REPAIR-MECHANISMS AND RADIATION-INDUCED MUTATIONS IN FISSION YEAST

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MUTAGENIC treatments of an adenine-requiring red strain of the fission yeast, *Schizosaccharomyces pombe*, lead to the production of two kinds of mutant colonies in which mutation has led to loss of red pigment. One of these is wholly white and these are called complete mutant colonies. The other contains white sectors together with red sectors of the original parental type and these are called mosaic mutant colonies.

In a previous publication (NASIM and AUERBACH 1967) four possible mechanisms were discussed in detail for the induction of complete mutants by mutagenic treatments which presumably alter just one of the two strands of DNA at a mutating locus. These appeared to encompass all of the more likely possibilities. Two of the four mechanisms i.e. the "lethal hit" and the "master strand" hypothesis were shown by this earlier evidence to be inadequate to explain the production of all of the complete mutants, leaving two remaining possible mechanisms still to be tested, i.e. the "repair-hypothesis" and the "dual-mechanism hypothesis".

The present study was designed to test the so-called repair-hypothesis which postulates that cellular repair mechanisms may alter either the mutated strand of DNA to match the nonmutated strand or alternatively may change the nonmutated strand to match the mutated strand. In the latter case a potential mosaic mutation would be transformed into a complete mutation. One of the many ways to test this hypothesis is to study the ratios of complete to mosaic mutants in the radiation-sensitive strains which lack the ability to repair the lethal damage inflicted by ultraviolet irradiation. In studies with such sensitive strains one could make two predictions: (a) that in the sensitive strains fewer potential mosaics will be transformed into completes, since this is assumed to involve a repair mechanism; and (b) that there will be a higher overall mutation frequency, since fewer of the potential mosaics will have reverted to nonmutants.

The ratios of mosaics to complete mutants, and the overall mutation frequencies have been studied following ultraviolet (UV) irradiation in three strains showing different degrees of sensitivities to the lethal action of ultraviolet and have been compared with those for the normal red strain. A range of UV doses giving widely different survivals was used. These comparisons have yielded correlations that bear both on the hypothesis under test, and on the possible presence of more than one kind of repair.

MATERIALS AND METHODS

For the isolation of the uvr⁻ (radiation-sensitive) strains the parental red Adn-7 407h⁻ strain was treated with the monofunctional alkylating agent ethyl methane sulphonate. The survivors were replicated on to two sets of yeast extract agar plates and one of the series exposed to UV irradiation. By a comparison of the plates in control and treated series the "probable" UV sensitive strains were picked and then retested in quantitative UV dose-effect experiments. On screening nearly 4,000 colonies the two sensitive strains uvr⁻ N_1 and N_2 were isolated. The strain 50/1a/uvs-1 was very kindly provided by PROF. U. LEUPOLD.

The composition of the media and method of scoring mutants have been described previously (NASIM and AUERBACH 1967; NASIM and CLARKE 1965). Cells for treatment were taken from the purple adenine-7-strain 407h⁻, grown on the YEA medium slope for 48 hours at 30°C. A forward mutation at any one of the five antecedent loci in the adenine pathway would result in complete or mosaic mutant colonies. A G.E. germicidal lamp was used for irradiation of all strains and doses were measured with an International Light Dosimeter. The UV source was at a distance of about 60 cm from the cell suspension with an output of 15 ergs/mm²/sec. For all treatments, 6 ml of cell suspension in distilled water with 1.6×10^8 cells per ml were exposed in a 10 cm diameter Petri dish with continuous agitation during irradiation.

After treatment cells were diluted to a given level in distilled water and plated onto YEA medium. Plates were incubated at 30°C for 5 days and then mutant colonies appearing either as completely white or bearing white sectors within the parental red colonies were scored. Survival and overall mutation frequency were scored on the same plates.

RESULTS

The survival curves for the normal and the three sensitive strains are shown in Figure 1. The dose-reduction factor of the sensitive strain 50/1a/uvs-1 was approximately 6. The uvr⁻ N_1 and N_2 strains were less sensitive to the lethal effects of UV than 50/1a/uvs-1, the dose-reduction factor being about 1.5.

The ratios of complete and mosaic mutants were studied mainly over survivals ranging from 100–1% in all four strains. Results are presented in Table 1.

In the normal strain (Table 1) the proportion of mosaic mutants decreased from 54% to 27% at the two extreme values of survival.

The mutational pattern within the two strains of intermediate sensitivity, N_1 and N_2, differed from that of the normal strain. The following observations could be made from the data in Table 1:

i) The proportion of mosaic mutants among the total mutants in the two sensitive strains was significantly higher at all survival levels. At the two lowest survival ranges (1–40%) there were only 34 and 27% mosaic mutants in the normal strain. In contrast these values were 65 and 61 in N_1 and 72 and 65 in N_2. Statistical tests showed that these ratios are significantly different. The P value ranged between <0.03 and <0.001. ii) The overall mutation frequency per 10^3 survivors was 1.3–1.8 times higher in the two sensitive strains than in the normal strain, within survival range of 1–40%, where there were sufficient mutants for a meaningful comparison of the data. iii) The proportion of mosaic mutants did not decrease at lower survivals for either of these sensitive strains. In both strains the proportion of mosaics remained fairly constant at 61–72% within a survival range of 60–1%. In the normal strain, however, there was a decrease from 54% to 27% in the proportion of mosaics with a decrease in survival.

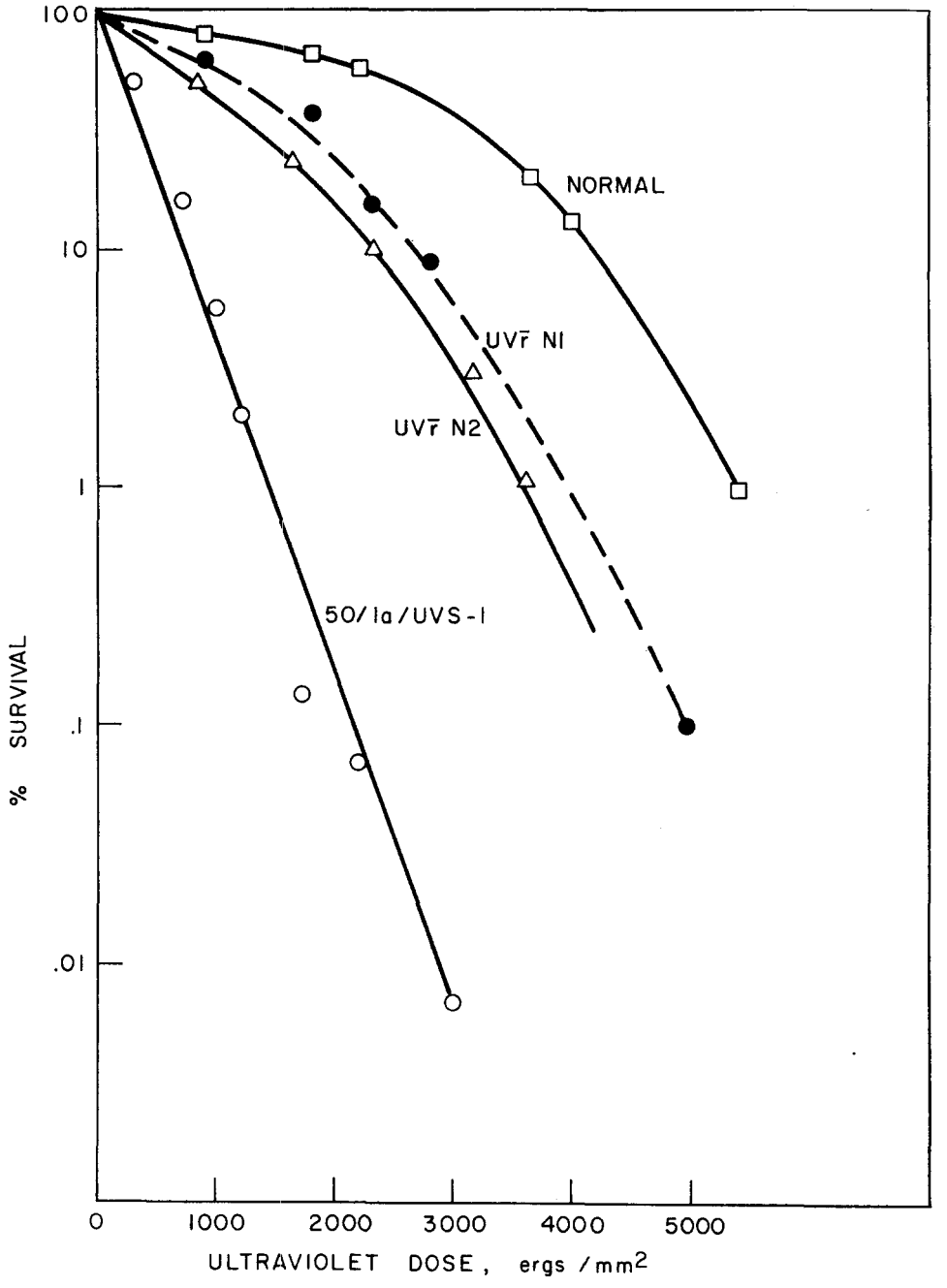

FIGURE 1.—Survival curves for the normal and three radiation-sensitive strains of fission yeast.

TABLE 1

The induction of complete and mosaic mutants by UV irradiation of the normal and radiation-sensitive strains of S. pombe

	Percent survival range						
	100	80–100	60–80	40–60	20–40	1–20	< 1%
a. Normal Strain							
Total Colonies Examined	43,173	4,861	6,747	8,802	7,524	74,040	.....
Mutation Freq per 10^3 Colonies	.14	2.8	4.7	4.9	7.3	11.3	.....
Proportion of Mosaics	33	54	40	40.5	34	27	.....
b. uvr-N1							
Total Colonies Examined	14,426	1,456	393	542	7,739	47,475	.....
Mutation Freq per 10^3 Colonies	.21	3.5	5	6	13.2	14.7	.....
Proportion of Mosaics	33	60	50	66	65	61	.....
c. uvr-N2							
Total Colonies Examined	18,376	.....	1,891	10,766	8,510	55,542	.....
Mutation Freq per 10^3 Colonies	.1	.....	3.7	7.6	12.3	17	.....
Proportion of Mosaics	.....	.....	57	66	72	65	.....
d. 50/1a/uvs-1							
Total Colonies Examined	21,602	.....	.....	2,112	4,625	13,767	125,197
Mutation Freq per 10^3 Colonies	.14	.....	.....	.....	.....	0.8	0.9
Proportion of Mosaics	.....	.....	.....	.....	.....	45	38

The results of studies with the strain 50/1a/uvs-1 which shows the highest degree of sensitivity to the lethal action of UV showed a significant decrease in the overall mutation frequency per survivor. The overall induced mutation frequency was at least 10 times lower than that of the normal strain. The total number of mutants produced by this strain within survival values ranging from 100–1% was not enough to warrant any conclusions about the proportions of complete and mosaic mutants. On the other hand, at very low survivals (<1%) the data indicated that the proportion of mosaics was higher than in the normal strain. Data regarding mosaicism at such low survival values may be complicated by a very high degree of lethal sectoring (HAEFNER 1966, 1967b; JAMES and WERNER 1966).

Any corrections for lethal sectoring and its bearing on the frequency of mosaic and complete mutants would only be possible if these different strains were examined for lethal sectoring at different survivals. All the data available presently indicate that radiation-sensitive strains of *E. coli* and *S. pombe* have a much higher degree of spontaneous and induced lethal-sectoring (HAEFNER and STRIEBECK 1967, NASIM and SAUNDERS unpublished results). An expected increase in lethal sectoring, especially at high doses, would make these values even more divergent.

It is possible to compare the total mutation frequency of different strains in two ways, either at UV doses *equated* for survival values or for the *same* total UV dose. Results of such comparisons are presented in Tables 2 and 3. UV doses

TABLE 2

A comparison of the total mutation frequency in normal and uvr⁻ strains at UV doses equated *for survival*

Strain	Total colonies examined	Complete whites	Mosaics	Mutants per 10^3 survivors	Percent mosaics
Normal	28,326	193	81	9.7	30
uvr⁻-N1	15,212	86	115	13.6	57
uvr⁻-N2	23,387	124	226	14.9	66
50/1a/uvs-1	10,947	6	3	0.82	33

were adjusted to obtain about 10% survival in all four strains. The comparisons showed that the two sensitive strains, N_1 and N_2, had a greater proportion of mosaic mutants and a higher total mutation frequency, whereas the strain 50/1a/uvs-1 had a much lower total mutation frequency. Similarly, when a total UV dose of approximately 4000 ergs/mm² was given to the different strains, the data showed the same trends as outlined above. Since the proportion of mosaic mutants in the normal strain was dose dependent, comparisons of the proportion of mosaic mutants in different strains should be based on Table 1. Tables 2 and 3 present mainly a comparison of the total mutation frequencies at the *same* survival value and at the *same* total UV dose. Statistical tests were carried out using MULLER's method (MULLER, OSTER and ZIMMERING 1963) to compare the overall mutation frequencies in the normal and the two sensitive N_1 and N_2 strains. For the data from both Tables 2 and 3 these tests showed that the mutation frequencies in these three strains were significantly different and the P values ranged between <0.003 and 0.0009.

DISCUSSION

The present investigation supports the repair hypothesis for the production of pure clones, but also emphasizes the existence of secondary mechanisms which may influence the total expressed mutational damage. The results with both strains, N_1 and N_2, were in very good agreement with those predicted from the repair-hypothesis, outlined in the INTRODUCTION. These two strains showed a higher mutation frequency and also a much higher proportion of mosaic mutant colonies at all survival levels (Table 1).

TABLE 3

A comparison of the total mutation frequency in normal and uvr⁻ strains at the same *total UV dose*

Strain	Total colonies examined	Complete whites	Mosaics	Mutants per 10^3 survivors	Percent mosaics
Normal	30,154	143	71	7.2	33
uvr⁻-N1	24,906	89	153	9.7	67
uvr⁻-N2	34,485	146	302	13	66
50/1a/uvs-1	35,037	16	19	1	54

It is pertinent to consider the influence of lethal-sectoring on the data since it has been reported by HAEFNER (HAEFNER and STRIEBECK 1967) that sensitive strains of *E. coli* show a higher degree of lethal-sectoring. For fission yeast the bearing of this phenomenon on the frequency of UV induced pure clones has also been discussed recently (HAEFNER 1967b; AUERBACH 1967). Since all the available evidence suggests that the radiation-sensitive strains show a higher degree of lethal sectoring, this would in fact increase the fraction of completes and it thus seems likely that the difference between these sensitive strains and the normal strain is even more significant than the data imply.

The data from strain 50/1a/uvs-1 did not conform to the predictions of the repair-hypothesis. There was a marked decrease in the total mutation frequency. The estimated proportion of mosaics was higher in this strain, though these results were perhaps unreliable due to scarcity of data. At very low survivals (<1%) the ratios could be complicated by lethal sectoring which would transform potential mosaics into pure mutant clones.

The fact that some sensitive strains do not conform to the repair-hypothesis is of particular interest, since it suggests that more than one mechanism may be influencing the sensitivity to lethal and mutational damage. Such mechanisms may include the selective repair of the mutated strand, the selective killing of the mutants, the failure to express induced mutations due to some changes in the macromolecular metabolic processes. An example of the last possibility is furnished by the "mutation frequency decline" resulting from the inhibition of protein synthesis in bacteria. (WITKIN 1966b.)

She has also recently reported similar observations regarding the independence of the repair of lethal and mutational damage (WITKIN 1966a, and 1967) in bacteria where different strains of *E. coli* B which differ in sensitivity to the lethal action of UV show both "mutation-proof" and mutation-prone modes of survival. The reduced mutation frequency in sensitive strains was attributed to an efficient and specific repair of the mutational damage.

More recently there has been evidence from various sources supporting the repair-hypothesis for the production of pure clones. HAEFNER published data (HAEFNER 1967b) obtained by pedigree analyses of fission yeast, which showed that pure mutant clones were obtained even without the occurrence of lethal-sectoring and perhaps result either from the repair in the non-mutated strand or action of the mutagen on both strands of DNA. The pure mutant clones were attributed to repair by FREESE and FREESE, with experiments on phage T_4V+ and a UV sensitive mutant of T_4 (FREESE and FREESE 1966). These workers attributed the conversion of the mosaic mutants to pure mutant clones to the ability of the phages to excise and repair their own UV lesions.

The choice of these strains was perhaps fortunate in the sense that the study not only supported the repair-hypothesis but also indicated the complexity of the situation regarding ability of strains to repair mutational and lethal damage. A marked decrease in survival after irradiation may not be a true indication of the absence of different kinds of repair-mechanisms influencing expression of mutational damage.

SUMMARY

The influence of repair-mechanisms on mutation induction by UV irradiation has been studied in fission yeast. A normal adenine-requiring red strain and three UV sensitive strains of independent origin were used. The relative frequencies of mosaic and complete mutants were compared in these four strains to test the repair-hypothesis for the production of pure clones. The two slightly sensitive strains uvr$^-$ N_1 and uvr$^-$ N_2 showed a much higher proportion of mosaic mutants and also a higher total mutation frequency as compared with the normal strain. These findings agree with the predictions from the repair-hypothesis for the production of pure mutant clones.—A third highly sensitive strain did not conform to the predictions of the repair-hypothesis. In this strain increased killing was accompanied by a marked decrease in the overall mutation frequency per survivors. This points to the existence of more than one mechanism which may influence killing and mutation differentially. The implications of these findings are discussed as they relate to the different molecular mechanisms proposed for the production of pure mutant clones.

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