GENETIC CONTROL OF RADIATION SENSITIVITY IN *SCHIZOSACCHAROMYCES POMBE*

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ABSTRACT

Genetic analysis of a large number of radiation-sensitive mutants of *S. pombe*, isolated in different laboratories, showed that these isolates represent 22 non-allelic loci. The mutants were shown to fall into three distinct classes concerning response to UV and ionizing radiation, including two mutants which are primarily sensitive to ionizing radiation but not to UV. Single-gene mutants were crossed to obtain supersensitive double mutants. Such double mutants showed a marked increase in sensitivity to a variety of inactivating agents as compared to the parental strains. The isolation of three classes of radiation-sensitive mutants and the construction of double mutants implies the presence of multiple pathways in *S. pombe* for repair of radiation-induced damage. The bearing of these data on cellular repair mechanisms in eukaryotes is discussed.

THE isolation and genetic analysis of radiation-sensitive mutants of bacteria have contributed substantially to an understanding of the cellular mechanisms involved in the repair of radiation damage (reviewed by ADLER 1966). Similar studies in fungi have made it apparent that the genetic control of radiation sensitivity in eukaryotes is much more complex. This complexity is reflected by the large number of radiation-sensitive mutants which have been isolated in different fungal species (CHANG and TUVESON 1968; COX and PARRY 1968; DAVIES 1967; RESNICK 1969; SCHROEDER 1970; SCHÜPBACH 1971; SNOW 1967; WRIGHT and PATEMAN 1970). In *Saccharomyces cerevisiae* a very detailed genetic study of this phenomenon has been carried out by COX and PARRY (1968).

The fission yeast *Schizosaccharomyces pombe* has been widely used in studies related to the genetic control and repair of radiation-induced damage. Some of the earlier data pertaining to liquid holding responses of this organism have shown that there are important differences between repair systems of *S. pombe* and those of other fungi including *S. cerevisiae* (HARM and HAEFNER 1968; SHAHIN, GENTNER and NASIM 1973; SHAHIN and NASIM 1973). In particular, the relatively high degree of radiation resistance shown by the wild-type cell is

indicative of some very efficient dark repair systems and makes it a particularly attractive system for radiobiological studies. A recent biochemical study of the excision of pyrimidine dimers in *S. pombe* (BIRNBOIM and NASIM, manuscript in preparation) has further indicated that there are important differences between the repair systems of this organism and those of *S. cerevisiae*.

There have been some reports describing the isolation and characteristics of UV-sensitive mutants of *S. pombe* (HAEFNER and HOWEREY 1967; SCHÜPBACH 1971). The present study was undertaken to isolate additional UV-sensitive mutants and extend the allelism tests to all presently available strains. Two new mutants primarily sensitive to γ-rays were isolated and characterized. The isolation of such mutants is of particular relevance in attempts to understand the nature of repair systems in this organism. In addition, by crossing single-gene mutants in various combinations, six supersensitive double mutants were obtained and two of these were shown to be highly sensitive to a variety of physical and chemical agents.

MATERIALS AND METHODS

Strains: Different strains were obtained from various labs in addition to those isolated in our laboratory. The source of different mutants is indicated at the bottom of Table 2. Detailed procedures for the isolation of UV-sensitive mutants by replica plating and all other related genetic tests were the same as previously used by SCHÜPBACH (see SCHÜPBACH 1971).

All isolates were tested for UV sensitivity by exposure to 600 ergs and only the highly sensitive mutants were included in further genetic analysis.

Growth media: Cells were routinely grown in YEL (yeast extract 0.3%, peptone 0.5% and glucose 3%) and also plated on the same medium supplemented with 2% agar. For crosses, malt extract agar was used (malt extract 3%, agar 2%).

Procedures for genetic crosses, allelism tests, random spore analysis, and detection of recombinants have been described previously (LEUPOLD 1970; SCHÜPBACH 1971).

Ultraviolet irradiation source: A General Electric 15-W germicidal lamp with output wavelength mainly at 253 nm was used. Doses were measured with a UV 254 dosimeter manufactured by International Light. Other experimental conditions such as dose rate were varied according to the needs of any particular test.

Isolation of γ-ray sensitive mutants: A stationary phase culture of the wild-type strain 972h⁻ was mutagenized with nitrosoguanidine; (MNNG) 2 mg/ml for 15 minutes at 30°, resulting in 25% colony forming ability. A total of 48,750 colonies resulting from this treatment were replica plated and the replicas were exposed to a dose of 66 kR from a Co 60 Gammacell 220 manufactured by Atomic Energy of Canada Limited. This radiation facility was particularly suitable for such experiments since 10 plates could be exposed simultaneously to a uniform dose. Strains which failed to grow or showed markedly diminished growth on the irradiated plates were reisolated from the master plate and subjected to further tests.

Tests for cross sensitivity of single and double mutants: Single and double radiation-sensitive mutants were tested for cross sensitivity to UV, γ-rays, Ethyl methane sulfonate (EMS), Methyl methane sulfonate (MMS) and Nitrosoguanidine (MNNG). For such tests mutants *rad1*, *rad10*, *rad13*, *rad22* and the double mutants derived from these strains were used. In each experiment the two parental single mutants and the double mutant were exposed to a given mutagen using experimental conditions outlined below:

Ultraviolet:	Doses ranging between 50 and 150 ergs/mm², dose rate 20 ergs/mm²/sec.
γ-rays:	Exposures between 10 kR and 40 kR. Dose rate 5 kR/minute.
EMS:	0.4% v/v, phosphate buffer pH 7.5, 30°.
MMS:	0.01 M, treatment carried out in distilled water at 30°, dilutions made in 5% sodium thiosulphate to stop further reaction.
MNNG:	100 μg/ml of the mutagen, acetate buffer pH 5, 30°.

RESULTS

A total of 67 UV-sensitive strains were obtained from various labs. All strains with the designation uvs were isolated by M. SCHÜPBACH; mutant uvs A was isolated by FABRE; mutants B–Y were isolated in Chalk River and those designated M–3 to M–36 were supplied by R. MEGNET. Among these, 17 different alleles have already been shown to represent 10 independent loci (SCHÜPBACH 1971). In preliminary experiments, the remaining 50 strains were used for UV and γ-ray sensitivity and only strains showing a high degree of sensitivity were used in further analysis. In nearly 200 genetic crosses carried out, 5 mutants showed very poor sporulation and these were not included in further analysis. Reduced or poor sporulation seems to be a common feature of radiation-sensitive mutants of *S. pombe* and has also been observed by Cox and PARRY (1968) in the UV-sensitive mutants of *S. cerevisiae*.

By pairwise crosses in various combinations some of the newly isolated mutants were shown to be allelic to each other or allelic to mutants isolated earlier by SCHÜPBACH. Allelism was indicated by the absence of any recombinants showing a wild-type response to UV in approximately 500 spore colonies resulting from a cross. A complete list of all the mutants with a new locus designation and their

TABLE 1

Data from allelism tests in S. pombe

New locus designation*	Alleles representing the locus
rad1	uvs1–1
rad2	uvs2–44, uvs–513, I, J, O
rad3	uvs3–136, uvs3–143, uvs3–183, uvs3–194, uvs3–202, uvs3–207, uvs3–211, B, D, E, M–3, M–18, M–19, M–22, M–31, M–32, M–39
rad4	uvs4–138
rad5	uvs5–158, uvs–407, M–7, M–33
rad6	uvs6–165, M–15, M–23
rad7	uvs7–185
rad8	uvs8–190
rad9	uvs9–192
rad10	uvs10–198
rad11	uvs–404
rad12	uvs–502
rad13	A, 61
rad14	G
rad15	P, M–6
rad16	U, M–8, M–36
rad17	W, M–24
rad18	X, M–34
rad19	M–9
rad20	M–25

* Proposed by PROF. U. LEUPOLD.

All mutants designated uvs were isolated by SCHÜPBACH. Mutant A was isolated by FABRE. M–3 to M–36 were isolated by R. MEGNET. The remaining mutants were isolated in our laboratory.

representative alleles is presented in Table 1. The 20 non-allelic UV-sensitive loci are represented by 51 different alleles, and since these include mutants isolated in four different laboratories, they are likely to include a broad spectrum of the genes controlling radiation sensitivity in *S. pombe*. It is noteworthy that as many as 10 of the new isolates were allelic to the locus *rad3*, which represents the highly UV-sensitive class. In earlier experiments SCHÜPBACH had observed a similar distribution when among 17 isolates, 7 were represented by UVS3–136 (now designated as *rad3*), whereas the remaining isolates were unique. As shown in Table 1, there are 8/20 loci where more than one allele has been isolated, and so the present collection of mutants is likely to represent a major fraction of the genes controlling radiation sensitivity in this organism.

TABLE 2

Genetic control of radiation sensitivity in S. pombe

Locus and allele designation*	Relative UV sensitivity	Relative γ-rays sensitivity
wild type	—†	—
rad1–1	+++	++
rad2–44	++	—
rad3–136	+++	++++
rad4–138	++	++
rad5–158	++	—
rad6–165	++	++
rad7–185	+	—
rad8–190	++	++
rad9–192	+++	++++
rad10–198	+++	++
rad11–404	+++	++
rad12–502	+++	—
rad13–A	++	—
rad14–G	++	—
rad15–P	+++	+
rad16–U	+++	+
rad17–W	++	+++
rad18–X	+	++
rad19–M9	+++	++
rad20–M25	++	+
rad21–45	—	++
rad22–67	—	+

* The loci *rad1–rad22* have all been shown to be non-allelic in allelism tests. The alleles indicated in the table have been isolated by M. SCHÜPBACH (*rad1–rad12*), F. FABRE (*rad13*), A. NASIM and B. P. SMITH (*rad14–rad18* and *rad21*, *rad22*) and R. MEGNET (*rad19*, *rad20*). The locus designations *rad1–rad10* and *rad13* replace the previous locus designations *uvs1–uvs10* (SCHÜPBACH 1971) and *uvsA* (FABRE 1971).

† — Normal sensitivity corresponding to that of the wild type.
\+ Increasing degrees of sensitivity as compared to that
++ of the wild type. Semiquantitative classification based
+++ on quantitative determination of the survival as a
++++ function of dose.

By tetrad analysis and dissection of at least 5 asci it was shown that radiation sensitivity in mutants *rad11* to *rad22* has resulted from a single-gene mutation which segregates 2:2 in crosses with the wild-type strain. Mutants *rad1* to *rad10* have already been shown by SCHÜPBACH to result from single-gene mutations.

The 2 γ-ray-sensitive mutants isolated later (see MATERIALS AND METHODS for details) were not tested for allelism to the UV-sensitive mutants. However, in view of the phenotypic characteristics they are unlikely to be allelic to any of the other mutants. The two, however, have been shown to be nonallelic to each other.

Table 2 shows the relative sensitivity to UV and γ-rays of all the 22 loci which have been given a new locus designation according to a system of nomenclature proposed by PROF. U. LEUPOLD. Unlike the system used in Saccharomyces (GAME and Cox 1971), the loci have been assigned numbers from *rad1* to *rad22* regardless of the phenotypes concerning UV or γ-ray sensitivity. Any additional mutants isolated in future would be designated *rad23* onwards, depending upon the time of isolation.

The frequencies of recombinants which show wild-type UV sensitivity obtained in the random spore analysis are shown in Table 3. In a majority of the crosses a high frequency of recombinants was obtained. This would imply that these loci are unlinked, although for any such conclusion a detailed study using tetrad analysis is essential.

γ-ray-sensitive mutants: Mutants *rad21* and *rad22*, which are primarily sensitive to γ-rays, represent a class of mutants which has previously not been reported for *S. pombe*. Although these mutants show some UV sensitivity, especially at the higher doses, they are primarily sensitive to γ-rays. The UV and γ-ray inactivation curves for the two mutants, as well as the double mutant obtained by crossing *rad21* and *rad22*, are presented in Figures 1 and 2. It should be noted that when these two mutants were crossed with each other, the resulting double mutant was much more sensitive to γ-rays and also showed an enhanced UV sensitivity compared to the parental strains. Such interactions have been investigated earlier and interpreted as an indication that at least two repair pathways operate in the wild-type yeast cell (GAME and Cox 1972, 1973; KHAN, BRENDEL and HAYNES 1970; NAKAI and MATSUMOTO 1967). In the present experiments this possibility was further explored by constructing various double mutants by crossing single-gene mutants. The detailed characteristics and reasons for the choice of single mutants used in such crosses are published elsewhere (NASIM and SMITH 1974). The response of two double mutants *rad1–rad10* and *rad21–rad22* to UV, γ-rays, EMS, MMS and MNNG was investigated in detail. It is to be expected that if the lesions caused by these different agents are repaired by more than one repair pathway, then the double mutants would be highly sensitive to these different agents. This indeed was found to be the case, and the typical response is shown in Table 4. These data strongly support the idea of multiple repair pathways for the repair of lesions caused by a variety of mutagens.

TABLE 3

Data from random ascospore analysis of crosses among various radiation-sensitive mutants of S. pombe

	RAD 11	RAD 12	RAD 13	RAD 14	RAD 15	RAD 16	RAD 17	RAD 18	RAD 19	RAD 20
RAD 1	44/190 23.2	70/270 25.9	120/678 17.7	44/126 34.9	44/263 16.7	65/182 35.7	55/233 23.6	50/134 37.3	77/224 34.4	46/290 15.8
RAD 2	55/223 24.7	60/512 11.7	49/335 14.6	50/110 45.4	55/234 23.5	49/120 40.8	67/213 31.5	44/178 24.7	34/116 29.3	60/167 35.9
RAD 3	84/424 19.8	22/72 30.6	85/393 21.6	69/134 51.5	56/135 41.5	108/628 17.2	25/114 21.9	216/800 27.0	68/238 28.6	70/175 40.0
RAD 4	50/176 28.4	17/49 34.7	12/63 19.0	45/155 29.0	35/138 25.4	24/97 24.7	33/104 31.7	57/189 30.2	35/90 38.9	79/194 40.7
RAD 5	67/263 25.5	45/300 15.0	49/152 32.2	104/255 40.8	30/102 29.4	64/235 27.2	41/204 20.1	116/398 29.1	70/330 21.2	52/200 26.0
RAD 6	39/134 29.1	64/116 55.2	48/228 21.1	128/227 56.4	51/125 40.8	76/210 36.2	19/54 35.2	69/440 15.7	99/570 17.4	112/750 14.9
RAD 7	71/188 37.8	17/222 7.6	21/66 31.8	54/178 30.3	35/118 29.7	42/172 24.4	22/155 14.2	55/160 34.4	33/157 21.0	76/196 38.8
RAD 8	67/225 29.8	66/323 20.4	72/223 32.3	61/216 28.2	11/39 28.2	73/238 30.7	62/381 16.3	105/550 19.1	69/296 23.3	72/275 26.2
RAD 9	35/150 23.3	56/405 13.8	70/416 16.8	40/70 57.1	41/290 14.1	85/505 16.8	57/154 37.0	59/184 32.1	51/181 28.2	37/344 10.7
RAD 10	61/242 25.2	24/88 27.3	21/51 41.2	59/136 43.4	92/450 20.4	15/78 19.2	139/465 29.9	51/222 23.0	45/126 35.7	45/134 33.6
RAD 11		39/117 33.3	27/120 22.5	61/144 42.4	42/124 33.9	116/458 25.3	47/129 36.4	82/234 35.0	40/134 29.9	49/117 41.9
RAD 12			44/178 24.7	110/502 21.9	48/109 44.0	42/130 32.3	97/270 35.9	42/102 41.2	53/450 11.8	56/152 36.8
RAD 13				67/216 31.0	93/252 36.9	90/500 18.0	45/133 33.8	74/240 30.8	117/265 44.2	40/153 26.1
RAD 14					86/198 43.4	15/40 37.5	66/124 53.2	62/153 40.5	67/161 41.6	69/136 50.7
RAD 15						48/155 30.9	116/750 15.5	31/77 40.3	46/108 42.6	51/103 49.5
RAD 16							47/144 32.6	39/110 35.5	49/172 28.5	35/111 31.5
RAD 17								44/205 21.5	16/48 33.3	33/106 31.1
RAD 18									22/230 9.6	25/155 16.1
RAD 19										36/280 12.9
RAD 20										

Figures represent the numbers of *uvs*+ recombinants/total number of colonies examined, and the percentages of *uvs*+ recombinants. The recombination data from crosses between *RAD1* to *RAD10* have been published earlier (SCHÜPBACH 1971).

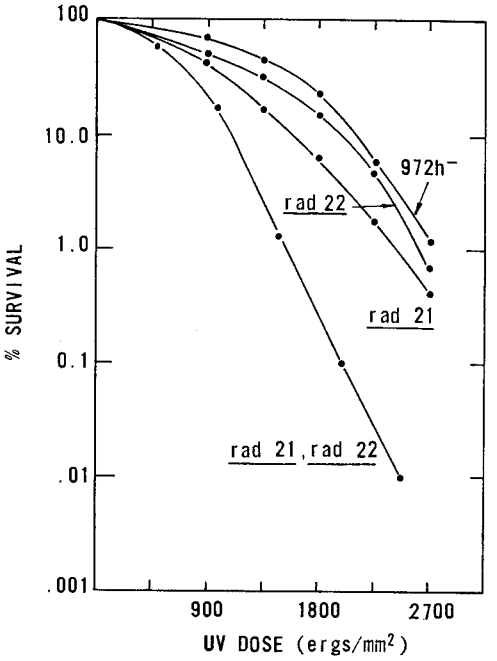

FIGURE 1:—Ultraviolet inactivation curves for the wild type and various mutant strains of *S. pombe*.

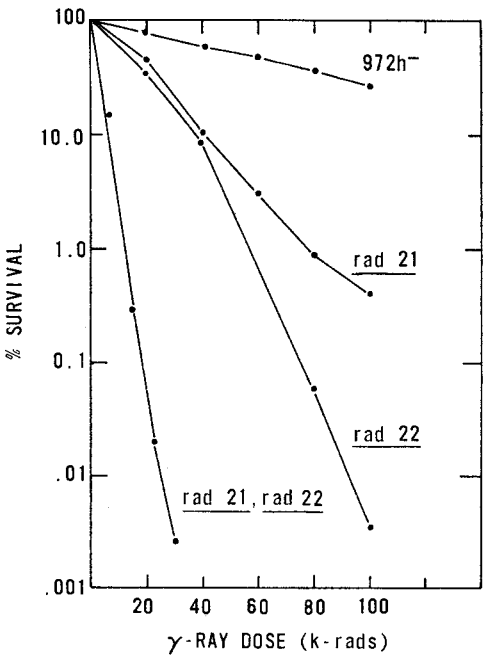

FIGURE 2.—γ-ray inactivation curves for the wild type and various mutant strains of S. pombe.

TABLE 4

Cross sensitivity of a double mutant to variety of inactivating agents

Strain tested	UV	γ-rays	MMS	EMS	MNNG
Wild type	—	—	—	—	—
rad1–1	++	++	++	+	+
rad10–198	++	++	+	++	+
rad1–rad10	++++	++++	++++	++++	++++

Various other single and double mutants tested showed a similar response, i.e., the double mutant showed a significantly enhanced sensitivity compared with the parental single mutants, indicating the presence of multiple repair pathways (see text for details).

DISCUSSION

In various eukaryotic organisms a large number of radiation-sensitive mutants have been isolated and characterized. *S. pombe* is no exception, and all possible types of radiation-sensitive mutants exist in this yeast. The present collection of mutants includes the three possible phenotypic classes, (1) mutants like *rad2* which are sensitive to UV but show an inactivation similar to wild type for γ-rays, (2) mutants like *rad21* which are sensitive to γ-rays but are only slightly UV sensitive and (3) mutants which are sensitive to both kinds of radiation. Some of these mutants have been characterized previously for other aspects such as mutator activity, spontaneous lethal sectoring and levels of induced mutability (LOPRIENO 1970; NASIM 1968; NASIM and SAUNDERS 1968). These studies revealed that at least some of these genes are involved in the control of cellular functions like DNA replication, or the production of spontaneous and UV-induced mutations. The present study is a comprehensive survey of a majority of the radiation-sensitive mutants in this organism. The fact that all possible phenotypic classes have been isolated (Table 2) indicates that there exist multiple and at least partially independent repair pathways in *S. pombe* to cope with the damage caused by a variety of agents. For Saccharomyces (GAME and Cox 1973), an extensive genetic analysis involving the construction of highly sensitive triple mutants has shown that there are at least three different pathways for repair of UV-induced lesions. For *S. pombe* we have constructed various double mutants and shown that these are highly sensitive to different mutagens. Although efforts to obtain supersensitive triple mutants were not successful, it is quite possible that such mutants can be obtained by isolating additional UV-sensitive mutants.

A related aspect of such studies is the investigation of the mutational response of the different radiation-sensitive mutants after treatment with various inactivating agents. One of the mutants, *rad1*, shown to have reduced recombination ability (analogous to *rec*$^-$ strains of bacteria) (FABRE 1972) had earlier been shown to exhibit reduced UV mutability (NASIM 1968). Such correlations in yeast support the earlier notion postulated for bacteria (WITKIN 1967) that recombination may be involved in the production of ultraviolet-induced mutations. Further characterization of different classes of radiation-sensitive mutants

should lead to a better understanding of the role of repair processes in the production of mutations by UV and other inactivating agents.

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LITERATURE CITED

ADLER, H. I., 1966 The genetic control of radiation sensitivity in microorganisms. pp. 167–191. In: *Advances in Radiation Biology*, Vol. 2. Edited by L. G. Augenstein, R. Mason and M. R. Zelle. Academic Press, New York.

CHANG, L. and R. W. TUVESON, 1968 Ultraviolet-sensitive mutants in *Neurospora crassa*. Genetics **56**: 801–810.

COX, B. S. and J. M. PARRY, 1968 The isolation, genetics and survival characteristics of ultraviolet light-sensitive mutants in yeast. Mutation Res. **6**: 37–55.

DAVIES, R. D., 1967 UV-sensitive mutants of *Chlamydomonas reinhardi*. Mutation Res. **4**: 765–770.

FABRE, F., 1972 Relation between repair mechanisms and induced mitotic recombination after UV irradiation in the yeast *Schizosaccharomyces pombe*: effects of caffeine. Molec. Gen. Genet. **117**: 153–166.

GAME, J. C. and B. S. COX, 1971 Allelism tests of mutants affecting sensitivity to radiation in yeast and a proposed nomenclature. Mutation Res. **12**: 328–331. ——, 1972 Epistatic interactions between four rad loci in yeast. Mutation Res. **16**: 353–362. ——, 1973 Synergistic interactions between RAD mutations in yeast. Mutation Res. **20**: 35–44.

HAEFNER, K. and L. HOWEREY, 1967 Gene controlled UV sensitivity in *Schizosaccharomyces pombe*. Mutation Res. **4**: 219–221.

HARM, W. and K. HAEFNER, 1968 Decreased survival resulting from liquid holding of UV-irradiated *Escherichia coli C* and *Schizosaccharomyces pombe*. Photochem. Photobiol. **8**: 179–192.

KHAN, N. A., M. BRENDEL and R. H. HAYNES, 1970 Supersensitive double-mutants in yeast. Molec. Gen. Genet. **107**: 376–378.

LEUPOLD, U., 1970 Genetical methods for *Schizosaccharomyces pombe*. pp. 169–177. In: *Methods in Cell Physiology*, Vol. 4. Edited by D. M. PRESCOTT. Academic Press, New York.

LOPRIENO, N., 1970 UV radiation-sensitivity and mutator activity in yeast *Schizosaccharomyces pombe*. Proc. 4th Intern. Cong. Radiation Res.

NAKAI, S. and S. MATSUMOTO, 1967 Two types of radiation-sensitive mutants in yeast. Mutation Res. **4**: 129–136.

NASIM, A., 1968 Repair-mechanisms and radiation-induced mutations in fission yeast. Genetics **59**: 327–333.

NASIM, A. and A. S. SAUNDERS, 1968 Spontaneous frequencies of lethal-sectoring and mutation in radiation-sensitive strains of *Schizosaccharomyces pombe*. Mutation Rees. **6**: 475–478.

NASIM, A. and B. P. SMITH, 1974 Dark repair inhibitors and pathways for repair of radiation damage in *Schizosaccharomyces pombe*. Molec. Gen. Genet. **132**: 13–22.

RESNICK, M. A., 1969 Genetic control of radiation sensitivity in *Saccharomyces cerevisiae*. Genetics **62**: 519–531.

SCHROEDER, A. L., 1970 Ultraviolet sensitive mutants of *Neurospora*. I. Genetic basis and effect on recombination. Molec. Gen. Genet. **107**: 291–304. ——, 1970 II. Ultraviolet sensitive mutants of *Neurospora*. II. Radiation studies. Molec. Gen. Genet. **107**: 305–320.

SCHÜPBACH, M., 1971 The isolation and genetic classification of UV-sensitive mutants of *Schizosaccharomyces pombe*. Mutation Res. **11**: 361–371.

SHAHIN, M. M., N. E. GENTNER and A. NASIM, 1973. The effect of liquid holding in *Schizosaccharomyces prombe* strains after gamma and ultraviolet irradiation. Radiation Res. **53**: 216–225.

SHAHIN, M. M. and A. NASIM, 1973 The effect of radiation sensitivity and cell stage on liquid holding response in *Schizosaccharomyces pombe*. Molec. Gen. Genet. **122**: 331–338.

SNOW, R., 1967 Mutants of yeast sensitive to ultraviolet light. J. Bacteriol. **94**: 571–575.

WITKIN, E. M., 1967 Mutation-proof and mutation-prone modes of survival in derivatives of *Escherichia coli B* differing in sensitivity to ultraviolet light. Brookhaven Symp. Biol. **20**: 17–55.

WRIGHT, P. J. and J. A. PATEMAN, 1970 Ultraviolet light sensitive mutants of *Aspergillus nidulans*. Mutation Res. **9**: 579–587.

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