frequencies were calculated by dividing the number of Rif′ or Str′ cells by that of total cells. For each strain to be examined, we repeated 20 times the determination of mutation frequency, and the median of the mutation frequencies was obtained from the data. DNA sequence analysis of Rif′ and Str′ mutations was performed by a direct sequencing method (Kanie et al., 2007; Hasegawa et al., 2008) using primer sets shown in Supplementary Materials, Supplementary Table S2. Twenty independent mutant colonies obtained from ΔmutMY cell populations grown in different conditions were subjected to sequence analysis to determine the type of base substitution mutation.

**Assay for rpsL forward mutation and allelic recombination** A hemi-diploid rpsL strain, MK9547, was used for the assay (Supplementary Fig. S1). Similar to the assay for Rif′ and Str′ mutations, cells grown on agar plates were harvested in 5 ml LB, and appropriate dilutions were spread on LB plates to determine the number of total cells and on LB plates containing streptomycin to determine the number of Str′ cells. Colonies formed on the plates were counted after incubation for 24 h at 37 °C. The Str′ mutation frequency was calculated by dividing the number of Str′ cells by that of total cells. To determine whether the Str′ phenotype was due to forward mutation in the transgenic rpsL gene (non-recombination events) or allelic recombination between the authentic rpsL128 allele and the transgenic rpsL allele (recombination events), 20 colonies were picked from the LB-Str plates and examined by PCR for A to C base substitution at position 128 in the transgenic rpsL sequence (Supplementary Fig. S2). PCR conditions are described in Supplementary Materials. Using the ratio determined between recombination events and non-recombination events, frequencies of recombination events and non-
recombination events were calculated. For each strain to be examined, we repeated 6–10 times the determination of frequencies of non-recombination and recombination events, and average frequencies were calculated, except that unusually high frequencies caused by jackpot mutations or recombination events were excluded from the calculation. Statistical analysis was performed using Graphpad Prism 7 software.

RESULTS

Cellular level of 8-oxoguanine in DNA varies with different nutrition conditions Using the Rif<sup>r</sup> mutation assay, which allows us to accurately determine the frequency of base substitution mutation (Hasegawa et al., 2008), we found that base substitutions occurring in a wild-type E. coli strain, MG1655, were affected little, if at all, by different growth media (Fig. 1A, Wild-type strain). However, a ΔmutM ΔmutY (ΔmutMY) strain, MK7180, showed about 9-fold higher mutation frequency when grown in a synthetic medium, M9-glucose, than when grown in LB medium (Fig. 1A, ΔmutMY strain). We observed a similar increase in frequency of Str<sup>r</sup> mutation in the ΔmutMY strain grown in M9-glucose (Supplementary Fig. S3). The Rif<sup>r</sup> and Str<sup>r</sup> mutations detected in the ΔmutMY cells grown in LB and M9-glucose were exclusively G:C→T:A transversion mutations in the coding sequence of the rpoB and rpsL gene, respectively, suggesting that some nutrient factor changes the cellular level of 8-oxoG in DNA. Growth of the ΔmutMY cells in M9 media containing unfermentable and poorly fermentable carbon sources, succinic acid and glycerol, respectively, resulted in much higher frequencies of Rif<sup>r</sup> mutation (Fig. 1A). On the other hand, the mutation frequency in the wild-type strain was unchanged when the cells were grown in M9-succinic acid and M9-glycerol, confirming the great capacity of MutM and MutY proteins for suppression of mutagenesis caused by 8-oxoG in DNA. These observations indicate that the cellular level of 8-oxoG in DNA varies greatly among growth media containing different carbon sources and other nutrition, with a maximum level 63-fold (when grown in M9-glycerol) higher than that in cells grown in LB.

Cellular level of 8-oxoG in DNA increases under hypoxia The frequency of G:C→T:A base substitution caused by 8-oxoG in DNA has been shown to decrease to an undetectable level when ΔmutMY cells were grown in LB under strictly anaerobic conditions (Sakai et al., 2006). In that study, strictly anaerobic conditions were achieved by using LB agar plates containing Oxyrase (a crude enzyme fraction that converts O<sub>2</sub> molecules to H<sub>2</sub>O), an anaeropack (a gas-tight plastic bag with O<sub>2</sub> absorbent), and an anaerobic chamber. All operations including pre-incubation of the LB+Oxyrase plate, spreading cells on the plate, inserting it into the anaeropack, and incubation of the plate were carried out in an anaerobic chamber where air was replaced with N<sub>2</sub>+H<sub>2</sub> gas and residual O<sub>2</sub> molecules were converted to H<sub>2</sub>O very effectively by a special catalyst. O<sub>2</sub> concentration in the anaerobic chamber was too low to be detected by an O<sub>2</sub> monitor. On the other hand, when an LB plate without Oxyrase was put into the anaeropack, which was then placed in the atmosphere, we found that the concentration of O<sub>2</sub> in the anaeropack was about 0.1% (hypoxic conditions). Chemical oxygen absorbers used for the anaeropack are powerful enough to reduce the oxygen concentration from 20% to 0.1%; the oxygen con-
centration in the anaeropack reached 0.1% in several hours after tight sealing of the bag and was maintained at 0.1% for several days. Unexpectedly, the mutation frequency of the ΔmutMY cells increased sharply when the cells were grown in these hypoxic conditions (Fig. 1B; Supplementary Fig. S4). The addition of Oxyrase to the LB agar plate decreased the frequency of Rifr mutation in ΔmutMY cells grown in the anaeropack to about 1/300 of that without Oxyrase (Supplementary Fig. S4). Thus, we considered that the amount of 8-oxoG in DNA was elevated in cells grown under hypoxia. While the cellular level of 8-oxoG in DNA was about 14-fold higher in hypoxic than in aerobic growth conditions (Fig. 1B), the mutation frequency of wild-type cells was unchanged in the hypoxic conditions, indicating a surplus capacity of MutM and MutY proteins.

**Frequency of spontaneous events of allelic recombination increases when cells are grown in conditions that induce more 8-oxoG DNA damage** 8-oxoG in DNA is caused by the hydroxyl radical, which also produces various kinds of oxidative DNA damage other than 8-oxoG (Hutchinson, 1985). In a previous study, 8-oxoG was estimated to represent about 5% of the total oxidative DNA damage (Richter et al., 1988). Therefore, it seems very likely that various species of oxidative DNA damage are produced much more abundantly in cells grown in minimal media or under hypoxia than in those grown in LB in the atmosphere. Some types of oxidative DNA damage, such as 8-oxoG and 5-hydroxycytosine (Shibutani et al., 1991; Purmal et al., 1994), are the miscoding type that does not block DNA replication but induces base substitution mutation, whereas many others are the replication-blocking type that stops chain elongation of DNA by DNA polymerase. The miscoding type of oxidative DNA damage is efficiently and accurately repaired in E. coli cells because the frequency of base substitution is unchanged even when cells are grown in conditions that induce more 8-oxoG in DNA. Previous studies have revealed multiple pathways of DNA repair for the replication-blocking type of oxidative DNA damage (Imlay, 2008). However, it is still unclear how the repair pathways participate in counteracting the blockage of DNA replication by oxidative DNA damage in normally growing cells. In particular, an important question is to what extent the recombination repair pathway plays a role in the process. If other repair pathways completely remove the replication-blocking damage, the recombination repair process, which initiates after the blockage of DNA replication, would not necessarily work.

Using a hemi-diploid rpsL strain, MK9547 (Supplementary Fig. S1), we examined the frequency of spontaneous events of allelic recombination, a consequence of recombination repair, in cells grown in conditions that induce more 8-oxoG in DNA. Strr derivatives emerging from the hemi-diploid rpsL strain result from the gene conversion type of allelic recombination between the authentic copy, rpsL128 (Strs), and the transgenic copy, rpsL+ (Strr), or from the loss-of-function type of genetic alterations in the transgenic rpsL gene (Sakai et al., 2006; Kanie et al., 2007) (Supplementary Fig. S5). The former type of genetic changes (recombination events) and the latter type (non-recombination events) can be accurately distinguished by PCR analysis with a primer set that detects a change caused by allelic recombination (Supplementary Fig. S2). As shown in Fig. 2A, the frequency of recombination events in MK9547 cells grown in M9-glucose was about 4.3-fold higher than that in LB, and increased further to 7.3-fold when the cells were grown in M9-glycerol. On the other hand, the frequency of non-recombination events was unchanged in cells grown in M9-glucose and M9-glycerol. This is consistent with the observation that the frequency of base substitution mutation in wild-type cells was not changed in these minimal media (Fig. 1A). It should be noted that 65% of non-recombination events in the rpsL target sequence were base substitutions (Kanie et al., 2007).

Cells grown in LB in hypoxic conditions also showed about a 3.5-fold higher recombination frequency than those grown in aerobic conditions (Fig. 2B). The addition of Oxyrase to LB significantly decreased the recombination frequency in cells grown under hypoxia, suggesting that oxidative DNA damage is involved in the increase of recombination frequency in hypoxic conditions. Furthermore, the frequency of spontaneous allelic recombination under these conditions (LB+Oxyrase, hypoxia) was about half that in aerobic conditions. The frequency of non-recombination events in hypoxic conditions showed no significant difference from that in aerobic conditions, whereas the addition of Oxyrase to LB significantly decreased the non-recombination frequency, consistent with the previous observation that the hot-spot type of base substitution in the rpsL target sequence disappeared when cells were grown in strictly anaerobic conditions (Sakai et al., 2006).

From the results described above, we concluded that, unlike spontaneous base substitution mutagenesis in wild-type cells, the occurrence of spontaneous recombination events is affected by environmental factors such as nutrition and oxygen concentration in E. coli.

**Cells defective in SOS response induction show the same frequencies of spontaneous recombination as wild-type cells in normal and higher 8-oxoG DNA damage-inducing conditions.** It seems plausible that the replication-blocking type of oxidative DNA damage or double-strand DNA breaks should increase in cells growing in conditions that produce more 8-oxoG in DNA. This is one of the possible explanations for the elevated frequencies of spontaneous recombination events observed with
However, we cannot rule out other possible explanations for the elevated recombination frequencies. It is unclear what is the limiting factor(s) for spontaneous recombination events. Besides recombinogenic DNA damage, the level of recombination activity or, more simply, the expression level of recombination proteins may be a limiting factor. The expression of RecA protein, a major recombinase in *E. coli*, is regulated by the SOS response mechanism and could be elevated when cells grow in minimal media or hypoxic conditions. To examine whether this is the case, we introduced the *lexA3* mutation into the MK9547 strain and determined the frequency of spontaneous recombination events in the resulting strain, MK9597, under different growth conditions (Fig. 3). In the *lexA3* mutant strain, induction of the SOS response is totally suppressed, and the expression level of RecA protein is unchanged even when the chromosomal DNA is severely damaged (Walker, 1984; Pennington and Rosenberg, 2007). When the cells were grown on LB plates in the atmosphere (normal conditions), frequencies of spontaneous recombination were almost the same between the wild-type and *lexA3* strains. In hypoxic conditions, the recombination frequency increased in the *lexA3* strain in a manner similar to the wild-type strain. Frequencies of non-recombination events decreased in both strains under hypoxia.

Fig. 2. Effects of nutrient and oxygen conditions on spontaneous allelic recombination events in wild-type *E. coli* strain. Wild-type (MG1655) strain was grown in different conditions as indicated, and frequencies of recombination and non-recombination events were determined for six independent cultures of cells grown in each condition. The data were obtained from an average of at least six independent experiments. Black and gray bars indicate frequencies of recombination and non-recombination events, respectively. Error bars indicate standard deviations. Values are indicated as means. P values were calculated by a Student’s *t*-test for the indicated combinations of frequencies of recombination events in different growth conditions. (A) Frequencies of recombination and non-recombination events in different nutrition conditions. M9 Glc and M9 Gly are M9 medium containing 0.2% glucose and 0.2% glycerol, respectively. (B) Frequencies of recombination and non-recombination events in different oxygen conditions.

Fig. 3. Effects of nutrient and oxygen conditions on spontaneous allelic recombination events in a high RecA-expressing strain of *E. coli*. *lexA3* (MK9597) strain was grown in different conditions as indicated, and frequencies of recombination and non-recombination events were determined for six independent cultures of cells grown in each condition. The data were obtained from an average of at least six independent experiments. Data for the wild-type (MG1655) strain in LB are the same as those shown in Fig. 2. Black and gray bars indicate frequencies of recombination and non-recombination events, respectively. Error bars indicate standard deviations. Values are indicated as means. P values were calculated by a Student’s *t*-test for the indicated combinations of frequencies of recombination events in different growth conditions.
DISCUSSION

Here we show for the first time that environmental factors, namely nutritional condition and oxygen concentration, have the potential to change the frequency of chromosomal rearrangement but not the frequency of base substitution mutation in *Escherichia coli*. Whereas the frequency of spontaneous base substitution is maintained at a constant level in various different growth environments, the occurrence of chromosomal rearrangement is changeable in different growth environments. We should therefore consider that some environmental factors can change the rate of genetic alteration, at least in *E. coli*. It is of interest to know whether other factors in the common growth environment for cells or organisms, such as temperature and pH, have similar effects. To answer this question, we have studied effects of growth temperature and pH of the growth medium on genetic alteration in *E. coli* (S. Nunose, R. Miyahara and H. Maki, unpublished data), and the results will be reported elsewhere.

By determining Rif\(^r\) mutation frequency in *ΔmutMY* cells growing in different growth conditions, cellular levels of 8-oxoG in DNA were assessed to measure the effects of different conditions in the present study. This approach is based on the fact that MutM and MutY are exclusively responsible for suppression of G:C→T:A base substitutions caused by the 8-oxoG DNA lesion, and most (more than 99%) of the Rif\(^r\) mutations that occur in *ΔmutMY* cells are G:C→T:A base substitutions. We consider that 8-oxoG produced by hydroxyl radicals in DNA remains unrepaired in *ΔmutMY* cells and leads to highly efficient induction of G:C→T:A base substitution in the cells. Thus, the frequency of Rif\(^r\) mutations should be a good measure of how frequently the 8-oxoG lesion is produced in chromosomal DNA. We found that the cellular level of 8-oxoG DNA lesions increased strikingly when the cells grew in minimal media or in hypoxic conditions. Why this elevation of 8-oxoG in DNA occurs is unclear and under investigation in our laboratory. Part of the reason could be an expansion of the doubling time of cells, which is about 3.0-, 3.0-, 3.7- and 4.6-fold longer in M9-glucose, M9-succinic acid, M9-glycerol and hypoxic conditions, respectively, than in LB. If the rate of 8-oxoG production is constant under these different conditions, the amount of 8-oxoG in DNA produced in a round of cell division should correlate with the doubling time. However, the levels of 8-oxoG in DNA relative to that estimated for cells grown in LB were 8.9, 42, 63 and 14 for cells grown in M9-glucose, M9-succinic acid, M9-glycerol and under hypoxia, respectively (Fig. 1A and 1B). Therefore, we concluded that the rate of 8-oxoG production varies with different nutrition conditions, likely due to considerable variation in the concentration of hydroxyl radicals in cells grown under these conditions.

Despite such a wide range in the amount of 8-oxoG in DNA in different growth conditions, wild-type *E. coli* cells maintain the frequency of spontaneous base substitution at a very low level, demonstrating a great capacity of MutM and MutY proteins to suppress mutagenesis caused by 8-oxoG in DNA. The level of 8-oxoG in DNA increased up to 63-fold in minimal media compared with that in the standard LB medium, while Rif\(^r\) mutation frequencies in wild-type cells in both conditions were almost the same. Thus, the G:C→T:A base substitution occurring with very low frequency in repair-proficient wild-type *E. coli* cells does not result from a stochastic escape of 8-oxoG DNA damage from the repair process by MutM and MutY proteins. We previously reported that hotspot-type base substitutions, which account for 80% of total base substitutions occurring in aerobic growth conditions, were oxygen-dependent but not related to 8-oxoG in DNA or 8-oxodGTP. Sequence-independent G:C→C:G and A:T→T:A base substitutions, accounting for 9% of the total base substitutions, were also oxygen-dependent. We proposed that these mutations might be derived from an infrequent type of oxidative DNA damage that could not be repaired by the existing repair mechanisms but would become fixed as mutations by the replicative DNA polymerase (Sakai et al., 2006). The remaining sequence-independent base substitutions might be derived from infrequent non-oxidative DNA damage that would also be unrepairable by any repair mechanisms.

The molecular basis underlying the growth condition-dependent increment of spontaneous recombination frequency is unknown at present. The recombination event studied in this work is RecA-dependent homologous recombination, which has been extensively investigated and documented (Lusetti and Cox, 2002). In normally growing *E. coli* cells, homologous recombination occurs as a repair process to overcome a stalled replication fork and/or double-strand break caused by the replication-blocking type of DNA damage. The capacity of the RecA-dependent recombination mechanism is strengthened when cells induce the SOS response, whereas the uninduced basal capacity is sufficient to rescue a stalled replication fork and to repair a double-strand break spontaneously occurring in a normally growing cell. Therefore, it is reasonable that the frequency of spontaneous recombination events correlates with how frequently replication forks stall and double-strand breaks occur in normally growing cells. The result obtained with *lexA3* cells supports this notion. If this is the case, the increase of spontaneous recombination frequency in cells grown in minimal media or under hypoxia could be due to an increase of the amount of replication-blocking type of DNA damage and/or the number of double-strand breaks in the cells. As described earlier, we expected that various kinds of oxidative DNA damage would be produced, to levels as high as that of 8-oxoG, in cells grown in mini-
normal media or hypoxic conditions. Most of the oxidative DNA damage is the replication-blocking type, and it is well known that double-strand breaks are induced when DNA is attacked by hydroxyl radicals that also produce 8-oxoG (Hutchinson, 1985; Richter et al., 1988). The growth condition-dependent increment of spontaneous recombination frequency was relatively small, up to about a 7-fold difference as far as we observed. This may be due to very effective action of other pathways of DNA repair to counteract the replication-blocking type of oxidative DNA damage before the replication fork reaches the damaged site. Most recently, we observed that the spontaneous recombination frequency in cells defective in both nucleotide excision repair and base excision repair was 10-fold higher than that in wild-type cells in normal growth conditions, while it increased sharply to about 700-fold in M9-glucose (L. A. T. Le and H. Maki, unpublished data). This finding will be published elsewhere and clearly indicates that huge amounts of recombination events that cause various types of chromosomal rearrangement.

Although growth conditions that stimulate E. coli cells to produce higher levels of oxidative DNA damage, i.e., low nutrition and low oxygen concentration, are unfavorable for rapid cell propagation, they resemble the primary living environment for E. coli, namely the mammalian and avian colon, where the conditions are nutritionally poor and hypoxic. Therefore, it seems reasonable that E. coli possesses such an excessive DNA repair capacity, which almost completely eliminates the miscoding type of spontaneous oxidative DNA damage even at levels two orders of magnitude higher than that typically produced in cells grown under standard laboratory conditions. It is also conceivable that in E. coli cells living in nature, spontaneous events of homologous recombination occur more frequently than we might expect from studies in the laboratory. In general, cells in the human body are maintained in a stable environment where nutrition and oxygen concentration are suitably controlled by elaborate mechanisms. However, the micro-environment of cancer cells is usually poor in nutrition and hypoxic (Bissell and Hines, 2011). Although there are many differences between bacterial cells and human cells, it is possible that cellular levels of oxidative DNA damage and the occurrence of chromosomal rearrangement are affected by the micro-environment in a similar manner for both E. coli and cancer cells. To answer this question, it is necessary to monitor the level of oxidative DNA damage and its genetic consequences in cancer cells grown in different conditions. The approach that we took in this study may help in the development of new methodologies that are applicable to cancer cells.

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**CONFLICT OF INTEREST**

There is no conflict of interest.

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