JOURNAL OF BACTERIOLOGY, Nov. 1976, p. 536-539

Vol. 128, No. 2
Printed in U.S.A.

Temperature-Sensitive Mutant of *Schizosaccharomyces pombe* Exhibiting Enhanced Radiation Sensitivity[1]

P. DUCK,[2]* A. NASIM, AND A. P. JAMES

Division of Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada

Received for publication 28 June 1976

A conditional lethal and radiation-sensitive mutant of *Schizosaccharomyces pombe* is described in which both characteristics result from a single gene mutation. Confirmation of the pleiotropic nature of this mutation was obtained by tetrad analysis and by testing the radiation sensitivity of a large number of revertants that grew normally at the restrictive temperature. The colony-forming ability of the mutant after ultraviolet radiation, γ radiation, and ethyl methane sulfonate treatment is considerably altered by the post-treatment incubation temperature, showing higher survival at 25 than at 30°C. The radiosensitivity of the mutant is also influenced by the stage of growth. The difference in radiation sensitivity between the wild type and mutant is greater when log-phase cultures are compared. The characteristics of this mutant suggest that it is defective in a step common to both deoxyribonucleic acid replication and repair.

Conditional lethal mutants, in which growth and other metabolic features can be controlled simply by altering conditions such as temperature, have proved to be very powerful tools in genetic and biochemical studies (1, 6, 8, 15). In *Schizosaccharomyces pombe*, a large number of temperature-sensitive mutants have been isolated and characterized (3). The existence of numerous genes that control radiation sensitivity has also been reported in this organism (10). However, the isolation of mutants in which both features result from a single gene mutation has not been documented. Such a mutant is a useful experimental tool for studying the enzymatic basis for the repair of radiation-induced damage in eukaryotes. A similar approach in *Escherichia coli* resulted in a mutant in which it was shown that varying degrees of radiation sensitivity at different post-irradiation incubation temperatures were directly related to a thermosensitive ligase activity (5, 12, 13). It has also been observed that recovery from irradiation damage is influenced by temperature (14) and that cross-sensitivity exists between inactivation by radiation and elevated temperatures (7). A mutant with temperature-dependent radiation sensitivity has also been described in *Saccharomyces cerevisiae* (11). The present study demonstrates that in the *S. pombe* mutant *rad4* both radiation sensitivity and temperature sensitivity result from a single gene mutation. Various other aspects of the behavior of this mutant, such as its growth rate and the influence of post-irradiation incubation temperature on inactivation by various mutagenic agents, have been reported.

[1] National Research Council of Canada publication no. 15535.

[2] Present address: Department of Health and Welfare, Lab Centre for Disease Control, Antimicrobials, Tunney's Pasture, Ottawa, Canada K1A 0L2.

MATERIALS AND METHODS

Yeast strains. All of the radiation-sensitive mutants of *S. pombe*, *rad1* to *rad22*, have been described in detail elsewhere (10). For control experiments and genetic crosses, the wild-type strains 972h$^-$ and 975h$^+$ were used.

Growth media. Cultures were grown in yeast extract medium YEL (0.3% yeast extract, 0.5% peptone and 2% dextrose). For plating experiments the same medium was supplemented with 2% agar. Genetic crosses were carried out on malt extract agar medium prepared by dissolving 30 g of malt extract in 1 liter of distilled water, supplemented with 1.5% agar.

Growth curves were obtained by measuring the optical density in a Coleman model 295 spectrophotometer at 660 nm and taking hemacytometer cell counts as a function of time after inoculation. In each experiment, cells were grown in flasks in a shaking water bath at the desired temperature. For comparisons of the radiation sensitivity of wild-type and *rad4* strains, cells were harvested from the same stage of growth, i.e., log or stationary phase of the growth cycle.

UV irradiation. Cells were centrifuged, washed twice with distilled water, and adjusted to a cell density of 1×10^7 to 2×10^7 cells/ml before being exposed to an ultraviolet (UV) fluence at a rate of approximately 1 J/m^2 per s, with continuous shaking during irradiation. The fluence was measured by an International light UV dosimeter. Detailed dosimetry has been described previously (9).

γ-Irradiation. Cells were irradiated in metal cap-

sules at a dose rate of ~3.5 kR/min; other details are the same as described in a previous publication (9).

EMS treatment. Cells were treated in phosphate buffer (pH 7.5)-2% ethyl methane sulfonate (EMS) (vol/vol). The reaction was stopped by treating with a 5% solution of sodium thiosulfate.

Plating and incubation. Treated cells were appropriately diluted in distilled water and portions were plated on yeast extract medium YEL supplemented with 2% agar. Special precautions were taken concerning post-treatment incubation temperatures, and plates were routinely prewarmed to the desired level. The plates were incubated for 5 to 7 days and then scored for visible colonies to determine survival.

RESULTS AND DISCUSSION

All of the available radiation-sensitive mutants of *S. pombe* and their alleles were tested for their ability to grow at 30 and 37°C. Among the nearly 50 isolates plated, 7 failed to form colonies at the higher temperature. These were crossed to the wild type, tetrads were dissected, and the spore colonies were tested for both UV and temperature sensitivity. In all of the isolates except *rad4* it became apparent from the analysis of only a few tetrads (Table 1) that the two characteristics segregated independently, a circumstance that demonstrates control by separate genes. This contradicts an earlier speculation (4) that in the mutant *rad2* the two characteristics result from the same mutation.

Since the *rad4* results indicated that the pleiotropic behavior of this mutant could be due to a single gene mutation, various other properties were extensively investigated.

(i) Growth. Figure 1 presents a comparison of the growth rate of the wild-type and *rad4* strains as determined by both optical density and cell count measurements. From the figure, generation times of approximately 1.7 and 2.7 h can be calculated for the wild type and *rad4*, respectively. Hence, both the growth rate and radiation sensitivity of the *rad4* mutant differ from the wild type.

(ii) Temperature reactivation. Inactivation curves for wild type and *rad4* after UV, γ rays, and EMS (Fig. 2, 3, and 4) show that in *rad4* post-treatment incubation at 25°C results in an increase in colony-forming ability as compared with 30°C. This temperature effect is dose dependent and becomes more pronounced at higher doses, which is to be expected since the cell must cope with an even larger number of induced lesions at higher doses. Since low-temperature reactivation is observed with all of the mutagens tested, it may be inferred that the defect is in a step common to the repair of deoxyribonucleic acid (DNA) damage caused by a variety of inactivating agents. In fact, a logical postulate is that like the *E. coli* mutant (12, 13), *rad4* has a temperature-sensitive ligase that is nonfunctional at 37°C and, therefore, results in lethality through an inability to complete normal DNA replication. This enzyme is known to be involved in the final sealing step in the repair of DNA damage caused by UV as well as ionizing radiation. With a temperature-dependent efficiency, cells would be expected to be least sensitive at 25°C, more so at 30°C, and inviable at 37°C. Temperature reactivation is a unique property of *rad4*; it was not observed in the wild type or in any of the other radiation-sensitive mutants tested.

(iii) Effect of growth phase on radiation sensitivity. Figure 5 shows the UV inactivation curves for wild type and *rad4* in both log and stationary growth phases. Unlike the wild type, the *rad4* mutant is more sensitive to UV during the log phase. Since nondividing, sta-

TABLE 1. *Segregation of the temperature-sensitive and UV-sensitive characteristics in crosses between each of seven temperature-sensitive and UV-sensitive mutants and the wild-type 972h⁻ strain*

Mutant strain tested[a]	No. of tetrads analyzed	No. of parental ditype tetrads
rad2 (UVS-2-44)	5	1
rad2 (UVS-O)	3	0
rad3 (UVS-D)	7	0
rad3 (UVS-E)	4	0
rad4 (UVS-4-138)	26	26
rad11 (UVS-404)	2	0
rad13 (UVS-61)	6	0

[a] Nomenclature in parentheses is the original designation.

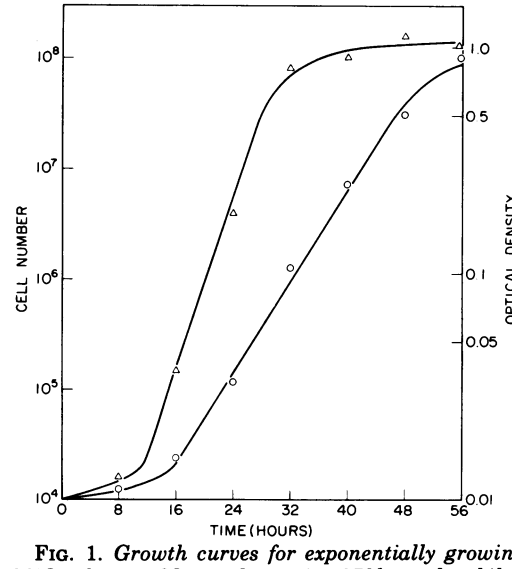

FIG. 1. *Growth curves for exponentially growing 30°C cultures of S. pombe strains 972h⁻ and rad4h⁻. Symbols: △, 972h⁻; ○, rad4h⁻.*

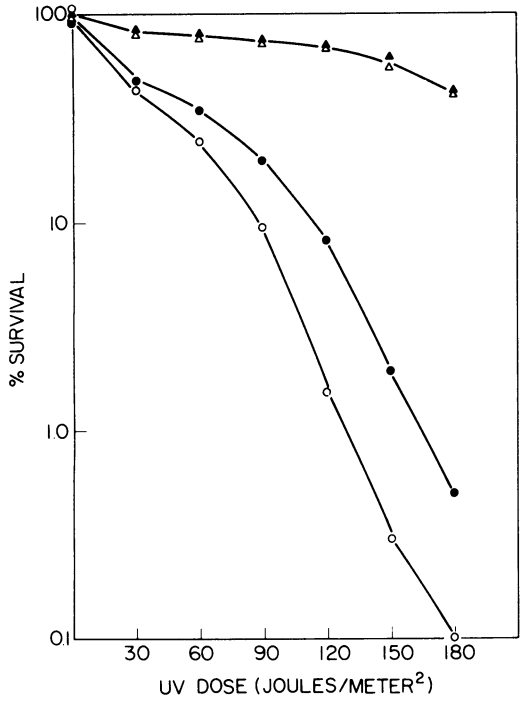

FIG. 2. *UV inactivation curves for log-phase cultures of strains 972h⁻ and rad4h⁻ incubated at 25 and 30°C. Symbols: △, 972h⁻, 30°C; ▲, 972h⁻, 25°C; ○, rad4h⁻, 30°C; ●, rad4h⁻, 25°C.*

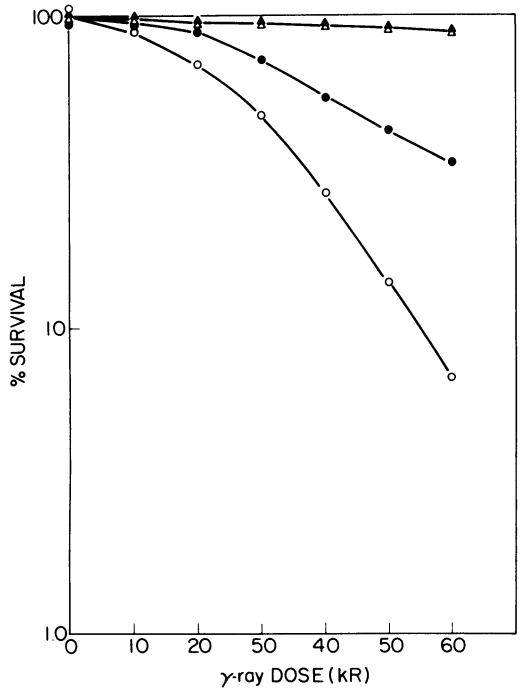

FIG. 3. *γ-Ray inactivation curves for log-phase cultures of strains 972h⁻ and rad4h⁻ incubated at 25 and 30°C. Symbols: △, 972h⁻, 30°C; ▲, 972h⁻, 25°C; ○, rad4h⁻, 30°C; ●, rad4h⁻, 25°C.*

tionary-phase cells have considerably reduced metabolic activity, the available enzymatic activity of the mutant could be exclusively committed to the repair of radiation-induced lesions, resulting in decreased radiosensitivity in this growth phase. During the log phase, however, the mutant activity would be required to simultaneously cope with both repair and DNA replication, thereby resulting in reduced repair efficiency. Clearly reduced repair ability in log-phase cells but enhanced repair ability in stationary-phase cells is consistent with the notion that the *rad4* gene product is concerned with more than one cellular function.

(iv) Single gene control. Having demonstrated that the *rad4* mutant differs from the wild type in several respects, it is necessary to establish that these differences are attributable to a single gene function. Therefore, additional crosses were carried out to confirm that radiation sensitivity and temperature sensitivity result from a single gene mutation. Pooled data from all such crosses are presented in Table 1. In 26 asci from crosses of strain *rad4h⁺* with strain 972h⁻, temperature sensitivity and UV sensitivity segregated together. To exclude the

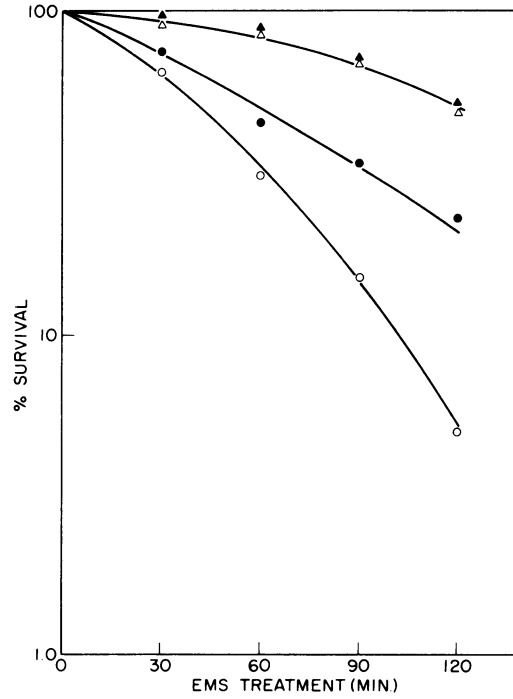

FIG. 4. *EMS inactivation curves for log-phase cultures of strains 972h⁻ and rad4h⁻ incubated at 25 and 30°C. Symbols: △, 972h⁻, 30°C; ▲, 972h⁻, 25°C; ○, rad4h⁻, 30°C; ●, rad4h⁻, 25°C.*

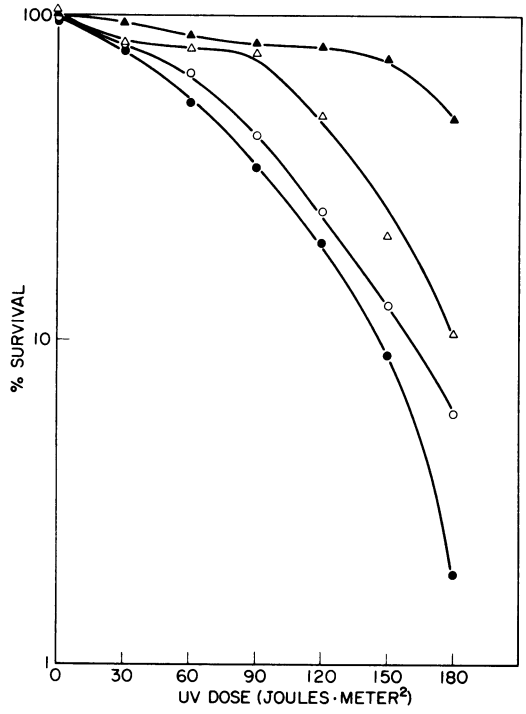

FIG. 5. *UV inactivation curves for strains 972h⁻ and rad4h⁻ in both log and stationary growth phases. Symbols: △, 972h⁻, stationary; ▲, 972h⁻, log; ○, rad4h⁻, stationary; ●, rad4h⁻, log.*

possibility of two very closely linked genes controlling these features, random spore analysis was carried out, the progeny being examined for recombinants that carried only one of the two phenotypes. These tests were carried out in four independent experiments, in which the progeny were tested by replica plating for the presence of UV-sensitive and temperature-normal or UV-normal and temperature-sensitive recombinants. No recombinants were found among a total of 6,281 spores examined, a fact that clearly indicates that a single gene controls these two characteristics in the *rad4* mutant. This conclusion was further strengthened by an additional test in which spontaneous and EMS-induced revertants from temperature-sensitive to temperature-normal cells were tested for a concomitant reversion to wild-type UV sensitivity. This is a rapid means for obtaining revertants since a large number of cells can be plated and incubated at the restrictive temperature. Of 48 temperature-normal revertants tested, all were shown to have wild-type radiation sensitivity. In addition, some revertants were checked for growth rate and temperature reactivation. Unlike the parental *rad4* strain, the revertants exhibited a normal growth rate and did not show the characteristic temperature-dependent radiation sensitivity after either UV or γ rays. A reasonable inference from this result is that the modified growth rate, the temperature reactivation characteristics, and the radiation sensitivity are all a result of the same single gene mutation.

The biochemical characterization of radiation-sensitive mutants in *S. pombe* is rather limited, and it is not known whether *rad4* can excise UV-induced pyrimidine dimers (2). Temperature-dependent radiation sensitivity is not a common phenomenon in *S. pombe*, and we expect that this mutant will be useful for investigating the enzymatic basis of DNA repair functions in this organism.

LITERATURE CITED

1. **Bandas, E. L., M. L. Bekker, L. A. Luchkina, V. P. Tkatchenko, and I. A. Zakharov.** 1973. Temperature sensitive radiosensitive mutants of the yeast *Saccharomyces paradoxus*. Mol. Gen. Genet. 126:153-164.
2. **Birnboim, H. C., and A. Nasim.** 1975. Excision of pyrimidine dimers by several UV-sensitive mutants of *S. pombe*. Mol. Gen. Genet. 136:1-8.
3. **Bonatti, S., M. Simili, and A. Abbondandolo.** 1972. Isolation of temperature-sensitive mutants of *Schizosaccharomyces pombe*. J. Bacteriol. 109:484-491.
4. **Corti, G., R. Guglielminetti, M. Nozzolini, and S. Simi.** 1970. UV-sensibilita' e letalita' condizionata in un ceppo di *Schizosaccharomyces pombe*. Atti Ass. Genet. Ital. XV:157-158.
5. **Dean, C., and C. Pauling.** 1970. Properties of a deoxyribonucleic acid ligase mutant of *Escherichia coli*: X-ray sensitivity. J. Bacteriol. 102:588-589.
6. **Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour, M. Susman, G. H. Denhardt, and A. Liflausis.** 1963. Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. 28:375-394.
7. **Evans, W. E., and J. M. Parry.** 1972. The cross sensitivity to radiations, chemical mutagens and heat treatment of X-ray sensitive mutants of yeast. Mol. Gen. Genet. 118:261-271.
8. **Hartwell, L. H.** 1967. Macromolecular synthesis in temperature-sensitive mutants of yeast. J. Bacteriol. 93:1662-1670.
9. **Nasim, A., and B. P. Smith.** 1974. Dark repair inhibitors and pathways for repair of radiation damage in *Schizosaccharomyces pombe*. Mol. Gen. Genet. 132:13-22.
10. **Nasim, A., and B. P. Smith.** 1975. Genetic control of radiation sensitivity in *Schizosaccharomyces pombe*. Genetics 79:573-582.
11. **Parry, E. M., and J. M. Parry.** 1972. New type of ultraviolet light-sensitive mutation in yeast. J. Bacteriol. 110:1206-1207.
12. **Pauling, C., and L. Hamm.** 1968. Properties of a temperature-sensitive mutant of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 60:1495-1502.
13. **Pauling, C., and L. Hamm.** 1969. Properties of a temperature-sensitive radiation-sensitive mutant of *Escherichia coli*. II. DNA replication. Proc. Natl. Acad. Sci. U.S.A. 64:1195-1202.
14. **Shimazu, Y., M. Morimyo, and K. Suzuki.** 1971. Temperature-sensitive recovery of a mutant of *Escherichia coli* K-12 irradiated with ultraviolet light. J. Bacteriol. 107:623-632.
15. **Unrau, P., and R. Holliday.** 1970. A search for temperature-sensitive mutants of *Ustilago maydis* blocked in DNA synthesis. Genet. Res. 15:157-169.