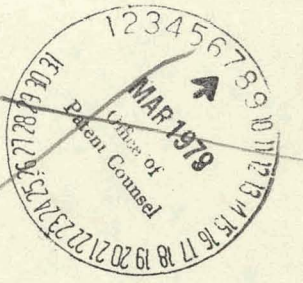
UR-3490-1571

EFFECTS OF THE *rad52* GENE ON RECOMBINATION IN *SACCHAROMYCES CEREVISIAE*

SATYA PRAKASH[1], LOUISE PRAKASH[2], WILLIAM BURKE[2], and BETH A. MONTELONE[1].



[1]Department of Biology
University of Rochester
Rochester, New York 14627

[2]Department of Radiation Biology and Biophysics
University of Rochester
School of Medicine & Dentistry
Rochester, New York 14642

DISCLAIMER

ABSTRACT

Effects of the *rad52* mutation in *Saccharomyces cerevisiae* on meiotic, γ-ray induced, UV induced and spontaneous mitotic recombination were studied. The *rad52/rad52* diploids undergo premeiotic DNA synthesis; sporulation occurs but inviable spores are produced. Intra- and intergenic recombination during meiosis were examined in cells transferred from sporulation medium to vegetative medium at different time intervals. No intragenic recombination was observed at the *his1-1/his1-315* and *trp5-2/trp5-48* heteroalleles. Gene-centromere recombination was also not observed in *rad52/rad52* diploids. No γ-ray induced intragenic mitotic recombination is seen in *rad52/rad52* diploids and UV induced intragenic recombination is greatly reduced. However, spontaneous mitotic recombination is not similarly affected. The *RAD52* gene thus functions in recombination in meiosis and in γ-ray and UV induced mitotic recombination but not in spontaneous mitotic recombination.

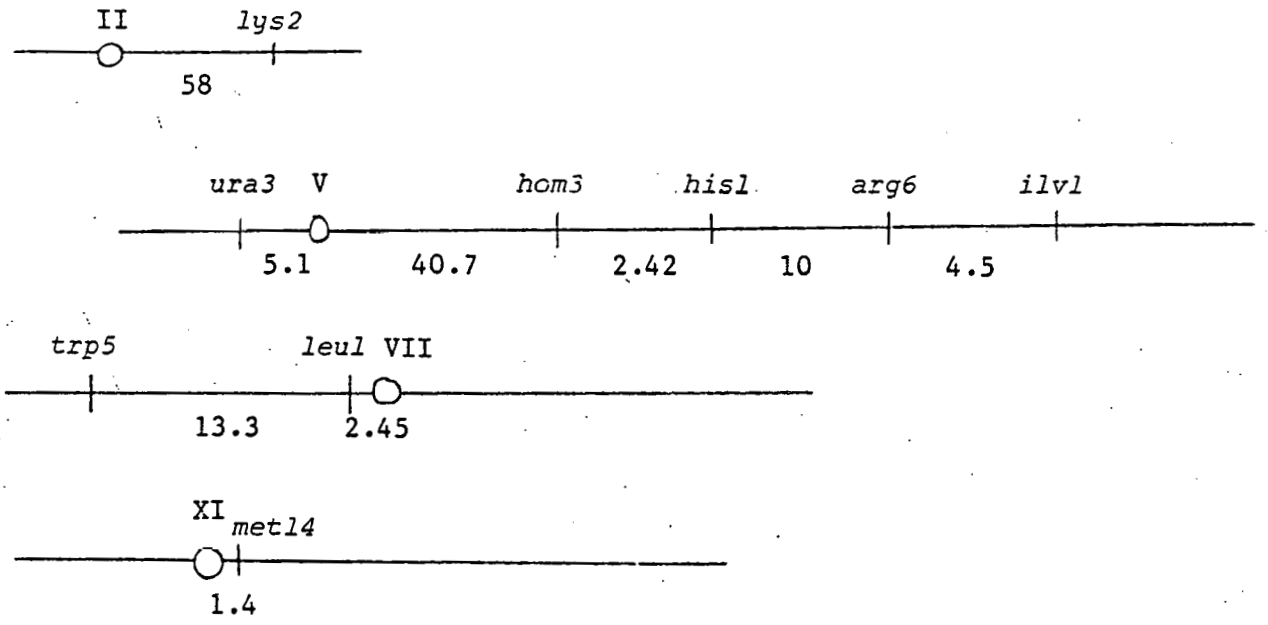
Major advances have been made in our understanding of the phenomenon of meiotic recombination in eucaryotes from genetic studies on fungi, including the yeast *Saccharomyces cerevisiae* (FINCHAM and DAY 1971; FOGEL and HURST 1967; FOGEL and MORTIMER 1971). Gene conversion in meiosis in yeast appears to involve the formation of asymmetrical heteroduplexes which maybe 1000 nucleotide pairs long (FOGEL and MORTIMER 1969, 1970). Mismatch repair of heteroduplexes results in gene conversion and the lack of mismatch repair leads to post-meiotic segregation. Gene conversion has been found to be associated with a high frequency of crossing over of flanking markers and this associated crossing over can sufficiently account for all of the exchanges occurring in meiosis in yeast (HURST, FOGEL and MORTIMER 1972).

Mitotic recombination, on the other hand, is not as well understood. From recent work in *S. cerevisiae* it appears that spontaneous mitotic recombination may involve formation of very long symmetrical heteroduplexes in G_1 which are resolved by DNA replication (ESPOSITO 1978). X-ray and UV induced mitotic recombinants have also been reported to arise during the G_1 phase of the cell cycle in *S. cerevisiae* (WILDENBERG 1970; FABRE 1978). In order to understand the role of various gene products in meiotic and mitotic recombination, it is necessary to obtain mutants affecting these processes. Since the proposed models of recombination involve nicking of DNA, formation of heteroduplexes and repair synthesis (HOLLIDAY 1964; MESELSON and RADDING 1975) and since many mutants of *Escherichia coli* which are defective in DNA repair are also recombination deficient (CLARK 1973; EISENSTOCK 1977; HOWARD-FLANDERS 1968; ROTHMAN, KATO and CLARK 1975), we have begun studies on mutants of the yeast *S. cerevisiae* which are defective in DNA repair and in sporulation (GAME and MORTIMER 1974). Prominent among these mutants are the radiation sensitive mutants *rad50, rad51, rad52, rad53, rad54, rad55, rad56, rad57* and *rad6*. We report here studies on mitotic and meiotic recombination

in *rad52* mutants and show that the *RAD52* gene is involved in meiotic and γ-ray and UV induced mitotic recombination but not in spontaneous mitotic recombination.

MATERIALS AND METHODS

Strains: The genotypes of the strains used in this study are given in Table I. The map distances, in centimorgans, of markers are indicated below and are compiled from HAWTHORNE and MORTIMER (1960), FOGEL and HURST (1967) and MORTIMER and HAWTHORNE (1966, 1973). Chromosomes are designated by Roman numerals.



The *rad52-1* mutant was obtained from M. RESNICK; other strains were obtained from S. FOGEL and R. ROTHSTEIN.

Media: The following media were used: YPD, 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose, solidified with 2% Bacto-agar; YPA, 2% Bacto-peptone, 1% yeast extract, 1% potassium acetate; sporulation medium (SPM), 2% potassium acetate, pH adjusted to 7.0 with acetic acid and supplements required by the particular strain used added to a final concentration of 75 μg/ml. Synthetic complete and synthetic omission media were used to score for prototrophs and consisted of the following:

synthetic complete (SC), 0.67% Difco yeast nitrogen base without amino acids, 2% dextrose, 2% Bacto-agar, adenine sulfate 20 µg/ml, L-arginine HCl 20 µg/ml, L-histidine HCl 20 µg/ml, DL-homoserine 100 µg/ml, L-isoleucine 30 µg/ml, L-leucine 30 µg/ml, L-lysine HCl 30 µg/ml, L-methionine 20 µg/ml, L-phenylalanine 50 µg/ml, L-tyrosine 30 µg/ml, uracil 20µg/ml and L-valine 150 µg/ml. Synthetic complete minus histidine (SC-his) and synthetic complete minus tryptophan (SC-trp) were used to score for histidine and tryptophan prototrophs, respectively, and consisted of the above constituents lacking histidine and tryptophan, respectively.

Premeiotic DNA synthesis: Cells were grown in YPA medium containing 2 µCi/ml ^{14}C-uracil and 50 mg/liter of unlabeled uracil until they reached a density of 1 X 10^7 cells/ml. At that time, cells were washed and transferred to SPM at a density of 1 X 10^7 cells/ml. Cells were then incubated in SPM at 30°C and 0.5 ml aliquots were withdrawn at various times after transfer to SPM for a 48 hour period. The samples were then added to tubes on ice containing 0.5 ml 2 N NaOH, mixed well and incubated for 18 hours at 37°C. Samples were precipitated by the addition of 50 µg of carrier DNA and 0.5 ml 50% TCA per tube. Samples were collected by filtration onto glass fiber filters, washed 5 times with 5% TCA, rinsed once with cold distilled water and once with cold 95% ethanol, dried and counted in Liquifluor-toluene scintillation fluid (New England Nuclear) in a Beckman LS-250 liquid scintillation counter.

Meiotic prototrophy: Freshly mated diploids were used for all experiments. After subcloning on appropriate omission media, YPA medium was inoculated with three to four colonies of each strain. Cultures were incubated at 30°C for about 16 hours, or until the cell density had reached 1-2 X 10^7 cells/ml. At that time, cells were washed and resuspended in SPM at the same density. At various intervals after transfer to SPM, aliquots were withdrawn, sonicated and plated on SC for viability determinations and on SC-his and SC-trp for prototrophy

determinations. Sporulation was monitored by microscopic examination of samples.

Determination of rates of mitotic prototrophy to histidine-independence and tryptophan-independence: For every strain examined, freshly mated diploids were used since it was found that the frequency of mitotic prototrophy for either marker was higher even in diploids stored at 4°C for long periods of time than it was in freshly mated diploids. This was true of *his1-1/his1-315* and *trp5-2/trp5-48* diploids, whether they were heterozygous or homozygous for *rad52*. A series of culture tubes with 1 ml YPD medium were each inoculated with 100 cells from a single clone. The frequency of histidine-independent and tryptophan-independent cells in the inoculum was determined by plating about 2000 cells on a total of 10 SC-his and 10 SC-trp plates and counting colonies growing on each type of medium. Only those experiments which had an initial frequency of zero HIS+ and TRP+ cells in the inoculum were used in the determinations. Cultures were incubated at 30°C for 3 days, when the frequency of HIS+ and TRP+ clones in each culture was determined by plating appropriate dilutions on SC-his and SC-trp media and on SC to determine viability. SC plates were incubated for 3 days before scoring whereas SC-his and SC-trp plates were scored after 3 to 5 days of incubation at 30°C. Twenty five cultures were used per strain for determining the rate of mitotic prototrophy shown in Table 3. For determining rates of prototrophy, the mean number of histidine-independent or tryptophan-independent cells arising per culture was determined by the method of the median of LEA and COULSON (1949). Rates were obtained by dividing the mean number of histidine-independent or tryptophan independent cells by the mean number of cells in the cultures.

Determination of ultraviolet-light (UV) and γ-ray induced mitotic prototrophy: Freshly mated diploids were used and enough colonies, obtained from freshly subcloned diploids, were suspended in sterile distilled water to yield a density of 1 X 10^8 cells per ml. Cells in suspension were distributed into scintillation

vials, subjected to γ-ray irradiation, and plated on SC for viability determinations and on SC-his and SC-trp for prototroph determination.

The irradiation was from a 6000 Curie Cobalt 60 source whose dosimetry is given in MCKEE and LAWRENCE (1979). UV-irradiation was carried out by irradiating cells on the surface of plates at a fluence of 1 Jm^{-2} s^{-1}. The radiation source and its dosimetry are given in LAWRENCE and CHRISTENSEN (1976).

RESULTS

Effects in meiosis

Before determining the effect of the *rad52* mutation on recombination during meiosis, it was necessary to determine whether pre-meiotic DNA synthesis occurs in *rad52/rad52* diploids, since commitment to recombination is not observed in mutants which do not undergo pre-meiotic DNA synthesis (BAKER *et al.* 1976; ROTH and LUSNAK 1970). Figure 1 shows that the *rad52/rad52* diploid carries out as much premeiotic DNA synthesis as the wild type *RAD52/RAD52* diploid. Although results for only one strain of each genotype are given in Figure 1, two more strains of each genotype gave similar results.

Strains with two heteroallelic sites, *his1-1/his1-315* on chromosome V and *trp5-2/trp5-48* on chromosome VII were constructed in order to determine the effect of the *rad52* mutation on meiotic recombination. In addition, the strains were heterozygous for *hom3, arg6,* and *ilv1,* so that intergenic recombination could be monitored (see Table 1). The centromere markers *ura3, leu1* and *met14,* located on chromosomes V, VII and XI, respectively, were also present in the heterozygous state so that meiotic segregation could be monitored. The genotypes of the strains used are given in Table 1. Intragenic recombination at the *his1* and *trp5* loci was monitored by withdrawing cells from liquid sporulation medium at different time intervals and plating these cells on SC, SC-his and SC-trp media. The

frequency of histidine prototrophs and tryptophan prototrophs arising during meiosis in diploids homozygous for *rad52* (LP-854, LP-1383 and LP-1384), heterozygous for *rad52* (LP-855, LP-1415) and in a diploid homozygous for *RAD52* (LP-1416) is given in Figure 2 and Figure 3, respectively. Prototrophs of both types arise with essentially similar kinetics in *rad52/RAD52* and *RAD52/RAD52* diploids, where the frequency of histidine and tryptophan prototrophs begins to rise after about 5 hours in sporulation medium. In *rad52* homozygous diploids, on the other hand, there is essentially no increase in either histidine or tryptophan prototrophs during sporulation. The frequencies observed are at the levels found in mitotic cells. By 50 hours in sporulation medium, the frequency of histidine-independent colonies per 10^6 colony forming units has risen to about 6000 in the *RAD52/RAD52* and *rad52/RAD52* diploids, whereas in the *rad52/rad52* diploids, it is less then 10 (Figure 2). A similar pattern of prototroph accumulation is observed for the *trp5-2/trp5-48* heteroallelic pair (Figure 3).

In addition to the lack of prototroph accumulation during meiosis, the viability of *rad52* homozygous diploids decreases to about 10% after 50 hours in SPM whereas the viability of *rad52/RAD52* and *RAD52/RAD52* diploids remains constant throughout sporulation (Figure 4).

Commitment to meiosis and ascus formation: In order to determine whether the histidine and tryptophan prototrophs recovered from sporulation medium at different times were committed to meiotic chromosome segregation, 50-150 histidine and tryptophan prototrophs at each time point were tested for auxotrophy for the centromere-linked marker *leu1* in the *leu1/+* heterozygotes. Figure 5 gives the percent of histidine prototrophs committed to meiotic chromosome segregation and percent of asci at various times during sporulation of a diploid homozygous for *RAD52*. Cells committed to meiotic chromosome segregation begin to appear after 8 hours, whereas the intragenic recombinants begin to arise earlier, after 5 hours in sporulation media. These results agree with the observations of ESPOSITO

and ESPOSITO (1974) that commitment to intragenic recombination occurs before commitment to meiotic chromosome segregation. In the wild-type strain (i.e., diploid homozygous for *RAD52*), the final percent of cells committed to meiotic chromosome segregation and the percent of ascus formation was 50-60%. The tryptophan prototrophs also gave similar results for commitment to meiosis.

In the *rad52/rad52* homozygotes, meiotic chromosome segregation was studied in tryptophan prototrophs obtained from sporulation medium at 24 hours. Six subclones from each of the 130 TRP+ colonies were examined for auxotrophy for the centromere-linked marker *leu1*. Meiotic chromosome segregation was not observed in *rad52/rad52* diploids as determined by the lack of leucine auxotrophs. After 24 hours in sporulation medium, the wild-type homozygotes, on the other hand, have almost reached the plateau for commitment to meiotic segregation. The *rad52/rad52* homozygotes were variable in sporulation in different strains. The percent of asci formed in various *rad52/rad52* strains ranged from zero to 10%. The spores from *rad52/rad52* strains were found to be inviable.

Gene-centromere recombination: Histidine and tryptophan prototrophs obtained from sporulation medium at various times were examined for gene-centromere recombination. Since the strains were heterozygous for several loci, the appearance of auxotrophs for these heterozygous loci in cells uncommitted to meiotic segregation is due to recombination (ESPOSITO and ESPOSITO 1974; ESPOSITO, PLOTKIN and ESPOSITO 1974). Histidine and tryptophan prototrophs from *RAD52/RAD52* were replica-plated on to media lacking homoserine, arginine, lysine and leucine. Table 2 gives the gene-centromere recombination observed in tryptophan and histidine prototrophs of wild-type diploids uncommitted to meiotic chromosome segregation and taken from sporulation medium at 11 hours. At the *arg6* and *lys2* loci, we already observe the recombination level characteristic of meiotic cells in the prototrophs of wild-type diploids. Gene centromere recombination was

examined among tryptophan prototrophs from the *rad52/rad52* strain (LP-1384) at 24 hours in SPM. These TRP+ colonies of *rad52/rad52* were subcloned and six subclones of each colony were examined by replica plating onto media lacking homoserine, arginine, lysine and leucine. Since there is no intragenic recombination in *rad52/rad52* during meiosis, the prototrophs represent those arising during mitosis, therefore, subclones of prototrophs were examined in order to ensure the recovery of any auxotrophs arising due to intergenic recombination during meiosis. However, in *rad52/rad52* homozygotes, no gene-centromere recombination was observed even after 24 hours in sporulation medium.

Giemsa staining of *rad52/rad52* diploids: The *rad52/rad52* diploids were stained with Giemsa after 48 hours in sporulation medium. The percent of cells with one, two and four nuclei was 65%, 21% and 14%, respectively. A total of 400 cells were examined for these determinations.

Effects in mitotic cells

Spontaneous mitotic recombination: Since meiotic recombinants are not seen in *rad52/rad52* diploids, we determined whether the *rad52* mutation also affected mitotic recombination. Experiments were carried out as described in MATERIALS and METHODS. Table 3 gives the results for rates of spontaneous mitotic prototrophy at *his1-1/his1-315* and *trp5-2/trp5-48* heteroalleles in *rad52/rad52*, *rad52/RAD52* and *RAD52/RAD52* diploids. In *rad52/RAD52* heterozygotes, there is about a two-fold reduction in the rate of histidine prototrophy compared to the *RAD52/RAD52* homozygous diploid. Similar results are observed for the *trp5-2/trp5-48* heteroalleles. In the *rad52/rad52* homozygous diploids, however, the rate of prototrophy for the two markers in quite different. Histidine prototrophs are formed at about half the rate in the *rad52/RAD52* diploid but tryptophan prototrophs are formed at about 10-15 times the rate in the heterozygous diploid. While there

appears to be a reduction in HIS+ recombinants at the *his1* locus, TRP+ recombinants occur at an increased rate in *rad52/rad52* diploids. The *trp* alleles used in these strains are both ochre-suppressible, and *rad52* strains are spontaneous mutators for ochre-suppressible loci (VAN BORSTEL, CAIN and STEINBERG 1971). However, the enhanced spontaneous mutation rate is not sufficient to account for the rate of increase in prototrophy of these heteroalleles; the rate of mitotic prototrophy in *rad52/rad52* is 20 to 30 times greater than the mutation rate. It thus appears that there is a differential effect of the *rad52* mutation on the rate of prototrophy at the *his1* and *trp5* loci.

γ-ray and UV induced mitotic recombination: Figure 6A gives the survival following γ-ray irradiation and Figure 6B gives the survival following UV-irradiation while Figures 7 and 8 present the results of γ-ray and UV induced heteroallelic recombination at the *his1-1/his1-315* and *trp5-2/trp5-48* sites in *rad52/rad52*, *rad52/RAD52* and *RAD52/RAD52* diploids. No γ-ray induced prototrophs were recovered at the *his1* or *trp5* loci among the *rad52/rad52* diploids (Figure 7). The *rad52/rad52* diploids gave some UV induced prototrophs at the *his1* heteroallelic sites at UV fluences above 20 J/m^2. At 40 J/m^2, the frequency of UV induced prototrophs at *his1* was 350/10^7 colony forming units (cfu) in *rad52/rad52* diploids and over 8000/10^7 cfu in both *RAD52/RAD52* and *rad52/RAD52* diploids. At *trp5*, few UV induced prototrophs are observed in *rad52/rad52* diploids below 20 J/m^2. However, UV induced prototrophs do appear above 20 J/m^2 in *rad52/rad52* diploids. At 40 J/m^2, the frequency of UV induced prototrophs in *rad52/rad52* diploids is 1800/10^7 cfu while the frequency in the *rad52/RAD52* and *RAD52/RAD52* diploids varies from 9200 to 13,600 per 10^7 cfu. We conclude that γ-ray induced heteroallelic recombination is absent in *rad52/rad52* diploids and that UV induced heteroallelic recombination is greatly reduced in these diploids.

DISCUSSION

We have shown that *rad52* homozygous diploids undergo premeiotic DNA synthesis but no intragenic or intergenic recombinants or auxotrophs for the centromere-linked marker (*leu1*) are recovered in *rad52* homozygotes even after 24 hours in sporulation medium. Sporulation occurs to varying degrees in different *rad52* homozygotes and inviable spores are produced. GAME and MORTIMER (1974) also reported greatly reduced spore viability in *rad52* homozygous diploids. Giemsa staining of chromatin of *rad52* diploids during meiosis revealed the occurrence of first and second nuclear divisions. Defective meiotic recombination in *rad52* diploids could cause extensive chromosomal nondisjunction resulting in the production of inviable aneuploid spores (BAKER *et al.* 1976). Since first and second nuclear divisions and sporulation do take place in *rad52/rad52* diploids, the lack of auxotrophs for the centromere linked markers, suggesting the absence of meiotic segregation, can be explained by the inviability of cells due to extensive chromosome nondisjunction resulting from first meiotic division, and may account for some of the decline in viability of cells observed in sporulation media (Figure 4). However, since the loss in viability is apparent even after three hours in sporulation medium, *rad52* probably also affects some other function in meiotic cells probably involving DNA repair, in addition to the role in recombination, and the defect(s) present in *rad52* mutants cannot be repaired even after the cells are transferred to vegetative medium from spore medium.

Spontaneous, UV induced and γ-ray induced mitotic intragenic recombination are affected differentially in *rad52* homozygotes. The γ-ray induced heteroallelic recombination is abolished at both the *his1* and *trp5* loci. RESNICK (1975) observed a lack of γ-ray induced heteroallelic recombination at the *arg4* locus. On the other hand, UV induced recombinants occur at the *his1* and *trp5* loci in *rad52* homozygotes, but the frequency of recombinants is greatly lowered compared

to the wild-type homozygotes and the *rad52/RAD52* heterozygotes. Similar results have been reported for UV induced recombination at the *arg4* locus (RESNICK 1975). X-ray and UV induced intergenic recombination are absent in *rad52/rad52* (NAKAI, personal communication). Spontaneous mitotic recombination is not significantly lower in *rad52* homozygotes; in fact, an increase is observed at the *trp5* locus. These differences in effect of the *rad52* mutation on various kinds of recombination may be due to different genetic pathways controlling different kinds of recombination, with *RAD52* playing a recombinational role in some of these pathways and not in the others. One might imagine that the *RAD52* gene product is inducible and plays a role in γ-ray and UV induced recombination which may have an inducible component (FABRE and ROMAN 1977). The *RAD52* gene also plays a role in meiotic recombination, which itself may also be inducible. Spontaneous mitotic recombination may represent a constitutive recombinational system and may utilize different gene products than are used by the inducible recombinational system. An alternative, though not mutually exclusive explanation, is that presynaptic mechanisms (CLARK 1971) vary in spontaneous mitotic and UV, γ-ray induced mitotic and meiotic recombination. ZEIG, MAPLES and KUSHNER (1978) have reported that mutations in *lex, rep, uvrA, uvrD, uvrE, lig, polA, dam* and *xthA* loci in *E. coli* affect recombination differentially during conjugation, transduction and formation of Lac+ recombinants in nontandem duplications of two partially deleted lactose operons. According to these authors, differences in presynaptic events could be responsible for their results.

In addition to the effects on recombination, *rad52* strains show increased spontaneous mutations of ochre suppressors (VON BORSTEL, CAIN and STEINBERG 1971) and are defective in the repair of X-ray induced double strand breaks in DNA in haploids (HO 1975) and diploids (RESNICK and MARTIN 1976). UV and γ-ray induced mutations are not affected in *rad52* strains (LAWRENCE and CHRISTENSEN 1976; MCKEE

and LAWRENCE, 1979). Thus, the *RAD52* gene product appears to participate in error-free recombination repair of γ-ray and UV induced damage. But, since UV induced heteroallelic recombination is not totally absent in *rad52* homozygotes, alternate recombinational pathway(s) may exist for UV induced recombination in *S. cerevisiae*.

Mutants specifically defective in meiotic recombination in *S. cerevisiae* have been isolated and characterized; three mutants, *con1*, *con2* and *con3* allow premeiotic DNA synthesis but show no prototroph formation for heteroalleles at the *leu2* locus. The *con1* mutation blocked sporulation while *con2* and *con3* mutants produced inviable ascospores (ROTH and FOGEL 1971; FOGEL and ROTH 1974). The *rec1*, *rec2*, *rec3* and *rec4* mutants of *S. cerevisiae* are deficient in X-ray induced and the *rec1*, *rec3* and *rec4* mutants are also deficient in UV induced mitotic intragenic recombination (RODARTE-RAMON and MORTIMER 1972; RODARTE-RAMÓN 1972). The *rec4* mutant blocks single site conversions and increases the frequency of coconversions which include both mutant sites of the heteroalleles. Intergenic meiotic recombination or sporulation are not affected in *rec4* strains (SAN FILIPPO 1976). In contrast to the *rec4* mutation which affects the length of the conversion segment but not intergenic recombination or sporulation during meiosis, the *rad52* mutation affects both intragenic and intergenic meiotic recombination and inviable spores are formed during meiosis. The *rad52* mutation probably affects some step during recombination and not the length of the conversion segment.

The *rad52* mutation of yeast resembles the *recA* mutation of *E. coli* in its deficiency in recombination and in its inability to repair double strand breaks in DNA. However, the *RAD52* gene function does not seem to be involved in error prone repair of UV or X-ray induced damage to DNA whereas the *RECA* gene product is absolutely required for such repair (KONDO, *et. al*

1970; WITKIN 1969). Moreover, *recA* mutants lack all kinds of homologous recombination (ZIEG, MAPLES and KUSHNER 1978) while *rad52* mutants undergo spontaneous mitotic recombination and UV induced mitotic recombination is greatly reduced but not abolished.

ACKNOWLEDGEMENTS

We are grateful to Rod Rothstein and Shelly Esposito for various helpful discussions, and to Bob Mortimer for kindly providing the information on map distances. We thank Pat Terho for excellent technical assistance.

L. P. was supported in part by a U. S. Public Health Service Career Development Award (GM-00004) from the National Institutes of Health. This investigation was supported in part by the U. S. Public Health Service Research Grant GM-19261 from the National Institutes of Health and in part by the U.S. Department of Energy at the University of Rochester Department of Radiation Biology and Biophysics. This paper has been assigned Report No. UR-3490-1571.

LITERATURE CITED

BAKER, B. S., A. T. C. CARPENTER, M. S. ESPOSITO, R. E. ESPOSITO and L. SANDLER, 1976 The genetic control of meiosis. Ann. Rev. Genet. 10: 53-134.

CLARK, A. J., 1971 Toward a metabolic interpretation of genetic recombination of *E. coli* and its phages. Ann. Rev. Microbiol. 25: 437-464. _____, 1973 Recombination deficient mutants of *E. coli* and other bacteria. Ann. Rev. Genet. 7: 67-86.

EISENSTARK, A., 1977 Genetic recombination in bacteria. Ann. Rev. Genet. 11: 369-396.

ESPOSITO, M. S., 1978 Evidence that spontaneous mitotic recombination occurs at the two strand stage. Proc. Natl. Acad. Sci., U. S. 75: 4436-4440.

ESPOSITO, R. E. and M. S. ESPOSITO, 1974 Genetic recombination and commitment to meiosis in *Saccharomyces*. Proc. Natl. Acad. Sci., U. S. 71: 3172-3176.

ESPOSITO, R. E., D. J. PLOTKIN and M. S. ESPOSITO, 1974 The relationship between genetic recombination and commitment to chromosomal segregation at meiosis. In Mechanisms in Recombination, ed. R. F. Grell, pp. 277-285.

FABRE, F., 1978 Induced intragenic recombination in yeast can occur during G_1 mitotic phase. Nature 272: 795-798.

FABRE, F. and H. ROMAN, 1977 Genetic evidence for inducibility of recombination competence in yeast. Proc. Natl. Acad. Sci., U. S. 74: 1667-1671.

FINCHAM, J. R. S. and P. R. DAY, 1971 Fungal genetics, third edition. Blackwell Scientific publication. Oxford and Edinburgh.

FOGEL, S. and D. D. HURST, 1967 Meiotic gene conversion in yeast tetrads and the theory of recombination. Genetics 57: 455-481.

FOGEL, S. and R. K. MORTIMER, 1969 Informational transfer in meiotic gene conversion. Proc. Natl. Acad. Sci. U. S. 62: 96-103. _____, 1970 Fidelity of gene conversion in yeast. Mol. Gen. Genet. 109: 177-185. _____, 1971 Recombination in yeast. Ann. Rev. Genet. 5: 219-236.

FOGEL, S. and R. ROTH, 1974 Mutations affecting meiotic gene conversion in yeast. Mol. Gen. Genet. 130: 189-201.

GAME, J. C. and R. K. MORTIMER, 1974 A genetic study of X-ray sensitive mutants in yeast. Mutat. Res. 24: 281-292.

HAWTHORNE, D. C. and R. K. MORTIMER, 1960 Chromosome mapping in *Saccharomyces:* centromere linked genes. Genetics 45: 1085-1110.

HO, K. S. Y., 1975 Induction of DNA double strand breaks by X-rays in a radiosensitive strain of the yeast *Saccharomyces cerevisiae*. Mutat. Res. 30: 327-334.

HOLLIDAY, R., 1964 A mechanism for gene conversion in fungi. Genet. Res. 5: 282-304.

HOWARD-FLANDERS, P., 1968 DNA repair. Ann. Rev. Biochem. 37: 175-200.

HURST, D. D., S. FOGEL and R. K. MORTIMER, 1972 Conversion associated recombination in yeast. Proc. Natl. Acad. Sci., U. S. 69: 101-105.

KONDO, S., H. ICHIKAWA, K. IWO and T. KATO, 1970 Base change mutagenesis and prophage induction in strains of *Escherichia coli* with different DNA repair capacities. Genetics 66: 187-217.

LAWRENCE, C. W. and R. CHRISTENSEN, 1976 UV mutagenesis in radiation sensitive strains of yeast. Genetics 82: 207-232.

LEA, D. E. and C. A. COULSON, 1949 The distribution of the numbers of mutants in bacterial populations. Genetics 49: 264-285.

MCKEE, R. H. and C. L. LAWRENCE, 1979 Genetic analysis of gamma ray mutagenesis in yeast. I. Reversion in radiation-sensitive strains, to be submitted to Genetics.

MESELSON, M. S. and C. M. RADDING, 1975 A general model for genetic recombination. Proc. Natl. Acad. Sci., U. S. 72: 358-361.

MORTIMER, R. K. and D. C. HAWTHORNE, 1966 Genetic mapping in *Saccharomyces*. Genetics 53: 165-173. _____, 1973 Genetic mapping in *Saccharomyces*. IV. Mapping of temperature-sensitive genes and use of disomic strains in localizing genes. Genetics. 74: 33-54.

RESNICK, M. A., 1975 The repair of double strand breaks in chromosomal DNA of yeast. In Molecular Mechanisms for Repair of DNA. Part B. pp. 549-556. Ed. P. C. Hanawalt and R. B. Setlow. Plenum Press, New York.

RESNICK, M. A. and P. MARTIN, 1976 The repair of double strand breaks in the nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. Mol. Gen. Genet. 143: 119-129.

RODARTE-RAMÓN, U. S., 1972 Radiation induced recombination in *Saccharomyces:* The genetic control of recombination in mitosis and meiosis. Rad. Res. 49: 148-154.

RODARTE-RAMÓN, U. S. and R. K. MORTIMER, 1972 Radiation induced recombination in *Saccharomyces:* Isolation and genetic study of recombination deficient mutants. Rad. Res. 49: 133-147.

ROTH, R. and S. FOGEL, 1971 A system selective for yeast mutants deficient in meiotic recombination. Mol. Gen. Genet. 112: 295-305.

ROTH, R. and K. LUSNAK, 1970 DNA synthesis during yeast sporulation: Genetic control of an early developmental event. Science 168: 493-494.

ROTHMAN, R. H., T. KATO and A. J. CLARK, 1975 The beginning of an investigation of the role of *recF* in the pathways of metabolism of ultraviolet-irradiated DNA in *Escherichia coli*. In Molecular Mechanisms for Repair of DNA. Part A. pp. 283-291 Ed. P. C. Hanawalt and R. B. Setlow. Plenum Press, N. Y.

SAN FILIPPO, D. 1976 A mutational analysis of gene conversion and reciprocal recombination in the yeast, *Saccharomyces cerevisiae*. Ph.D. thesis, University of California, Berkeley.

VON BORSTEL, R. C., K. T. CAIN and C. M. STEINBERG, 1971 Inheritance of spontaneous mutability in yeast. Genetics. 69: 17-27.

WILDENBERG, J., 1970 The relation of mitotic recombination to DNA replication in yeast pedigrees. Genetics 66: 291-304.

WITKIN, E. 1969 The mutability toward ultraviolet light of recombination deficient strains of *Escherichia coli*. Mutat. Res. 8: 9-14.

ZEIG, J., V. F. MAPLES and S. R. KUSHNER, 1978 Recombination levels of *Escherichia coli* K-12 mutants deficient in various replication, recombination or repair genes. J. Bact. 134: 958-966.

TABLE 1. GENETOPYES OF STRAINS USED

Strain		Genotype
LP-854	α	*LYS2* *HOM3 his1-315* *arg6* *ILV1* *trp5-48* *rad52-1*
	a	*lys2-1* *hom3 his1-1* *ARG6* *ilv1-1* *trp5-2* *rad52-1*
LP-1383	α	*lys2-1* *hom3 his1-1* *ARG6* *ilv1-1* *trp5-2* *leu1-12* *rad52-1*
	a	*LYS2* *HOM3 his1-315* *arg6* *ILV1* *TRP5* *LEU1* *rad52-1*
LP-1384	α	*lys2-1* *hom3 his1-1* *ARG6* *ilv1-1* *trp5-2* *leu1-12* *rad52-1*
	a	*LYS2* *HOM3 his1-315* *arg6* *ILV1* *trp5-48* *LEU1* *rad52-1*
LP-855	α	*LYS2* *ura3 HOM3 his1-315 arg6 ILV1* *trp5-48* *met14* *RAD52*
	a	*lys2-1* *URA3 hom3 his1-1* *ARG6 ilv1-1* *trp5-2* *MET14* *rad52-1*
LP-1415	α	*HOM3 his1-315 arg6 ILV1* *trp5-48* *LEU1* *rad52-1*
	a	*hom3 his1-1* *ARG6 ilv1-1* *trp5-2* *leu1-12* *RAD52*
LP-1416	α	*LYS2* *ura3 HOM3 his1-315 arg6* *ILV1* *trp5-48 LEU1* *met14* *RAD52*
	a	*lys2-1* *URA3 hom3 his1-1* *ARG6* *ilv1-1* *trp5-2 leu1-12* *MET14* *RAD52*

TABLE 2.

GENE-CENTROMERE RECOMBINATION AMONG TRYPTOPHAN AND HISTIDINE PROTOTROPHS UNCOMMITTED TO MEIOSIS

			TRP+			His+		
			% Gene-Centromere Recombination*					
Strain	Genotype	# Tested	*hom3*	*arg6*	*lys2-1*	*hom3*	*arg6*	*lys2-1*
LP-1416	*RAD52*/*RAD52*	128†	9.0	43.0	36.0	14.0	31.0	25.0
LP-1384	*rad52*/*rad52*	130††	0.0	0.0	0.0			

* % Gene Centromere recombination was calculated as in Esposito and Esposito (1974)

† Prototrophs from LP-1416 were withdrawn from sporulation medium at 11 hours.

†† Prototrophs from LP-1384 were withdrawn from sporulation medium at 24 hours. TRP+ colonies were subcloned and 6 subclones from each colony were analyzed.

TABLE 3.

SPONTANEOUS MITOTIC PROTOTROPHY AT

his1-1/his1-315 AND *trp5-2/trp5-48* SITES

		His+			*TRP+*		
	mean # of cells per culture	median # *His+* per culture	mean # *His+* arising per culture	RATE (X 10^{-6})	median # *TRP+* per culture	mean # *TRP+* arising per culture	RATE (X 10^{-6})
RAD52/*RAD52* (LP-1416)	3.41 X 10^8	5050	653	1.92	2475	350	1.03
RAD52/*rad52* (LP-855)	3.46 X 10^8	1855	271	0.78	1150	179	0.5
(LP-1415)	2.83 X 10^8	1850	271	0.96	1288	197	0.7
rad52/*rad52* (LP-854)	3.4 X 10^7	65	16	0.47	1400	213	6.3
(LP-1384)	3.02 X 10^7	80	19	0.63	1925	281	9.3

Figure 1. Pre-meiotic DNA synthesis in *RAD+/RAD+* (●) and *rad52/rad52* (○) diploids. The number of counts remaining acid-precipitable after alkaline hydrolysis of samples taken at various times after transfer to sporulation medium was taken as a measure of the amount of DNA synthesized. The ratio of amount of DNA at a given time over the amount at 0hrs after transfer to spore media is plotted in the figure.

Figure 2. Frequency of histidine prototrophs as a function of time of incubation in sporulation medium, in LP-1416, *RAD+/RAD+* (○); LP-855 and LP-1415 *RAD+/rad52* (△ and ▲, respectively); LP-1383 and LP-1384, *rad52/rad52* (● and □, respectively). All strains contained *his1-1/his1-315* heteroalleles.

Figure 3. Frequency of tryptophan prototrophs as a function of time of incubation in sporulation medium in LP-1416, *RAD+/RAD+* (●); LP-1415 and LP-855 *RAD+/rad52* (□ and ■, respectively); and in LP-1384 and LP-854, *rad52/rad52* (△ and ▲, respectively). All strains contained *trp5-2/trp5-48* heteroalleles.

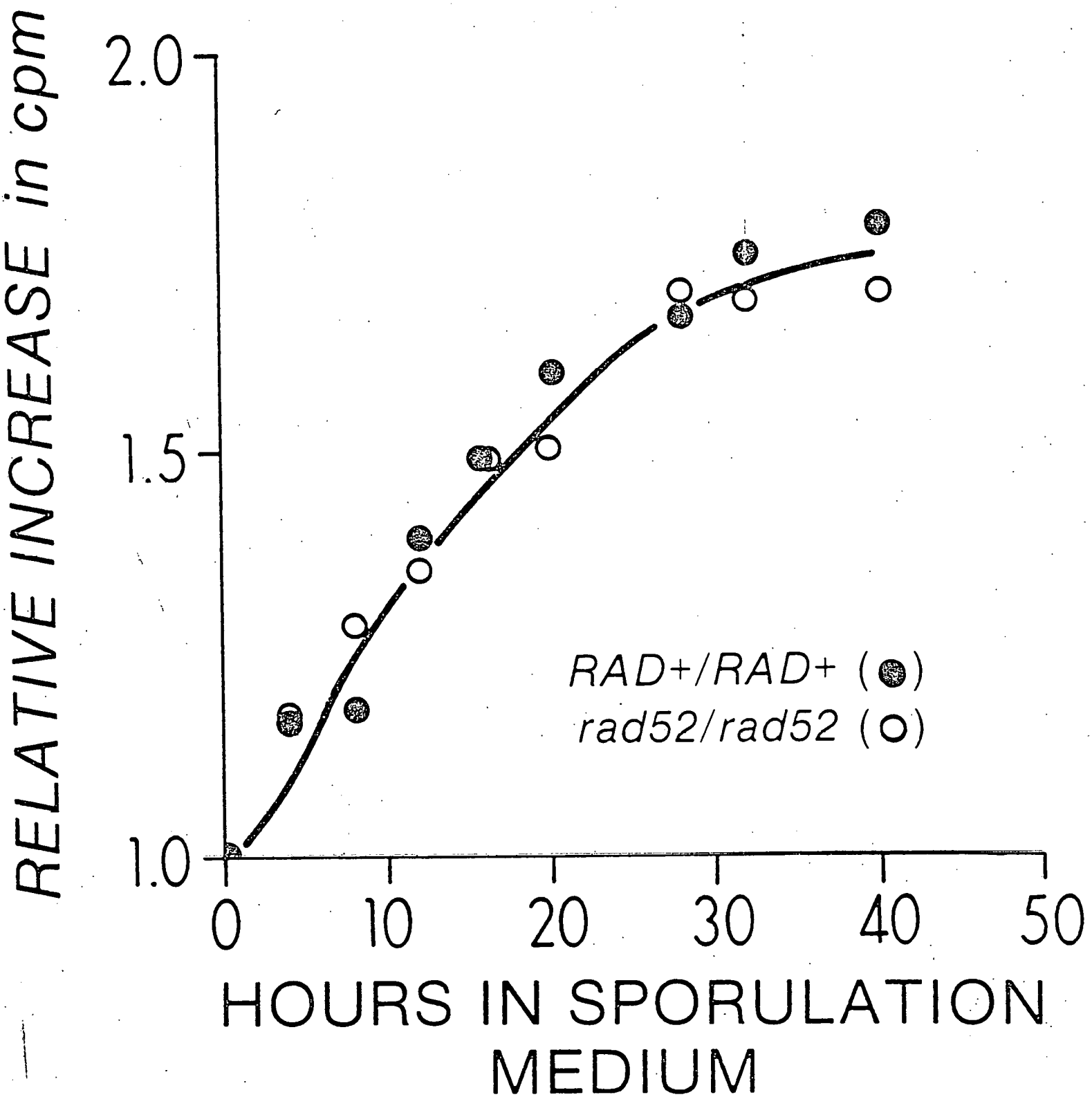
Figure 4. Viability, as determined by plating appropriate dilutions of cells on SC medium after various times of incubation in sporulation medium in LP-1416, *RAD+/RAD+* (○); LP-855 and LP-1415, *RAD+/rad52* (△ and ▲, respectively) and LP-854 and LP-1384, *rad52/rad52* (■ and □, respectively).

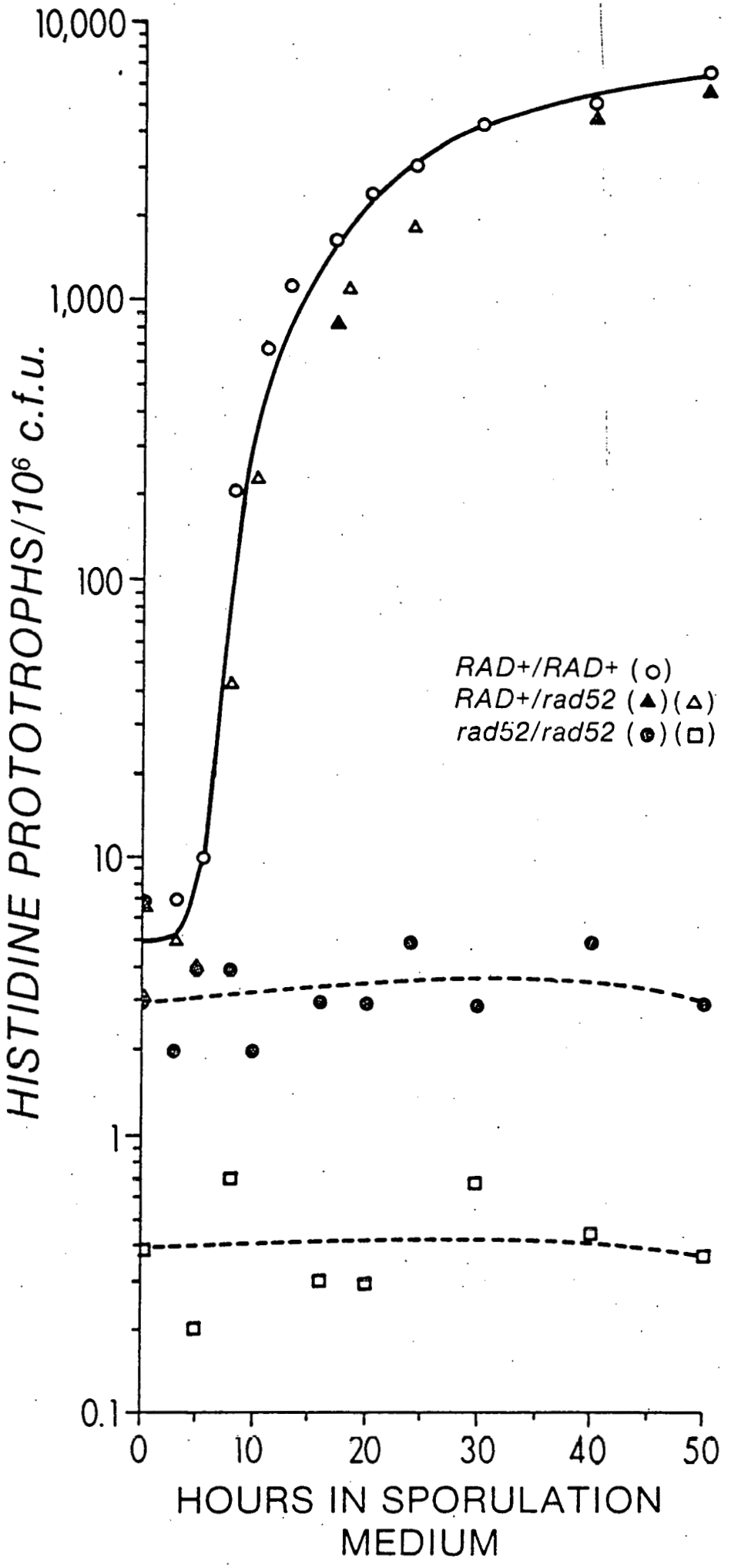
Figure 5. Meiotic chromosome segregation in a *RAD+/RAD+* diploid, LP-1416 among histidine prototrophs determined as twice the percent of centromere linked leucine (*leu1*) auxotrophs (●). Ascus formation in the *RAD+/RAD+* diploid (○).

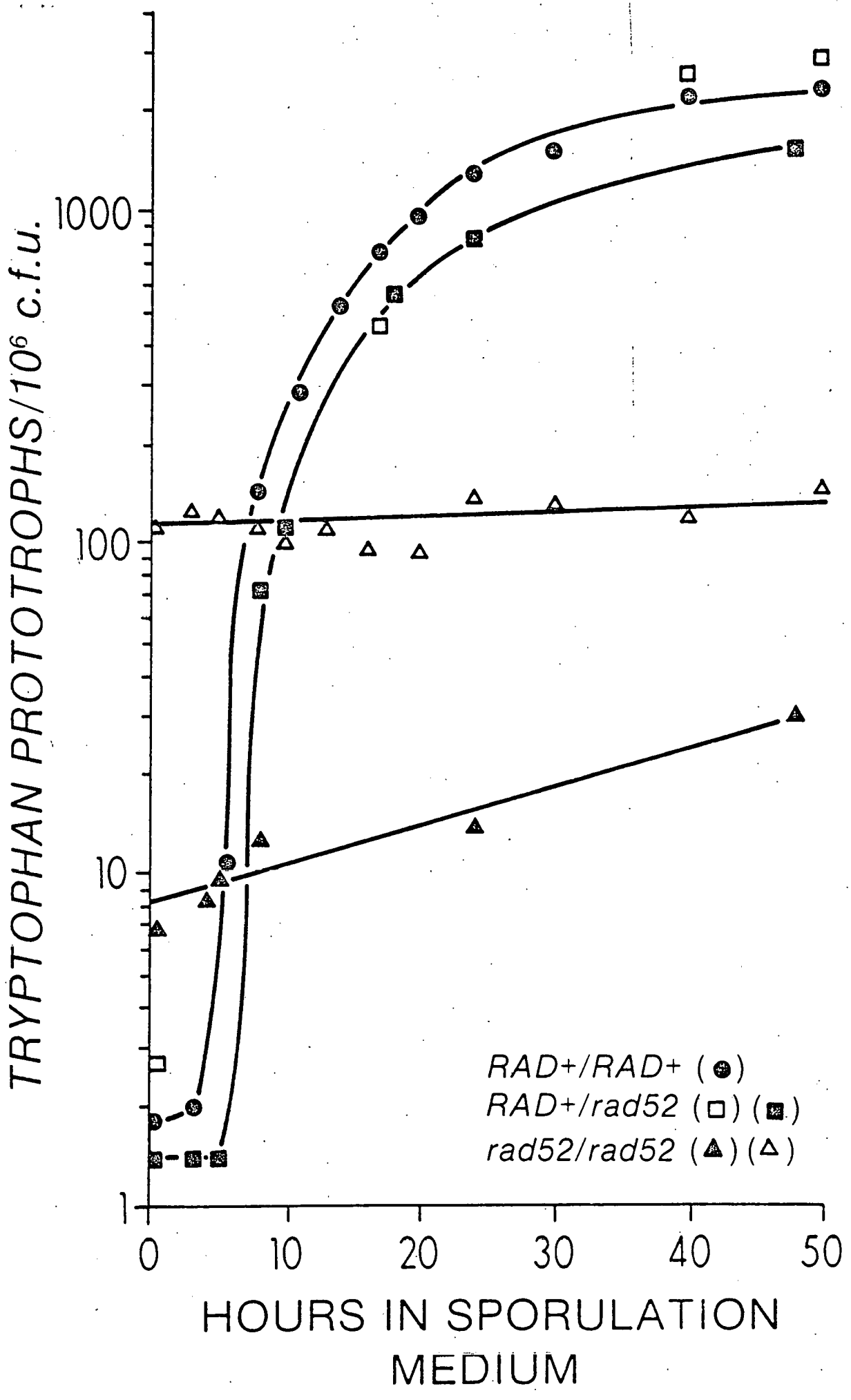
Figure 6. Survival curves following γ-ray irradiation (A) and UV-irradiation (B) in *RAD+/RAD+* (LP-1416), ● ; *RAD+/rad52* (LP-1415), △ ; and *rad52/rad52* (LP-1384), O, diploids.

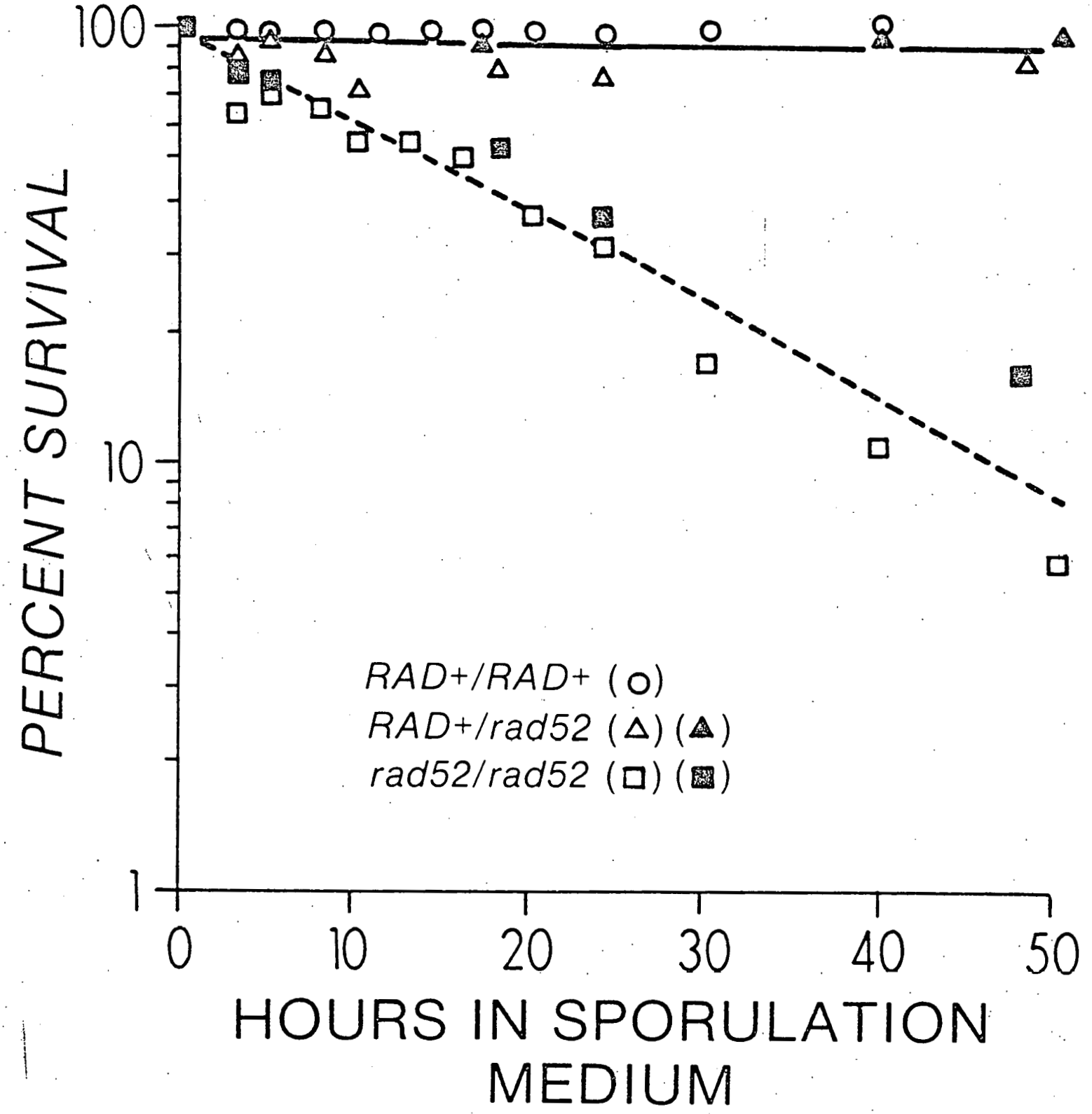
Figure 7. Dose-response curve for induction of histidine (A) and tryptophan (B) mitotic prototrophs following γ-ray irradiation of *RAD+/RAD+* (LP-1416), ● ; *RAD+/rad52* (LP-1415), △ ; and *rad52/rad52* (LP-1384), O.

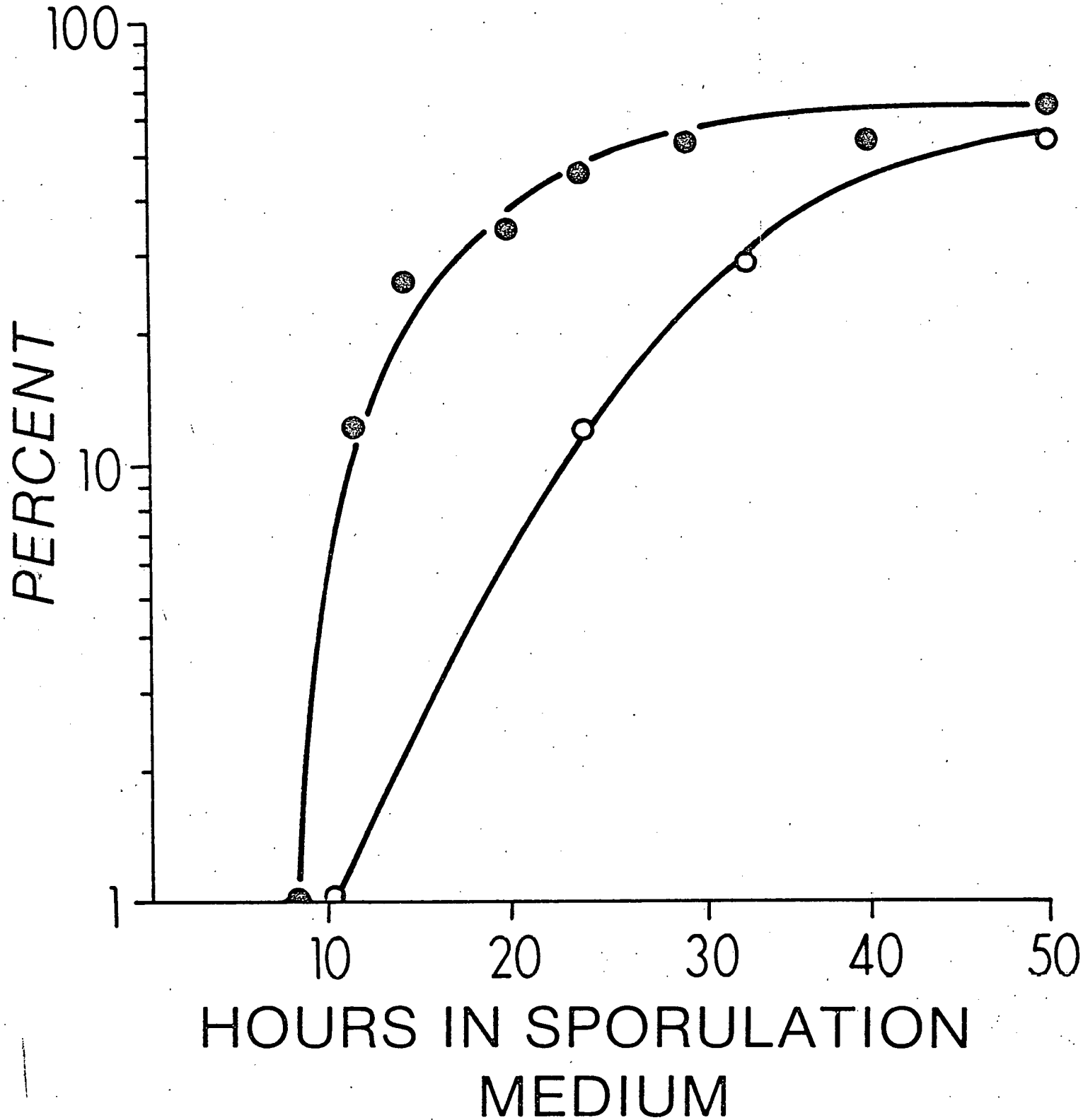
Figure 8. Fluence-response curve for induction of histidine (A) and tryptophan (B) mitotic prototrophs following UV-irradiation of *RAD+/RAD+* (LP-1416), ● ; *RAD+/rad52* (LP-1415), △ ; and *rad52/rad52* (LP-1384), O.

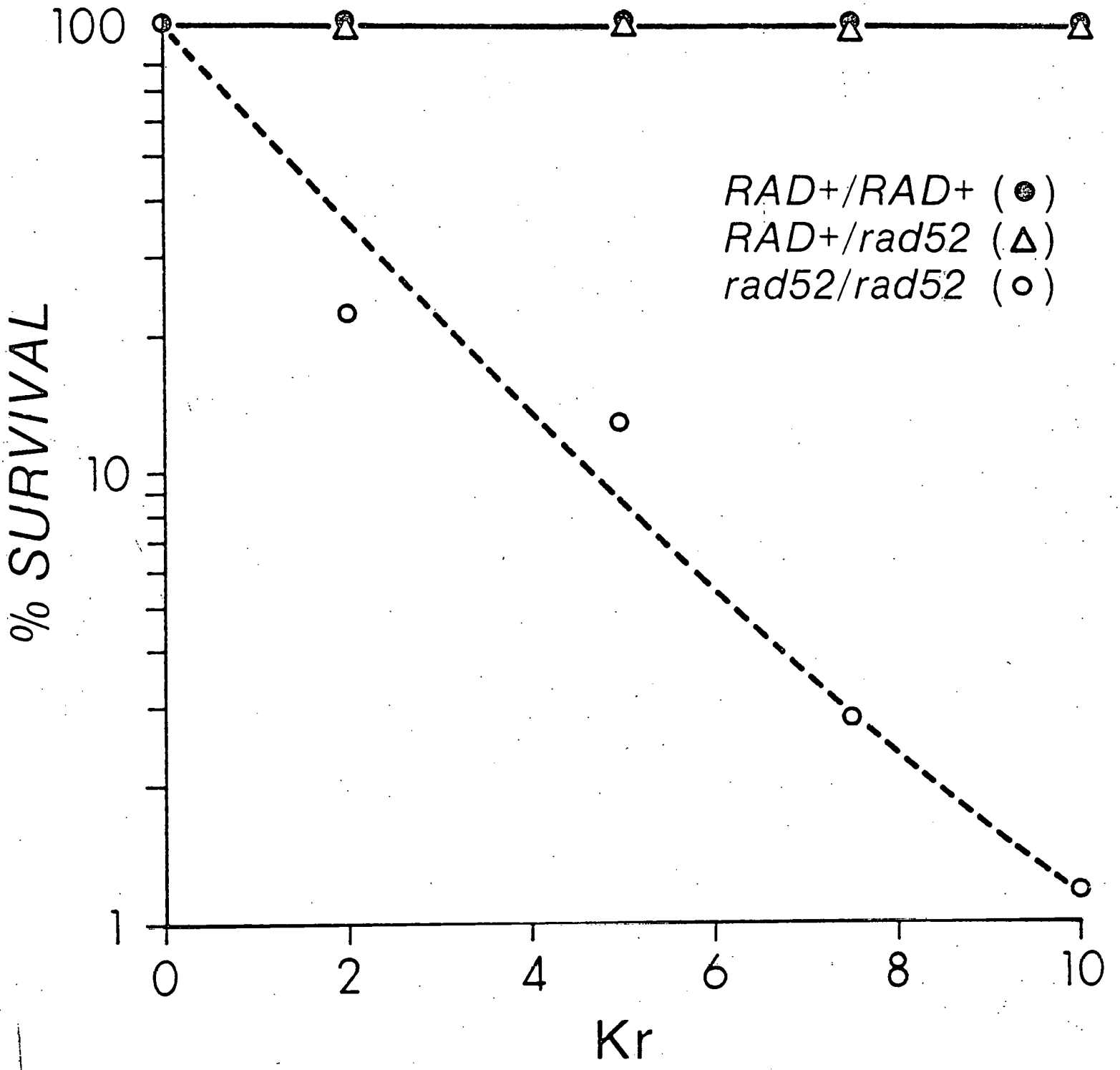

RELATIVE INCREASE in cpm
2.0
1.5
1.0
0
10
20
30
40
50
HOURS IN SPORULATION MEDIUM
RAD+/RAD+ (●)
rad52/rad52 (○)

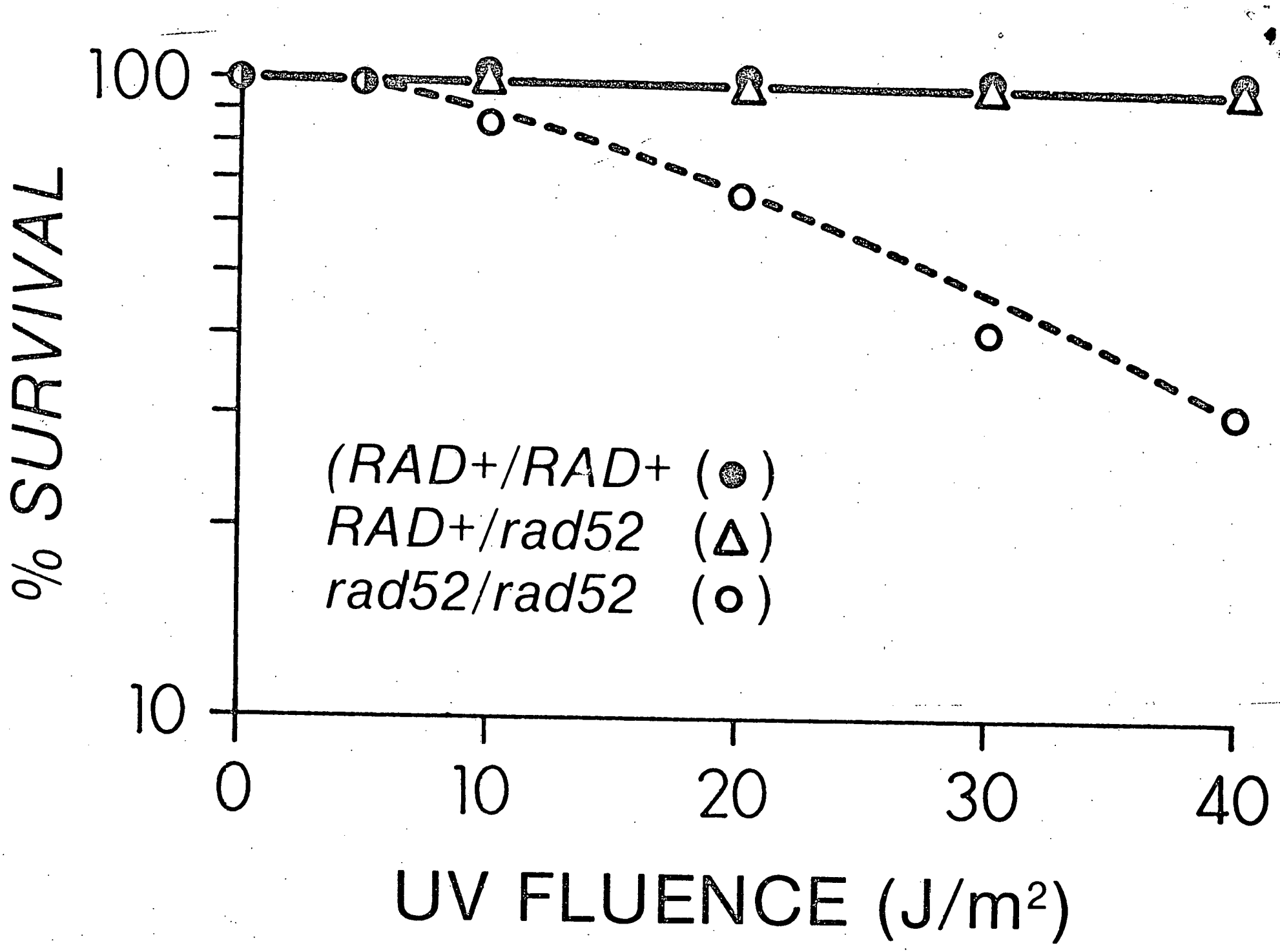

10,000
1,000
100
10
1
0.1
HISTIDINE PROTOTROPHS/10⁶ c.f.u.
RAD+/RAD+ (○)
RAD+/rad52 (▲)(△)
rad52/rad52 (⊙)(□)
0
10
20
30
40
50
HOURS IN SPORULATION MEDIUM

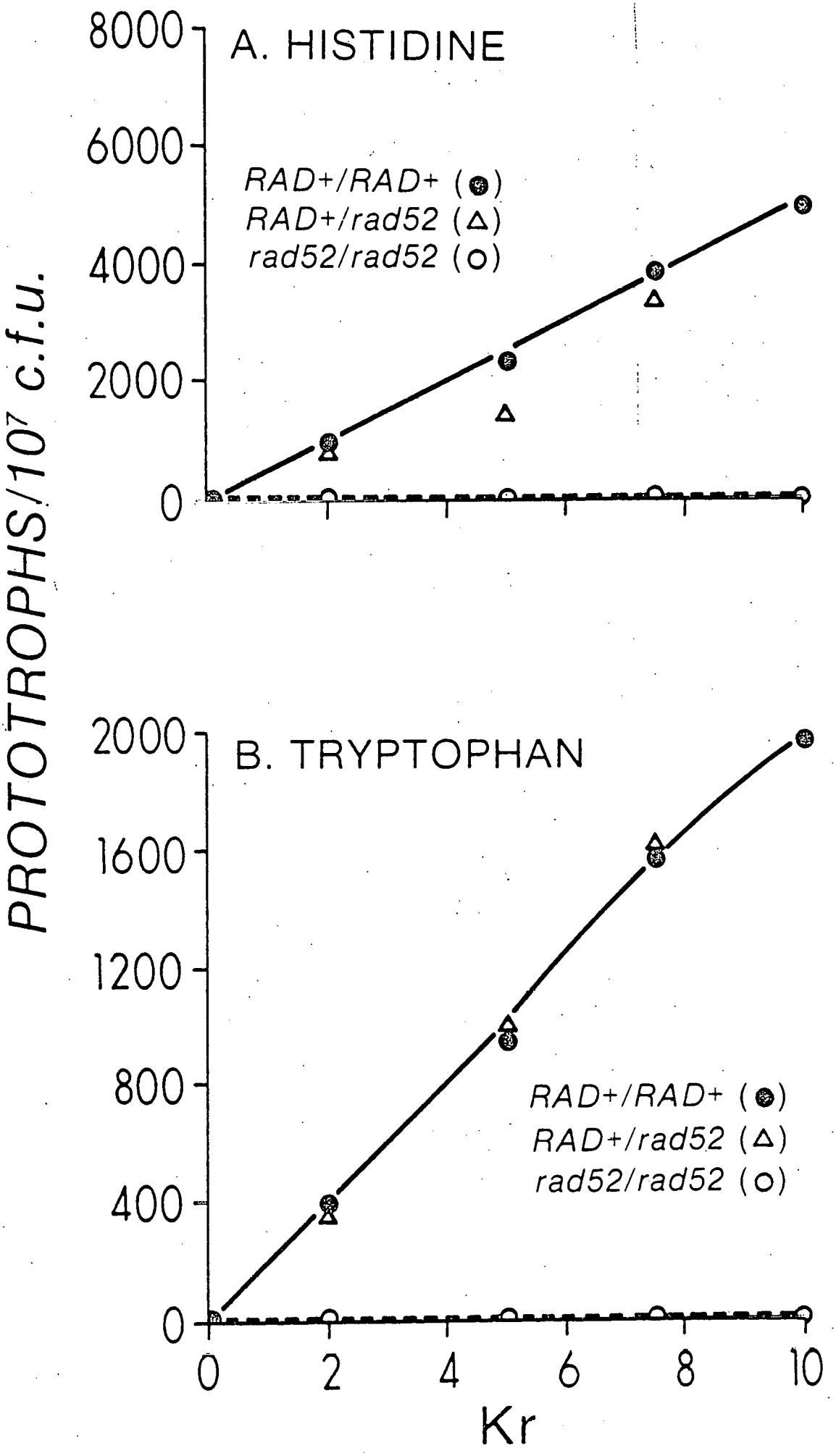

TRYPTOPHAN PROTOTROPHS/10⁶ c.f.u.
1000
100
10
1
0
10
20
30
40
50
HOURS IN SPORULATION MEDIUM
RAD+/RAD+ (●)
RAD+/rad52 (□) (■)
rad52/rad52 (▲) (△)

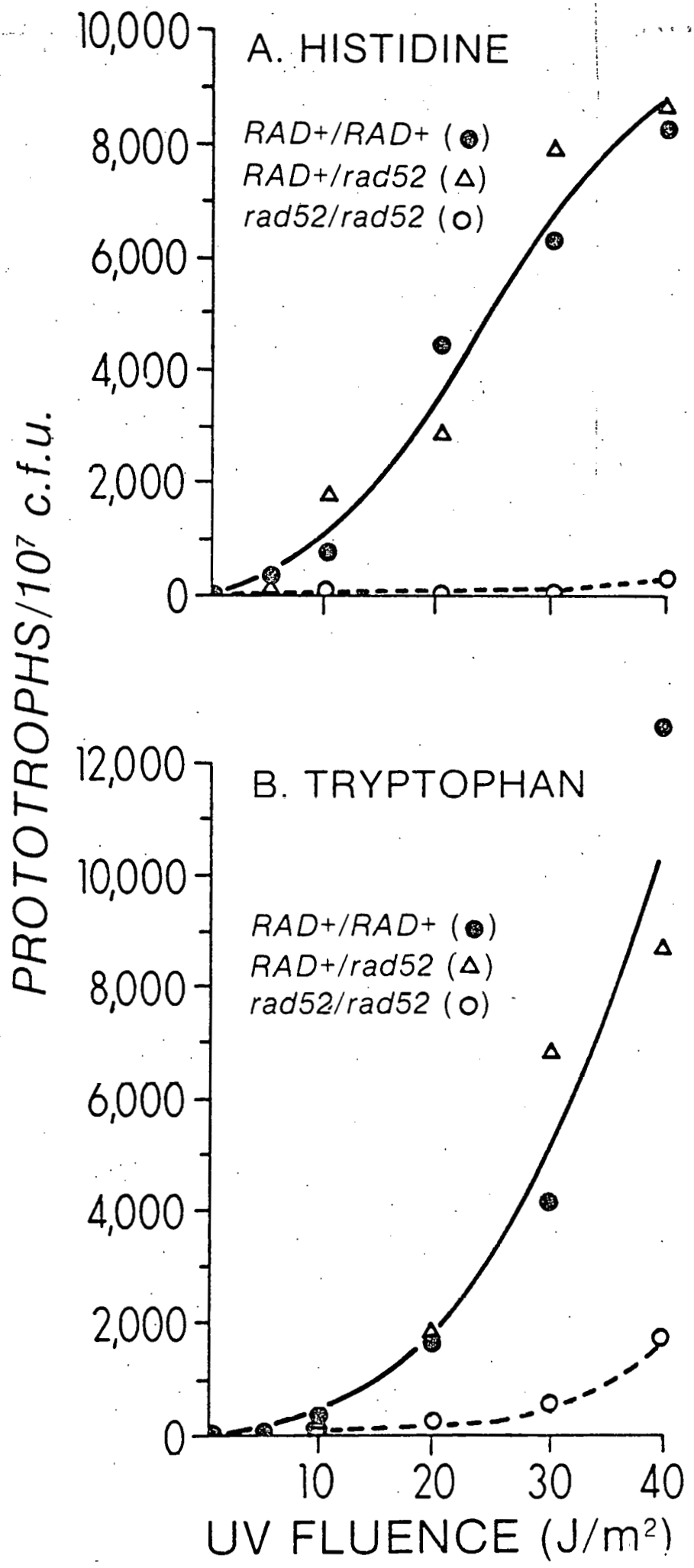

100
10
1
PERCENT SURVIVAL
0
10
20
30
40
50
HOURS IN SPORULATION MEDIUM
RAD+/RAD+ (○)
RAD+/rad52 (△) (▲)
rad52/rad52 (□) (■)


100
10
1
PERCENT
10
20
30
40
50
HOURS IN SPORULATION MEDIUM


100
10
1
% SURVIVAL
0
2
4
6
8
10
Kr
RAD+/RAD+ (●)
RAD+/rad52 (Δ)
rad52/rad52 (○)


100
10
% SURVIVAL
(RAD+/RAD+ (●)
RAD+/rad52 (Δ)
rad52/rad52 (o)
0
10
20
30
40
UV FLUENCE (J/m²)


A. HISTIDINE
8000
6000
4000
2000
0
RAD+/RAD+ (●)
RAD+/rad52 (△)
rad52/rad52 (○)
B. TRYPTOPHAN
2000
1600
1200
800
400
0
RAD+/RAD+ (●)
RAD+/rad52 (△)
rad52/rad52 (○)
0
2
4
6
8
10
Kr
PROTOTROPHS/10⁷ c.f.u.


A. HISTIDINE
RAD+/RAD+ (●)
RAD+/rad52 (△)
rad52/rad52 (○)
10,000
8,000
6,000
4,000
2,000
0
B. TRYPTOPHAN
RAD+/RAD+ (●)
RAD+/rad52 (△)
rad52/rad52 (○)
12,000
10,000
8,000
6,000
4,000
2,000
0
10
20
30
40
PROTOTROPHS/10⁷ c.f.u.
UV FLUENCE (J/m²)