DIFFERENTIAL MUTABILITIES TO TYPES OF MUTATIONS WITH ULTRAVIOLET LIGHT BETWEEN NORMAL AND UV-SENSITIVE MUTANT OF YEAST

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INTRODUCTION

Recent development in radiation biology has revealed that at least in bacteria, majority of UV-induced lethal damage which is predominantly due to pyrimidine dimers in DNA is repaired by two kinds of cellular repair systems, i.e., dark repair and photoreactivation. For mutation induction, Witkin (1966), Hill (1965), Bridges et al. (1967) and Kondo and Kato (1968) have demonstrated that UV-induced mutational damage in E. coli is also capable of repairing by both dark and photorepair systems in the cells. Effect of photoreactivation on mutation induction also reported in T4 phage (Drake 1966). In order to understand the mechanism of mutations, it is necessary to clarify repair specificity to premutational damages leading to various types of mutation. Photoreversibility to UV-induced mutations would not be specific for both base substitution and frame shift-type mutations in T4 phage (Drake 1966) and Neurospora (Kilbey and de Serres 1967). However, the differential photo-reversibility between super-suppressor and back mutation has been shown in the recent studies of Resnick (1968) in yeast.

There has been relatively little investigation of repair mechanism of mutation in eukaryotic organism (Chang et al. 1968; Nakai and Matsumoto 1967; Nasim 1968; Resnick 1968). A simple eukaryotic organism the yeast, Saccharomyces cerevisiae, makes it possible to study the repair mechanisms of the different kinds of genetic damages, which are responsible for conversion, mitotic intra- and inter-genic recombinations, and for cytoplasmic mutation. In this report we presented the data concerning the repairabilities of damages to different types of UV-induced mutations with special emphasis on base change type mutation produced at low dose range in yeast.

MATERIALS AND METHODS

Strain

Saccharomyces cerevisiae heterothallic strains obtained from Berkeley yeast culture collection were used in this experiment. Genetic markers are described by the symbols proposed by Dr. R. K. Mortimer at the convention of International Conference Yeast Genetics 1968 at Osaka, Japan. For the mutation study, following strains were constructed:
The six alleles, arg 4-17, his 5-2, lys 1-1, try 5-48, leu 1-11, ade 2-1, known to be nonsense-mutant alleles suppressed by the super-suppressor Sd, were obtained from Dr. R. K. Mortimer. Allele of his 1-1 known to be addition and deletion type mutation was introduced to our strain from the original stock of 1012 obtained by Dr. G. Magni by six time back crosses.

**Media**

Growth medium (YEPD) contained 1% Difco yeast extract, 2% Difco pepton, 2% dextrose. Synthetic complete medium (SC) contained 0.64% Difco yeast nitrogen base without amino acids, 1% dextrose 2% agar and following amino acids and purine base: adenine (AD) and histidine (HI) methionine (ME) tryptophan (TR), at 20 mg/litter leucine (LE) and lysine (LY) at 30 mg/litter, respectively.

For mutation studies, omission medium was used: a series of media in which one of the amino acids or bases was omitted from synthetic complete; e.g., (−AR) is SC minus arginine.

**UV-irradiation and microbiological techniques**

Stationary phase cells were harvested from liquid YEPD culture. After shaking at 30°C for 4 days, the cells were washed twice and then plated onto omission media to determine mutants or onto SC medium for scoring survivals. Thereafter, they were exposed to UV irradiation from a low-pressure mercury germicidal lamp (National Electric). The dose rates used for the high and low dose experiments were 30 erg/mm²/sec and 6 or 1 erg/mm²/sec, respectively. To avoid uncontrolled photoreactivation all the experiments were performed in a room illuminated with yellow fluorescent lamps. For photoreactivation treatments, six 40W day-light fluorescent lamps (National Electric) were used. The cells on the plates were illuminated for two hours at a distance of 20 cm at 25°C. This condition gave a maximum photoreactivation to UV-irradiated cells. After these treatments plates were incubated at 30°C. Mutants were scored on the 10th day, and survivals were counted on the 4th day after initiation of incubation.

In order to determine whether obtained prototrophs are due to super-suppressor mutation Hawthorne and Mortimer (1963) or true back-mutation, a replica method was employed (Gilmore and Mortimer 1966). Prototrophs grown on omission medium plates were picked up, restreaked on YEPD master plates, incubated for 2 days and then replica plated onto omission media −AR, −HI, −LY and −AD. These omission plates were incubated for 10 days and then scored. Cells grown on all kinds of omission medium were considered to possess super-suppressor, whereas cells grown on only one of the omission medium were considered as back mutants or mutants of specific suppressor.
Spontaneous frequencies of these kinds of mutations examined in \textit{uvs} 1 strain were almost equal to those in a wild-type strain. Frequencies of background for back mutation, super-suppressor and addition-deletion type mutation in wild type strain were $1\times10^{-8}$, $1\times10^{-7}$ and $1\times10^{-8}$, respectively. On the other hand background frequencies of those type of mutation in \textit{uvs} 1 strain were $1\times10^{-7}$, $1\times10^{-6}$ and $1\times10^{-7}$, respectively. Spontaneous frequencies of these mutatins did not depend on the number of cells plated. Thus, frequencies of induced mutations were calculated simply by subtracting spontaneous frequencies from frequencies of produced mutations.

\section*{RESULTS}

\textit{Action of UV-sensitive gene and repair}

It has been demonstrated with \textit{E. coli} that high UV-sensitivity of some mutants, such as \textit{B}_{-1} or \textit{hcr} strains is due to lack of dark repair ability for damaged DNA (Boyce and Howard-Flanders 1964; Hill 1965; Witkin 1966). Highly UV-sensitive mutants of yeast, \textit{uvs} 1 and \textit{uvs} 2, isolated in our laboratory, are controlled by different recessive genes (Nakai and Matsumoto 1967). Although there is no direct biochemical evidence, it may be assumed that actions of these UV-sensitive genes are associated with dark repair mechanisms to the UV-induced photoproducts such as pyrimidine dimers in a similar way to that found in \textit{E. coli} (Setlow and Carrier 1964). Figure 1 shows typical experimental

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig1.png}
\caption{Survivals of a wild type and two UV-sensitive strains of haploid yeast after a maximal photoreactivation treatment as a function of time of treatment with visible light after initiation of post-UV incubation. UV doses administered are 600 erg/mm$^2$ for a wild type strain and 30 erg/mm$^2$ for \textit{uvs} 1 and \textit{uvs} 2 strains, respectively.}
\end{figure}


results, levels of survivals in both uvs 1 and uvs 2 strains after photoreactivation did not change significantly until two hours after initiation of post-incubation. After two hours at which time first DNA replication after UV-irradiation might take place, survivals of uvs 1 and uvs 2 gradually decreased immediately after initiation of incubation. If we assume photoreactivable damage in yeast is pyrimidine dimers (Cook 1967), one can postulate that dimers are more susceptible to dark repair in repair sufficient—wild type strain. Accordingly a same level of reactivated survivals after treatment with visible light might be expected at any time before next DNA replication in repair-deficient strain, while survivals of UV-irradiated cells of the wild strain might decreased with increasing time between photoreactivation and initiation of incubation. Thus it would be concluded that actions of uvs 1 and uvs 2 gene are associated with dark repair mechanism, presumably excision-type repair. Dark-repair suppressing action of uvs 2 gene was found to be suppressed by super-suppressor produced after UV-irradiation. This strain is not suitable for UV studies. Therefore, uvs 1 gene was used as the repair deficient strain in the present experiment.

**UV-induced back mutation from suppressible alleles**

Suppressible alleles of arg 4-17, his 5-2 and lys 1-1 examined are assumed to be a nonsense-type mutation presumably due to UAA ochre codon (Gilmore and Mortimer 1968). Phenotypic reversions of these genes could arise either by super-suppressor or true-back mutation from nonsense mutation. By using poly-auxotrophic strains and replica method described in Materials and Methods, it is possible to discriminate between super-suppressor and true-back mutation. Comparison has been made between the strains with and without a UV-sensitive gene, both having nearly the same genotype.

Figure 2 shows the dose-response curves on a logarithmic scale for back mutation from suppressible alleles in haploid strains. It is evident that the yields of back mutation in the uvs 1 strain are more than 100 times higher than that in the wild type strain at equal doses. Since the action of uvs 1 gene is considered to be lacking the dark repair for lethal damage in DNA as mentioned previous section, low yield of induced mutation in the wild type strain could be explained by the function of repair of pre-mutational damage. In repairless uvs 1 strain, however, pre-mutational damage would be expressed more fully as a fixation of mutation. The shape of the dose-response curve for mutation induction in the wild-type

**Fig. 2.** Induction kinetics of true-back mutations from nonsense alleles, are 4-17 [○], his 5-2 [△] and lys 1-1 [■], by ultraviolet light in wild type and uvs 1 strains of haploid yeast. Closed symbols; dark; open symbols; light.
strain is almost linear over all the dose range studied. UV-hit number of this line was estimated to be ca. 1.5. While in the UV-sensitive-strain the curves seem to be divided into two components; one with a steeper curve in the high-UV dose range and the other with moderate slope in the UV dose range of about 70-100% survival. Former slope is almost same as that of wild type. UV-hit numbers of these components were estimated to be ca. 1.5 and 0.75, respectively. Thus it may be attributed that premutational damage for true back mutation consists of two components: One of the component produced at low doses is more dark repairable than the other component. It should be pointed out that in *uvs* 1 strain the yield of back mutation for lys 1-1 is relatively lower than that of arg 4-17 and his 5-2 at low dose range. However, these differential yields of mutations are not revealed under the condition of high dose irradiation in *uvs* 1 strain. The quantitatively different premutational damages produced at low UV-doses were found only in UV-sensitive and repairless strain. The effect of photoreactivation for mutation induction is revealed at high doses but not so evident at low doses.

Figure 3 shows the results of experiments for back mutation in diploid strain. General aspects of the dose-response curves for mutation induction are similar as those for the corresponding haploid strain; 1) production of high yields of mutation in repairless *uvs* 1 strain than the wild type strain at equal doses, 2) two-components curves of mutation induction in *uvs* 1, 3) increasing effect of photoreactivation on mutation at high UV doses, 4) allele dependence of mutability in *uvs* 1 at low doses. It seems that frequencies of induced mutation in diploid strains are only slightly higher than those in haploid strains even though survivals of diploid strains are markedly higher than those of haploid strains at the same UV doses.

*UV-induced super-suppressor mutation*

Figures 4 and 5 show the dose-response curves for induction of super-suppressor mutation in haploid and diploid strains, respectively. Shapes of these curves are clearly different from those for back mutation. Both the curves in UV-sensitive and wild-type strains seems to be linear. Slopes of the UV-sensitive strains are slightly less than those of back mutation. Frequency of mutation increased with approximately square of UV dose. Although the frequencies of induced super-suppressor mutation in *uvs* 1 strain are apparently higher than that in wild-type strain at the same doses, dark re-
pairability of premutational damage of super-suppressor estimated by the difference in mutation frequency between uvs 1 and wild-type strains is about half of that of back mutation. Effect of photoreactivation for suppressor mutation at high UV doses is much more pronounced than that of back mutation. Major part of the premutational damage of suppressor, unlike back mutation, is photorepairable, i.e., presumably pyrimidine dimers. General aspects of mutation induction of super-suppressors are the same in diploid and haploid cells.

**UV-induced addition-deletion type mutation**

Figure 6 shows results of experiment with reversion from his 1-1 allele. Based on the results conducted by Magni and Puglisi (1966), the nature of his 1-1 allele is considered as addition-deletion type mutation. Figure 6 presents the results of high yields of reversion of his 1 in uvs 1 strain compared with that in wild-type strain at the same dose. It will be concluded that UV-induced addition-deletion type mutation is also dark repairable. Photoreversibility for this type of mutation appeared markedly in uvs 1 but only slightly in the wild type. This suggests that pyrimidine dimers are primarily responsible for the addition-deletion type mutation in uvs 1 strain. The shape of dose response curve of this type of mutation consists of one component.

**Relation between induced mutation and survival**

Frequencies of different types of mutation plotted against survivals, instead of UV doses, are shown in Figure 7. It can be seen that frequencies of true back mutation in uvs 1 strain, unlike other types of mutations, are abruptly increased within the range
Fig. 6. Induction kinetics of reversion of his 1-1 allele by ultraviolet light in wild type and uvs 1 strains of haploid yeast.

Fig. 7. The relation between survivals and the frequencies of induced mutations in haploid yeast.

- •• 0-0 true back mutation of arg 4-17
- ■■ □□ super-suppressor mutation
- △△ △△ reversion of his 1-1, addition-deletion type mutation in uvs 1 strain.

Open symbols, UV-sensitive, uvs 1, strain, Closed symbols, wild type strain.
of 100–80% survivals. Below 80% survivals, the shape of survival curve frequency for back mutation almost similar to that for suppressor or addition-deletion type mutation. Frequencies of the latter two types of mutations decrease uniformly with increase in survivals above 80%. These results are consistent with the assumption that premutational damage of back mutation may consist of qualitatively different types of damage depending on UV dose. Unlike the result in *E. coli* by Witkin (1966), experiments show that frequencies of most induced mutations in repair-sufficient strain are much more higher than repairless strain except for back mutation in a range of 100–80% survivals. We may conclude that repairability of lethal damage is higher than that of premutational damage except for back mutation at very low doses. The experimental results mentioned above suggest that the nature of UV-induced premutational damage with regard to the dark-repairability be somewhat different with lethal damage.

**DISCUSSION**

It will be interesting to know whether repair system for premutational damage in eukaryotic-organism yeast similar to that in *E. coli*. It was found by many authors with suppressor mutation in bacteria that marked enhancement of UV-induced mutations occurs in UV-sensitive strain than that in wild-type strains at equal UV dose (Witkin 1966; Hill 1965; Bridges et al. 1967; Kondo and Kato 1968). While the studies of eukaryotic organism, such as *Neurospora crassa* by Change et al. (1968), and *Shizosaccharomyces pombe* by Nasim (1968), showed that UV-induced mutations in UV-sensitive strains do not increase significantly beyond wild type strain at equal doses. Disagreement between these previous results and present ones may have arisen the difference in the nature of UV-sensitive strains used. In fact, the *uvs* 1 strain used in the present experiments showed markedly higher yields of mutations of three types than those in a wild type strain at equal doses (Nakai and Yamaguchi 1967). This suggests UV-induced premutational damage in yeast is mostly dark-repairable irrespective of molecular types of mutations. Independent experiment conducted by Resnick (1968) in Berkeley using another UV-sensitive mutant of *Saccharomyces cerevisiae*, has led to the result completely in accord with this conclusion.

Present experiment suggests that at least two types of premutational damages for back mutation were induced with UV-light. Because the shapes of dose response curves for back mutation in repairless strain seem to consist of two components. Alteration in the slope of the curves in the UV-sensitive strain with UV dose can not be attributed to the effect of selective killing of mutants by the effect of high dose irradiation, since altering point at the mutation curve appeared within the sublethal dose range. Moreover, present results showed that at low doses, but not at high doses, yields of premutational damage of back mutation were highly allele specific indicating that production of this type of damage is dose dependent. Molecular nature of these two types of premutational damage is not known. It would however be worth to note that the highly dark-repairable damage produced at low UV doses was not susceptible to photoreactivation.

In the present study, it is apparent that UV-induced premutational damages lead-
ing to super-suppressor and addition-deletion type mutations are also dark repairable. Similair conclusion has been given by the studies of Resnick (1968) in yeast. Dark repair of suppressor mutation in *E. coli* also has been reported by Witkin (1966), Bridges *et al.* (1967) and Kondo and Kato (1968). The dark repairability of premutational damage seems to be decreased with the order of back mutation at low UV doses, super-suppressor and addition-deletion type mutation; ratios of the dose-reduction factors for these three-type mutations were about 16 : 8 : 7. Thus it is likely that there is rather good correlation between production and dark repairability for premutational damages to the different types of mutation. It may be concluded that different extent of molecular lesion which is responsible for different types of mutation, may reflect the efficiency of repairabilities.

Drake (1966) reported that in T4 phage, photoreversivility was not specific for both UV-induced transition (base chang type) and sign mutation (fram shift type). Similair conclusion was presented by Kilbey and de Serres (1967) in *Neurospora*. However, both of their results were obtained in repair sufficient, wild-type strains. Therefore, their conclusion may not be applicable in repairless condition for premutational damage. It would be reasonable to assume that in repairless condition all of the potential mutational damage should be expressed. However, if certain type of damage is more repairable, and such a damage is contributed predominantly to induce for particular type of mutation, absolute and relative frequency of this type of mutation should decrease in the repair-sufficient condition. Present experiment showed that yield of back mutation in the repairless strain, was much higher than that of super-suppressor or addition-deletion type mutation while in the repair sufficient strain, such difference diminished. Therefore preferential repair of particular type of premutational damage is postulated. Based on this postulation, the following hypothetical model may be proposed. Premutational damage constitutes two types of dark-repairable damages, that is, pyrimidine dimers and non-dimer type lesions. The latter lesions could be minor alterations in DNA and more preferentially dark repairable. Premutational damage for back mutation is mainly due to non-pyrimidine dimer-type lesions at low lesions, but due to pyrimidine dimers at high doses. Whereas addition-deletion type mutation arises mainly from pyrimidine dimers in all the UV dose range. More conclusive evidence supporting this model has been obtained by investigation of forward mutations of different molecular types (Nakai *et al.* in preparation).

The ratio of suppressor mutation to back mutation among UV-induced prototrophs in revertants from a nonsense alleles in UV-sensitive strains varied with the organism examined. Suppressor is the majority among prototrophs in *E. coli*, whereas suppressor is the minority in yeast. Although the reason for these contradictory results was not known, we must keep in mind that suppressor systems will be somewhat different between these two organisms.

**SUMMARY**

Experiment has been carried out using UV-sensitive mutant of *uvs* 1 of *Saccharomyces cerevisiae*, presumably lacking ability of dark repair, to investigate genetic nature of UV-induced premutational damages leading to three-different types of mutation under
the dark and illuminated conditions. Yields of these types of mutation in \textit{uvs} 1 are markedly higher than those in wild type at equal doses. At the dark condition, logarithmically plotted dose-response curves of true-back mutations from \textit{arg} 4-17, \textit{his} 5-2 and \textit{lys} 1-1 induced in \textit{uvs} 1 consist of two straight lines. Slopes of the curves at high doses are more steeper than those at low doses and almost the same as those in wild type in all the dose range. The curves for induced frequency of super-suppressor mutation and that of back mutation from \textit{his} 1-1, i.e., addition-deletion type mutation consists of one component, and their slopes are nearly equal for both \textit{uvs} 1 and wild-type strains. Furthermore, ratios of true back mutation yields between \textit{uvs} 1 and wild-type strains at equal doses are greater than those of super-suppressor mutations or reversion of addition-deletion type. To account for these results it is postulated that premutational damage leading to true-back mutation is \textit{preferentially repairable} compared with damages responsible for the other types of mutation. Molecular natures of the premutational damages leading to the three types of mutation are discussed.

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**LITERATURE CITED**


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