INFORMATION TO USERS

This dissertation was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

University Microfilms

300 North Zeeb Road
Ann Arbor, Michigan 48106

A Xerox Education Company

73-2828

BENATHEN, Isaiah Amiel, 1940-
THE GENETIC CONTROL OF X-RAY RESISTANCE IN BUDDING YEAST CELLS.

The City University of New York, Ph.D., 1972
Biology-Genetics

University Microfilms, A XEROX Company, Ann Arbor, Michigan

THE GENETIC CONTROL OF X-RAY RESISTANCE IN BUDDING YEAST CELLS

by

ISAIAH AMIEL BENATHEN

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1972

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

June 28, 1972
Date

Chairman of Examining Committee
Professor C. Beam, Brooklyn College

June 29, 1972
Date

Executive Officer

Prof. N. Eaton	Brooklyn College Institution
Prof. F. Zimmermann	Brooklyn College Institution
Prof. M. Green	Hunter College Institution
Prof. J. Marmur	Albert Einstein College of Medicine Institution
Prof. D. Bursick	Howard University Institution

The City University of New York

Acknowledgements

I wish to express my deepest appreciation and thanks to my doctoral thesis advisor, Professor Carl A. Beam, for his encouragement, instruction, and the numerous hours of fruitful discussion during the course of these investigations. His guidance in this and other projects is gratefully acknowledged and appreciated.

I also wish to express my appreciation and thanks to Professor Norman Eaton and Professor Friedrich K. Zimmermann for serving as members of my advisory committee and for their helpful advice and suggestions during these investigations.

I also wish to thank Professor Seymour Fogel for his advice and suggestions during these investigations.

I also wish to thank the Executive Officer, Professor Louis Moriber and his administrative assistant, Mrs. Jeanette Needleman for the many kindnesses extended to me during my tenure as a graduate student.

I also wish to thank the Department of Biology for their assistance and support.

I want to thank my mother, Mrs. Pauline Benathen, for her patience and effort in typing the manuscripts.

Table of Contents

List of Tables

List of Figures

Introduction

The present study is concerned with the intermittent high resistance to ionizing radiation occurring in budding yeasts and with its genetic control. Budding yeast cells are more resistant than nonbudded cells to inactivation by x-rays and alpha particles, and to a much lesser extent to inactivation by ultraviolet light (21, 22). In addition to inactivation, budding cells have been shown to be resistant to induction by ionizing radiation of recessive lethals (5), petites (68, 69) mitotic crossing over (68, 69) and heritable alterations in colony form and color (68, 69).

Evidence with respect to the timing of events in the cell cycle associated with budding cell resistance is somewhat conflicting. Williamson (85) and Hartwell (31) have shown that in their yeast strains DNA synthesis is initiated with the onset of budding and is completed by the first quarter of the cell cycle, while the bud is still quite small. In the strain employed most extensively in the present study (SC7K), replication of DNA, as followed by feulgen staining, is completed before budding begins and in the absence of observable changes in radiation sensitivity. The initiation of budding is, however, accompanied by the appearance of radiation resistance, but is without observable nuclear cytological changes (6). A high level of resistance is restricted to a brief portion of the budding cycle, during

which the bud is approximately one third to one half the size of the mother cell. Cells with smaller buds are less resistant, while those with larger buds (with two nuclei) respond as two interdivisional cells. In our strains of yeast, resistance appears after DNA synthesis and disappears at or before nuclear division (6). It seems, therefore, that resistance is not a direct consequence of DNA content or of its synthesis.

There is no reason to believe that the regulation of the radiation resistance of the budding yeast cell is induced by the radiation itself or by damaged DNA produced by it. Induction by ultraviolet light of uv resistance has been observed in uvs mutants of yeast (15) and of Chlamydomonas rheinhardii (16, 17). If resistance were inducible, one would expect that budding cell resistance would be submaximal at low doses (less damage to DNA) and would maximize with greater dose (more damage to DNA). In fact, budding cells are fully resistant to the lowest doses administered (1). Thus, it would appear that budding cell resistance is a normal consequence of events in the cell cycle and is not induced by the radiation itself and is not noticeably responsive to the amount of damage produced. Nor have the observations of Cox and Parry (15) and Davies (16, 17) of apparent uv induction of a repair process in the uvs 3 mutant of yeast and in

Chlamydomonas rheinhardii, respectively, been observed in any of our mutants. The mutants isolated in this investigation show reduced budding cell resistance or no resistance at all.

The question as to whether resistance arises as a result of chemical protection against radiation damage by sulfhydryl compounds or other scavengers of the radio chemical products (2) or whether resistance results from repair of damage inflicted by radiation has not been conclusively answered. If resistance resulted from the synthesis or release of protective metabolites within the cell during the budding phase of the cell cycle, one might expect that irradiation of budded cells in the presence of added sulfhydryl compounds or anoxia would provide little or no additive protection. Instead one finds that the degree of such protection is either unchanged from that observed in interdivisional cells (4) or only slightly diminished (2). These results argue against intracellular chemical protection and favor the repair of damage produced by radiation. A repair model for budding cell resistance to both nuclear and mitochondrial effects (58, 59) requires a repair system that is generally effective throughout the cell.

If the increased resistance of the budding cell results from the onset of a repair process, the inactivation of

budding cells could be due either to the induction of damage not subject to this repair or to inactivation of the repair mechanism itself. These two possibilities have been considered before (5) and lead to somewhat different predictions. In the first case, one may propose that the "non repairable damage" occurring in the budding cell occurred in interdivisional cells as well. Thus one would expect that the multihit x-ray survival curve seen for budding cells would be reflected in the interdivisional cell survival curve in an additive fashion along with any other inactivations. The result of such addition of a multihit component to presumed exponentials could never result in the exponential inactivation observed in haploid interdivisional cells. Thus, "non repairable damage" is either an attribute of budding cells only, or the budding cell survival curve requires another explanation.

If, on the other hand, the multihit inactivation of the budding cell results from inactivation of the repair process, one would expect the limiting exponential of this curve to be identical to the interdivisional cell exponential inactivation, which it never is (see figures 1-8). Another interpretation similar to that proposed by Haynes (33, 34) for other multihit survival curves, would stipulate that the limiting exponential at high doses represents not inactivation of the repair enzymes, but their saturation by accumulated substrate

(damage). Since the kinetics resulting from this saturation bears no relation to the exponential survival of interdivisional cells, the observed difference in survival kinetics would be expected. For the above reasons, the x-ray resistance of the budding cell will be viewed for purposes of the present investigation as resulting from an internally regulated repair process, the enzymes of which may be saturated at high doses.

The genetic control of radiation resistance has been investigated in a general way, and without consideration of the cell cycle by Cox and Parry (15) and others (60, 73, 79) in yeasts and by others in other eukaryotes (3, 12, 16, 37, 52, 75, 88), and more extensively in prokaryotes particularly Escherichia coli (1, 13, 18, 26, 29, 30, 45, 48, 55, 74). In prokaryotes, an intimate relationship has been demonstrated between resistance to radiation and the repair or removal of lethal radiation damage in nonsynchronous cultures and in cultures without regard to the state in the cell cycle (1). The isolation and characterization of sensitive mutants has shown these processes to be genetically controlled (1, 30, 48). Three types of such repair processes effective for uv damage have been studied in several prokaryotes, but most especially in E. coli. These are photoreactivation and two mechanisms of dark repair. The literature on photoreactivation

in various systems has been recently reviewed (76). In essence, the relevant facts are as follows: low doses of uv produce *in vivo* cyclobutane type pyrimidine dimers. These lesions, when not removed, contribute to cell killing (76). Illumination of the irradiated cells by visible light in the presence of an appropriate intracellular enzyme monomerizes the pyrimidine dimers *in situ* (76). Photoreactivationless mutants of *E. coli* have been isolated and shown to lack the appropriate enzyme activity (30) indicating that this process is under genetic control.

Two mechanisms of dark repair also eliminate uv induced pyrimidine dimers. They are excision repair and recombination repair (38, 70, 71). In excision repair, single stranded regions of DNA containing pyrimidine dimers (70) and other types of structural damage such as crosslinking (51) are excised enzymatically. This is followed by non-conservative DNA repair replication and the rejoining of the newly synthesized DNA to the original strands (70). Mutants known as *uvr* are defective in the initial excision step, and are unable to excise pyrimidine dimers after ultraviolet light treatment. These *uvr* mutants also show a decrease in the number of plaques formed following infection by uv irradiated T1 bacteriophage and therefore lack the normal host cell reactivation of irradiated virus (39, 40). Excision defective strains also show an

increased frequency of uv induced mutation (35, 86) and are more sensitive to chemical mutagens (40), but show normal recombination repair.

Another class of mutants, the rec mutants (39) of E. coli are sensitive to both X-rays and ultraviolet light and are defective in recombination. The rec A mutants degrade their DNA after ultraviolet light irradiation (38, 40) and show complex survival curves (71). Rec A bacteria are very sensitive to low doses of uv light, while after higher doses, the slope of the survival curve is similar to wild type. Radman et al (71) have shown that recombination repair is the principal repair mechanism after low doses of uv, while excision repair predominates after higher doses where DNA synthesis is blocked. Thus, the recombination defective rec A mutants appear to be defective in recombination repair, but show normal excision repair. These two types of repair seem to occur at different times in the cell cycle (71); recombination repair occurs after and/or during post radiation replication while excision repair occurs prior to replication. Excision repair would be the repair process available following high doses of uv light where DNA synthesis is inhibited (71). These results also suggest that excision and recombination repair are independent repair mechanisms. Using radiation sensitive mutants defective in excision repair, Rupp and Howard Flanders have demonstrated

a kind of repair involving recombination which they called postreplication repair (74). These mutants can synthesize DNA after uv irradiation, and the newly synthesized DNA contains single strand breaks opposite each pyrimidine dimer present in the parental strands. Postreplication incubation of the cells after irradiation results in conversion of the low molecular weight DNA fragments into high molecular weight DNA characteristic of the unirradiated control by means of exchanges between sister DNA duplexes. This repair process reconstructs at least one undamaged copy of the genome. Smith and Meun (78) have shown that rec A mutants, incapable of postreplication repair, are defective in genetic recombination. Rec B and Rec C mutants which retain some capacity for genetic recombination show some postreplication repair.

Photoreactivation of lethality (22) and other uv endpoints (58, 63) have been demonstrated in yeast. Elkind and Sutton (22) have demonstrated a greater rate of photoreactivation for budding cells than for non-budding cells after exposure to uv light. Little, however, is known about the basis of repair at the DNA level in fungi including yeast since specific labelling of their DNA is difficult (85). There is evidence (66, 67) of dark repair of x-ray damage in interdivisional diploid yeast cells. Parry and Parry (65) have demonstrated that representative haploid mutants from 21 of the 22

complementation groups of their uv sensitive interdivisional cell mutants, fall into four categories on the basis of their response to dark repair conditions and dark repair followed by photoreactivation. These four groups are believed to represent gene defects at different points in a repair pathway. Indirect evidence that this repair is excision repair is provided by the cross sensitivity of these uvs mutants to nitrous acid and methyl methane sulfonate (89). Brendal et al (9) using radiation sensitive mutants have reported correlations between sensitivity to uv light and nitrogen mustard as well as between sensitivity to X-rays and methyl methane sulfonate. Excision repair is associated with damage by these mutagenic substances because excision of crosslinked bases of DNA (51) and repair replication have been shown to occur in E. coli which had been treated with methylating agents (14).

The relevance of these various repair mechanisms to the budding cell resistance of yeast is unclear. Budding yeast cells show both liquid holding recovery (53) and agar holding recovery (3) after exposure to X-rays and uv light respectively. Haploid X-irradiated budding yeast cells have been shown by Perper (68) to show greater colony formation when posttreated on growth media different from the one used for irradiation. Interdivisional cells show little or none of this recovery (68). Such recovery processes which appear with the onset of

budding suggest the presence of a cell cycle dependent control of a resistance and repair mechanism, and suggest that an investigation of the genetic basis of resistance of the budding cell to radiation might help to clarify its nature. The present study deals with the induction, isolation and characterization of mutants deficient in or devoid of budding cell resistance. The mutants obtained permit a formal genetic description of the budding cell resistance phenotype and provide a basis for speculation as to the actual mechanisms involved.

Materials and Methods

A. YEAST STRAINS

Table I lists strains utilized in these studies. Genetically marked strains obtained from Dr. Robert K. Mortimer were used directly or were employed to construct new strains for use in these investigations. Genetic markers are described by conventions adopted at the Yeast Genetics Conference 1961 at Carbondale except that locus and allele numbers are printed on the same line as the gene designation. Lower and upper case letters for a gene symbol indicate the mutant and wild type genotypes respectively.

B. MEDIA

YEPD

Dextrose, 1.0%; yeast extract, 0.5%; peptone, 0.35%, agar 2% (modified from 21).

Synthetic Complete (ISC)

Dextrose 2%, Difco yeast nitrogen base without amino acids, 0.67%; and the following additions: adenine, 20 mg/l; arginine, 20 mg/l; histidine, 20 mg/l; methionine, 20 mg/l; tryptophan, 20 mg/l; uracil, 20 mg/l; leucine, 30 mg/l; lysine, 30 mg/l; threonine, 100 mg/l. (72).

Omissions (SC-)

Synthetic complete minus one of the above additions: (72)

Minimal Medium (Min.)

Dextrose 2%, Difco yeast nitrogen base without amino acids, 0.67%, agar 2% (72).

Petite Medium

Glycerol 3%, dextrose 0.025%, yeast extract 0.5%, Bacto peptone 0.35% and agar 2%; used for scoring the petite phenotype (inability to utilize glycerol as a carbon source (modified from 21 and 72).

Presporulation Medium (GNA)

Dextrose, 5%; yeast extract, 1%; Bacto nutrient broth, 5%; agar 2% (72).

Sporulation Media

Potassium acetate, 2%; yeast extract, 0.25%; dextrose, 0.1%; adenine, 20 mg/l; agar 2% (27).

Buffer

G. solution: KH_2PO4 2.65 mgs/liter; K_2HPO_4, 5.32 gms/liter, and sterilized separately and added after sterilization 10 cc. of 0.2M $MgSO_4 \cdot 7H_2O$ (21).

Starvation Medium

Dextrose, 5%; in 0.05 M KH_2PO_4 with no fixed nitrogen source (21).

C. Radiation Sources

Cells were irradiated with X-rays from a beryllium window tube (Machlett OEG-60) at a dose rate of 56 KR/min (50 Kv, 40 mA). The X-ray tube was calibrated with the aid of an

ionization extrapolation chamber constructed according to the design of Mortimer by Prof. Edward Green of the Department of Physics of Brooklyn College (28). The UV source was a G. E. Germicidal lamp calibrated with the aid of a Laterjet dosimeter. The distance between the source and the agar surface of the irradiated petri plate was such that the dose rate was 60 ergs/mm^2/sec.

D. Radiation Studies

1 - Inactivation by X-rays

Three well established methods (7, 68, 73) were employed in the estimation of survival.

a. Replica plate tests to score for radiation sensitivity.

b. Macroscopic colony production on the agar surface of Petri dishes and

c. Microcolony production following the micromanipulative isolation of suitably treated yeast cell.

Method a:

The replica plating test for X-ray sensitivity was used for the isolation of radiation sensitive mutants, for genetic analysis of radiation sensitivity in spore cultures derived from dissected asci, and for studies of allelism or complementation among mutants.

Details of the procedure employed are as follows: Single

colonies from YEPD plates of the strains to be studied were picked and transferred with sterile toothpicks to fresh YEPD master plates to give 50 colonies per plate. Streaks of SC7K and SC6 were also included for controls. The YEPD plates were incubated overnight at 30°C and were then replica plated to three YEPD plates. For the production of X-ray sensitive mutants, a glycerol agar replica plate for petite detection was also included, since it was desirable to study X-ray sensitivity in the absence of a petite phenotype. The yeast populations on the master and replica plates at this time contained approximately 20% budding cells. One YEPD replica was exposed to 196 KR of X-rays, a dose sufficient to inactivate essentially all interdivisional cells, but leave approximately 1% surviving budding cells. The other replica was exposed to 56 KR, while the third YEPD replica was retained as a control. After 48 hours of incubation, the replica imprints were examined for the presence or absence of growth.

For the production of XS mutants, replica imprints of colonies showing growth after 56 KR but no growth after 196 KR were regarded as presumptive partially sensitive budding cell mutants. Replica imprints of colonies showing no growth after both 56 KR and 196 KR were regarded as mutants lacking any budding cell resistance. Such presumptive mutants were reisolated from the control plate and were retested for

sensitivity by the replica plating procedure and by survival curves.

For genetic analysis of radiation sensitivity ascospore cultures to be tested for radiation sensitivity were picked to YEPD master plates. These incubated spore cultures from dissected asci were incubated overnight, and then treated in the manner previously described. Routinely 2:2 segregation was found for radiation sensitivity. All streaks from radiation sensitive segregants showed no growth after 196 KR. Those streaks from segregants from crosses involving mutants lacking any budding cell resistance showed no growth after 56 KR as well.

For allelism studies, single diploid colonies were transferred from YEPD plates to fresh YEPD master plates and treated in the manner described above. After 48 hours of incubation, the replicas were scored for growth which would indicate resistance and therefore complementation and thus nonallelism. For the studies of allelism of mutants, the replica plating test procedure was employed as a qualitative screening method for complementation, and survival curves were routinely completed to verify the results of this test. For these studies, diploid strains, the survival curves of which show 68% or greater of normal resistance at 50% or 1% survival on YEPD, exhibited growth on replicas irradiated

for 196 KR indistinguishable from wildtype. Such strains were designated as having normal resistance, full complementation or nonallelism of mutants (see discussion section II). However, diploid strains which showed 55% - 60% of normal resistance at these levels of survival, routinely exhibited sparse growth after incubation of replica plates irradiated for 196 KR. (see discussion section II). These latter diploids showed growth indistinguishable from wild type after 56 KR. Such diploids were classified as sensitive to radiation, and showing only partial complementation.

Method b:

Details of the procedure employing the second method are as follows:

A colony from a three day old YEPD streak plate of the strain to be studied was suspended in 10 ml. of YEPD broth and aerated overnight at 30°C on a gyratory shaker. This growth procedure gave rise to a culture containing between 10^7-10^8 cells per ml. with approximately 30% budding cells. The cells were then pelleted by centrifugation, washed twice with sterile chilled G. solution, and then suspended in 5 ml. of G. solution. Whenever clumps were observed in the washed cells, they were removed by low speed centrifugation. For some irradiation experiments, homogenous preparations of interdivisional cells were obtained by the following procedures: cells from an

overnight YEPD broth culture were harvested, pelleted by centrifugation, washed three times, then resuspended in 10 ml. of starvation medium and aerated for 48 hours at 30°C in order to reduce the budding cell fraction. After starvation, the cells were pelleted, washed three times, resuspended in 5 ml. of G. solution in a 15 ml. sterile glass centrifuge tube and centrifuged at 700 RPM for approximately 2-3 minutes. The necessary duration of low speed centrifugation was found to differ for each yeast strain under study. After suitable low speed centrifugation, a sample of the turbid supernate was microscopically examined for budding cells. If none were visible, the top three ml. of supernate was carefully pipetted into a sterile glass test tube. This suspension of interdivisional cells was used for experiments. This centrifugation procedure routinely results in a culture containing approximately 10^7 interdivisional cells/ml. with less than 0.5% budding cells.

A relatively pure population of budding cells was obtained in the following manner: an interdivisional cell suspension prepared as described above was pelleted and suspended in 10 ml. of YEPD broth and placed on a gyratory shaker at 30°C. Aliquots were removed after two hours, and at one half-hour intervals thereafter, and microscopically examined for the presence of budding cells. No cells having more than one bud

were observed. Thus all budded cells in these preparations arose from the formation by interdivisional cells of the first budding cells in 70-75% synchrony. At this time incubation was ended. The cells were pelleted by centrifugation, washed once in chilled G. solution and resuspended in 5 ml. of chilled G. solution. Cell density was in all cases determined with a hemocytometer; estimation of the percentage of budding cells was also routinely made. Routinely 20-30% budding cells for all strains from log phase cultures (overnight cultures) studied was observed. Synchronous cultures showed 75% budding cells. One tenth ml. aliquots of appropriate dilutions of the cells were then spread on agar in petri dishes and the dishes irradiated with the covers removed. After incubation at 30°C for 5 days to permit colony formation by all survivors, survival ratios were determined by comparing the number of colonies on irradiated and unirradiated plates.

The concept of the dose modifying factor (DMF), common to radiobiology, was employed in this study to aid interpretation of alteration of budding cell resistance in survival curves of sensitive mutants and mutant combinations in haploid and diploid state when compared to wild type. Experimental conditions often enhance or diminish the effectiveness of radiation. A DMF is a number which equates experimental and control doses of equal effectiveness. In the case of X-ray

sensitive mutants and mutant combinations, the DMF may be regarded as the fraction remaining of budding cell resistance function when compared to wild type. For example, the LD1 (dose producing 1% survival) for SC7K is 187 KR and that for the mutant XS9 is 131 KR. The DMF is 131/187 or 0.7 (see Figure 1).

Method c:

Micromanipulative studies were carried out as follows:

A loopful of cells either from a culture, or from a suspension in which experimental treatment had been conducted, was streaked on a YEPD agar slab which was placed on a dissection chamber. If the cells were to be irradiated, the chamber was located under the source with the agar surface facing the radiation. After exposure, the coverslip was inverted on the chamber and budded or non-budded cells isolated with the micromanipulator. The streak was then cut off, discarded, and the agar slab with 50 to 100 isolated cells in rows was then transferred to a plate of YEPD agar, which was then incubated for 48 hours. A cell giving rise to a colony of a diameter of 1 mm. or more was considered a survivor.

2. Non-Lethal Endpoints

In addition to inactivation, a non-lethal X-ray effect was also studied. This was the induction of recessive homozygosis for heterozygous input markers.

Recessive homozygosis was studied in clones arising from diploid cells irradiated as heterozygotes as reported by Hurst and Fogel (23). Replica plating of colonies from control and irradiated plates on to media deficient for a single nutrilite would indicate recessive homozygosis by the absence of growth on this media.

3. Production of XS Mutants

Cells of SC7K or culture SC7K-4D were streaked on a fresh YEPD plate. After two days growth, a single colony was suspended in 10 ml. of YEPD broth and aerated overnight at 30°C. The cells were then harvested, washed once by centrifugation and suspended in 10 ml. of starvation medium and grown on a shaker for 3-5 days. The procedure employed was a modification of that used by Elkind and Beam (21). This treatment was undertaken to reduce the budding cell fraction which might be expected to be resistant to mutagenesis and to impede detection of mutants by adding uselessly to the non-mutant population. After starvation, cells were pelleted by centrifugation, washed twice in chilled G. solution, diluted, plated on YEPD and exposed to sufficient UV to give 50% survivors, as estimated by the macrocolony procedure. After five days incubation at 30°C, colonies were transferred with sterile toothpicks to YEPD master plates to give 50 colonies per plate. Streaks of SC7K and SC6 were included for controls.

The YEPD master plates were then treated in the manner previously described for replica plate testing for X-ray sensitivity. (see section D-1).

E. Genetic Analysis

1. Genetic Properties of Mutants

Methods of mating, sporulating, and genetic analysis of yeast followed standard procedures which have been detailed elsewhere (73).

Mating of haploid strains was accomplished in either one of two fashions. In one case, the multiply marked parent haploid strains were mass-mated on YEPD agar. After 24 hours incubation, the cross was replica plated to minimal agar to select for growth of prototrophic heterozygote diploids. The diploid isolate was straked on to minimal agar to isolate single colonies. After 72 hours, single colonies were picked and streaked on to YEPD.

Mating was also accomplished by mixing freshly grown cultures of haploids of opposite mating type in YEPD broth. After approximately four hours, the zygotes were isolated by micromanipulation on YEPD. After 48 hours, the diploid colonies were picked and streaked on to fresh YEPD agar.

Presporulation was accomplished by transferring diploid colonies after 48 hours growth on a GNA plate to GNA master plate for overnight incubation. These were then replica

plated to petri dishes of sporulation medium which were incubated for three days, and then examined for asci. Sporulated cultures were suspended in a 1:6 dilution of snail enzyme (68) for five minutes and then diluted tenfold with sterile distilled water. After enzyme digestion of the ascus walls, the spore tetrads were dissected by the microdissection technique of Johnston and Mortimer (44).

Genetic analysis of tetrads for gene-gene linkage and gene centromere linkage was accomplished as follows: in a cross AB x a b the following ascus types can be distinguished: parental ditype (PD) AB AB ab ab, non parental ditype (NPD) Ab Ab aB aB and tetratype (T) AB ab aB ab. Gene-gene linkage is indicated by a PD: NPD ratio significantly greater than one. The second division segregation is determined relative to known centromere linked genes. Centromere linkage of a gene is indicated by a second division segregation frequency significantly less than 2/3. (32,56). Genetic analysis of radiation sensitivity was conducted as previously described. (See Section D-1).

2. Allelism and Sporulation of Mutants

For allelism tests, zygotes heterozygous in repulsion for two sensitivity genes were constructed as follows: the multiply marked single mutant haploid parent strains were mass mated on YEPD agar. After 24 hours incubation, the cross was replica

plated to minimal agar to select for growth of prototrophic heterozygote diploids. The diploid isolate was streaked on to minimal agar to isolate single colonies. After 72 hours, single colonies were picked and streaked on to YEPD agar for single colony isolation. Single colonies were then picked and transferred with a sterile toothpick to fresh YEPD master plates which were then incubated overnight at 30°C and tested in the manner previously described. (See section D-1).

As a further test of allelism to determine whether mutants recombine with one another, diploids of all mutant pairs were sporulated, dissected and the segregants of those asci showing survival of all 4 spores were tested for resistance. A single meiotic recombinational event placing the two different mutant genes in one genome should generate a complimentary wild type genome in one spore of the ascus.

3. Meiotic Recombination and Linkage Studies

Sporulation and dissection of diploids multiply heterozygous for standard nutritional markers, but in addition homozygous or heterozygous for sensitivity mutants were also performed to determine if XS genes influence meiosis and meiotic recombination.

To test for linkage between mutants, the segregants of several asci showing survival of all 4 spores were tested for resistance in the manner previously described. A PD ascus

would be expected to generate 4 sensitive ascospore cultures. An NPD ascus would generate two resistant ascospore cultures and two doubly mutant sensitive spore cultures. A T class ascus would produce one resistant spore culture and three sensitive cultures, one of which is a double mutant.

An examination after 48 hours of the irradiated spore culture replicas from the mutant x mutant intercrosses for the presence or absence of growth permitted a determination of the number of PD, NPD, and T asci. A PD: NPD ratio significantly greater than one indicates linkage. (table 5).

4. Double and Triple Mutant Hpaloid and Homozygous Diploid Studies

The NPD spore cultures were used to determine the survival to X-rays of haploid double mutants. In order to select for a triple mutant composed of the three class II mutants, the double mutant XS6 XS8 was crossed to the single mutant XS9. The diploid was sporulated, dissected and replicas of the spore cultures showing survival of all four spores were exposed to 224 KR to select for tetratype asci which would show one wild type spore, 2 parental spores, and the recombinant triple mutant. Replicas of the spore cultures of a tetratype ascus were then irradiated at a series of doses in order to select for the triple mutant class. Exposure of a replica to a dose of 74 KR was found to be sufficient to distinguish between

growth for parental and wild type and the absence of growth for the triple mutant. The triple mutant was isolated and streaked onto YEPD agar and retained for future use. A triple mutant diploid homozygote was isolated by mass mating of two triple mutant haploid strains on YEPD. The cross was replica plated to minimal agar to isolate the prototrophic heterozygote diploid. The diploid was streaked to minimal agar to isolate single colonies. After 72 hours, single colonies were picked and streaked to YEPD.

Results

MUTANTS SENSITIVE TO X-RAYS

A. Isolation of Mutants

Five X-ray sensitive (XS) mutants were isolated from approximately 10,000 colonies tested after UV treatment of starved cells of SC7K (x) or SC7K-4D tryl his2. The sensitive strains were called XS5, XS6, XS7, XS8, and XS9 in accordance with the designation of radiation sensitive mutant strains used by Resnick (72, 73). X^S1 was graciously donated by Nakai (60). The X-ray sensitive strains of other authors (8, 72) were not sought for this study since the effects of their mutational alterations on budding cell sensitivity is unclear. These mutants fall into two phenotype classes: class 1, in which budding cells lack any normal resistance and show exponential survival to ionizing radiation (X^S1 and XS5), and class II, in which the budding cell resistance is detectable but subnormal (see table 2). These mutants represent five distinct genetic loci (see allelism of mutants below).

B. Genetic Analysis

1. Genetic Properties of Mutants

To determine the genetic nature of these mutants, they were crossed with genetically marked resistant strains, the diploids sporulated, dissected and the spore clones were analyzed for segregation of radiation sensitivity and nutritional

markers. In all of these crosses 2:2 segregation for all traits was observed. The analyses of the asci from these crosses are given in table 3. None of the mutants was centromere linked inasmuch as second division segregation frequencies were not significantly less than 2/3 (table 4). None of the XS mutants were found to be linked to any other marker used in these studies.

2. Behavior of Heterozygotes

Diploids heterozygous for two mutant alleles were constructed by means of pairwise crosses in all possible combinations of all class I and class II mutants on YEPD agar followed by prototrophic diploid isolation on minimal agar. These heterozygous diploids were then tested for sporulation on acetate medium. All heterozygous mutant A x mutant B crosses except for XS6/XS7 showed good sporulation. No sporulation was found for the homozygous crosses X^S1/X^S1, XS6/XS6, XS7/XS7 and XS9/XS9 (table 5). Lack of sporulation in homozygous XS strains has been reported for X^S1 by Nakai and Matsumoto (60) and for the XS3 mutants isolated by Resnick (72). The XS1 strain of Resnick produces inviable spores. The observed lack of sporulation in the doubly heterozygous diploid XS6/XS7 suggests that XS6 and XS7 may represent either independent occurrences of the same mutation, or may be closely linked genes which have the same effect on the phenotypes of

budding cell resistance and sporulation. It is difficult to distinguish between these two alternatives. The lack of sporulation observed in homozygous mutants suggests that some genes affecting X-ray sensitivity are also essential to meiosis. This effect on meiosis like that upon X-ray sensitivity is recessive, since sporulation was found in all XS/+ strains.

3. Linkage of Mutants

Analysis of the frequency of PD, NPD and T asci, among the segregants of diploids derived from mutant x mutant crosses tested for sensitivity suggest linkage for the class I mutant XS5 and the class II mutant XS6 (see table 5). All other class I x class I, class I x class II, and class II x class II mutants when crossed together segregate independently (table 5).

4. Meiotic recombination and radiation sensitivity

The possible relationship between radiation sensitivity and defects in meiotic recombination was examined in wildtype and in the respective sporulating class I/class I and class II/class II homozygous mutant diploids XS5/XS5 and XS8/XS8. Meiotic recombination frequency was scored for the two linked markers leu 1 and try 5. Normal linkage for these genes was observed in the class I and class II homozygous single mutant diploid strains (see table 6). These data suggest that genes involved in budding cell resistance are not involved in meiotic genetic recombination.

5. Allelism of Mutants

The radiation sensitivity of the budding cells of diploids derived from the above pairwise crosses was examined to determine if any two mutants were at the same locus. This question was first studied by the replica plating test for sensitivity. In table 7, it can be seen that diploids homozygous for sensitivity exhibit no growth 48 hours after exposure to 196 KR, a dose chosen because it is sufficient to inactivate the budding cells of all mutants. In contrast, heavy growth was found on similarly exposed replica imprints of diploids composed of wildtype and any class II mutant, or of wildtype and the class I mutant XS5. The diploid composed of wildtype and the class I mutant $X^{S}1$ showed detectably less growth (see X-ray survival results Section C). Allelism, detected by the absence of growth on x-rayed imprints of mutant A x mutant B diploids was observed only for XS6 and XS7. Replica imprints of the diploid containing the two class I mutants $X^{S}1$ and XS5, after exposure to 196KR show only isolated colonies after 72 hours indicating only partial complementation. Confluent growth indistinguishable from that observed on imprints of wildtype cells was found after exposure to 56 KR, a dose from which each of the parent haploid strains show less than 10-3 survival (see table 7). A similar response is observed after exposure of replica imprints

of the X^S1/wildtype diploid to 56 KR. (see X-ray survival results below Section C).

As a second test for allelism, the ascospore cultures showing growth of all 4 spores were tested to determine whether the mutants were separable by recombination, that is, for the presence of at least one wildtype resistant segregant. At least one such spore culture was found in the asci tested from crosses from all heterozygous double mutant diploids, again with the exception of XS6/XS7 which failed to sporulate (table 5).

In addition, as a third test of allelism, the frequency of microcolony formation was determined for irradiated micromanipulated budding cells of wildtype SC6 and the following four heterozygous double mutant diploids exposed to 56 KR: X^S1/XS5, XS1/XS8, XS5/XS8 and XS6/XS9. The observed microcolony frequencies suggest the complementary resistant behavior of XS5/XS8, and XS6/XS9 budding cells and reveals the partial complementation occurring in budding cells derived from X^S1/XS5 or X^S1/XS8 heterozygous diploids (table 8). (see discussion). These allele tests collectively indicate that a minimum of five genetic loci are concerned with budding cell resistance (tables 5 and 7). Allelism will also be considered below when survival curves of various combinations of mutants are compared.

C. Inactivation by X-rays

1. Single Mutant Haploid and Diploid Studies

X-ray survival curves of the mutants and of wildtype are given in Figure 1. It can be seen that at low doses, survival of mutants is similar to that of wildtype indicating that the haploid interdivisional cell sensitivity is not markedly affected by the mutations. (Also see table 2). The budding cell component of the mutant survival curve is, however, reduced or absent. Since the frequency of budding cells in wildtype and all mutant cultures was 20% to 30%, which was sufficient to provide a conspicuous resistant component to a survival curve of the wildtype SC7K (figure 1), the mutant phenotypes must result from modification of genes controlling the X-ray resistance that appears during budding. These radiation survival data and the genetic data given above show that a single gene mutation can abolish budding cell resistance rendering the budding cell as sensitive as an interdivisional one (X^s1, XS5, see fig. 1) or subnormal in resistance (XS6, XS8, XS9 see fig. 1 - see also table 2).

The concept of the dose modifying factor (DMF), has been employed in this study to aid interpretation of alteration of budding cell resistance in sensitive mutants. As described in Materials and Methods, in the case of X-ray sensitive mutants, the DMF can be regarded as the fraction remaining of normal budding cell resistance. The tables inserted in

figure 1 and 2 give DMFS of one or more indicated survival ratios and the X-ray exposures required to produce them. For example, the LD1 (dose producing 1% survival) for SC7K is 187 KR, and that for XS9 is 131 KR. The DMF is 131/187 or 0.7. In these survival curves DMF fluctuates somewhat as a function of survival and dose, probably due to alterated inactivation kinetics in the mutant. However, the order among the mutants remains the same regardless of dose and serves as a means to express the severity of the mutational lesions. (see figure 1 and 2). In these terms, the class II mutants appear to differ in the degree of remaining function conferring budding cell resistance. For example, at LD1.0 the DMF ranges from 0.70 for XS9, to 0.48 for XS8 to about 0.39 for XS6. The class I mutant budding cells possess a minimal 0.10 of normal budding cell resistance. This number is no different than the DMF for wildtype interdivisional cells, (fig. 12 and table 2), and means that class I mutants show none of the resistance characteristic of normal budding cells.

The effect of the mutant genes upon diploid interdivisional cell sensitivity can be observed by comparing DMFs at 50% survival (fig. 2). These DMF values are reasonably representative of interdivisional cell survival, although budding cells which comprise 20-30% of the population contribute somewhat to overall survival, and the contribution is greater in those

populations in which they are more resistant. As the table in Fig. 2 shows, the sensitivity of diploid interdivisional cells of homozygous mutants representative of all classes is affected by the mutant genes. When the DMFs are presented in order of decreasing percentage of wildtype resistance, the values of 80%, 50%, 26%, 0.08% and 0.10% are found for XS6, XS9, XS8, XS5, and X^s1, respectively. The homozygous class I mutant possesses a sensitivity close to that of a haploid interdivisional cell. Thus class I mutants represent total blockage of both resistance conferred by the budding of a haploid interdivisional cell and that provided normally by the diploid condition (fig. 2). It should be noted that none of the mutant genes sensitizes haploid interdivisional cells.

A similar consideration for interdivisional cells of heterozygous single and double mutants representing all class combinations is presented in the tables in figures 3, 4, 5, 6 and 7. It can be seen that the DMFs at 50% survival for wild-type/mutant diploids and mutant/mutant heterozygous diploids does not differ significantly from unity. Therefore, the mutant genes are recessive in their expression in interdivisional cells, and complementation in interdivisional cells appears to be complete.

Comparison of the DMFs of budding cells of homozygous diploids and of haploids at 1% survival (fig. 2) reveals a

degree of consistency in the functional ordering of the mutants. With the exception of close similarities in DMF of XS6 and XS8 in either haploid or diploid state, the order of effectiveness (DMF) of mutant genes in budding cells is essentially independent of whether haploid or diploid. More significant is the substantial lowering of XS8 and XS9 DMFs when the lesions are in the homozygous diploid condition. For instance, XS9 has 70% of wild type resistance in the haploid state, but only 42% in the homozygous diploid condition. XS8 is about 50% as effective in the homozygous diploid as in the haploid. The similar response of the XS6 mutation in the haploid and diploid states is considered in the next section. In budding cells, regardless of ploidy, XS9 appears to be the least damaged in ability to sustain wild type function. The class I mutants $X^{8}1$ or XS5, deplete budding cell resistance to approximately the haploid interdivisional cell level. (see figures 1 and 12.)

Consideration of the relationship, previously reported by Resnick, between haploid budding cell resistance and the diploid interdivisional cell shoulder (72, 73) shows that in homozygous diploids, with the exception of XS6, the sensitivity of haploid budding cells is paralleled by the sensitivity of the diploid interdivisional cells. The diploid interdivisional cell shoulder is unaffected by mutation of the XS6 gene, but

is reduced when either XS8 or XS9 is present in the homozygous condition. The function of mutant XS6 genes in interdivisional diploid cell resistance may be correlated with the similarity of DMF at 1% survival in the budding cell region of the survival curves of a haploid and a homozygous diploid. Evidence will be presented later to show that a normal XS6 gene is required for the budding cell shoulder.

The mutant genes have also been studied in diploids in which thegenes are present in single and double heterozygotes including all class I . class I combinations. These results are presented in figures 3 - 7 and the accompanying tables. It can be seen that a DMF of 70-90% is observed for all diploids except for wild type $X^S/1$, $X^S1/XS5$ and all X^S1/class II diploids. Except for diploids bearing X^S1, complementation is approximately complete. The reasons for the DMF of 70-90% will be considered in the discussion of results. The budding cells of all X^S1 heterozygotes including $X^S1/XS5$ show about 55-60% of normal resistance. This incomplete complementation and incomplete dominance of the X^S1 locus may indicate either a gene dosage effect at the X^S1 locus, or that the active product of the normal X^S1 gene may be an aggregate molecule composed of two identical subunits. Two lines of evidence suggest that the two class I mutants, X^S1 and XS5 are not equivalent, and that a gene dosage effect may be present at

the X^S1 locus. Firstly, the XS5 heterozygote has a higher DMF than the X^S1 heterozygote (DMF of 0.70-0.90 versus DMF of 0.50). Secondly, the X^S1 single mutant heterozygote shows the same DMF as the XS1/XS5 double mutant heterozygote. A X^S1 single mutant ($X^S1/+$), or double mutant heterozygous diploid (X^S1/XS5) having only one of the normal complementary genes for this locus would be expected to show only 50% of remaining wild type function (DMF of 0.50-0.55). Thus, normal XS5 function, in the absence of the other class I mutant gene X^S1, is dose dependent in the heterozygous or homozygous diploid state. However, normal XS5 function is masked by the presence of a dosage effect at the X^S1 locus in the single mutant and double mutant heterozygous diploid X^S1/XS5. Therefore, X^S1 function is independent of the number of XS5 genes present; XS5 in contrast is strongly affected by the state of X^S1.

Multiple Mutant Studies

In order to clarify the roles of the various loci to the phenotype of budding cell resistance, it seemed essential to observe the effects of various gene combinations in a single haploid genome. Such strains were constructed as described in the Materials and Methods and were analyzed in the same fashion as indicated above. The results are presented in figures 8, 9 and 10, and the associated tables. Firstly, in the case of the class I mutants, $X^{s}1$ and XS5, neither their presence together in a single haploid genome, nor the addition to a single class I mutant of a class II mutation increases radiation sensitivity beyond that of class I single mutant. None of these strains is significantly more or less sensitive than a haploid interdivisional cell. Thus, a class I mutant would appear, at least in a haploid genome, to represent a block before or behind which no associated genetic competence or deficiency has access to the phenotype.

The class II mutants behave in an entirely different fashion. To recapitulate: in homozygous condition they represent different degrees of budding and interdivisional cell resistance; in heterozygous condition, all diploids show wild-type interdivisional cell responses. When class II mutants are placed together in a haploid nucleus, budding cell resistance is always reduced, while the interdivisional cells

show wild type sensitivity (fig. 10). Budding cell survivals of these class II . class II haploid double mutants are as follows: Type A (XS8 XS9) shows a shoulder similar to wild type budding cells but much reduced, which is followed quickly by a limiting exponential at higher doses. Type B (XS6 and XS6 XS8) lacks any budding shoulder and is exponential throughout. The limiting exponential survival component of XS8 XS9 is parallel to the survival curve of XS6 XS9. These results suggest that a normal XS6 gene is responsible for the budding cell shoulder. The average DMF of 0.23 for the three class II double mutant haploids is about half of the observed average DMF for the three single class II mutants at this survival. Addition of the second class II lesion results in a loss in an additive fashion of approximately 75% of wild type resistance.

The greatest reduction in budding cell resistance is found in the triple class II mutant composed of XS6, XS8 and XS9 in a single haploid genome. In figure 12, it can be seen that its budding cell survival is indistinguishable from that of a class I mutant budding cell, or an interdivisional cell; i.e. there is no budding cell resistance. The fact that the presence in a haploid of either a class I mutant, or all three class II mutations abolishes budding cell resistance suggests that these genes may represent the total genetic control of the phenotype. *

* We are aware of the improbability of finding in six mutants modifications of the five genes relevant to a phenotype.

In order to further define the roles of the X^s1 and XS5 genes, a mutant diploid homozygous for the three class II mutations XS6, XS8 and XS9 was synthesized and studied (see figure 12). The DMF of this triple mutant homozygote in the interdivisional cell region is 0.25, while the DMF for budding cells is 0.18. Thus, the normal X^s1 and XS5 genes together account for about 25% of normal diploid interdivisional cell resistance. The remaining 75% of this resistance may be attributable to the combined action of the normal class II genes. As observed with the triple class II haploid mutant, no additional resistance is conferred by budding. Thus, regardless of ploidy, functional class II genes are necessary for budding cell resistance.

The relationship between mutant budding cell sensitivity, and the haploid low dose shoulder has been mentioned. With the exception of the mutant homozygote XS6/XS6 which shows a DMF of 0.80, the more sensitive the budding cells are to X-rays, the lower is the interdivisional cell resistance (DMF_{50}). Therefore, the budding cell sensitivity genes which exert a major effect on the diploid shoulder include X^s1, XS5, XS8 and XS9. XS6 exerts only a minor influence on the diploid shoulder. In order to more clearly illustrate the role of class II genes on this relationship, class II double mutant diploids homozygous for either XS6 and XS8, XS6 and XS9, or

XS8 and XS9 were synthesized, irradiated, and the DMFs at 50% and 1% survival were computed and compared with those observed for the single class II mutant homozygotes. A 20% reduction in interdivisional diploid shoulder occurs when the XS9 gene is added in homozygous condition to the XS6 homozygote (see figure 11). The fact that the XS6 XS9/XS6 XS9 double homozygote is more sensitive than the XS6 XS8/XS6 XS8 and XS8 XS9/XS8 XS9 diploids suggests that the normal XS8 gene may be epistatic to the normal XS6 and XS9 genes (see figures 2 and 11). The XS8 XS9/XS8 XS9 mutant diploid shows a DMF at 50% survival for interdivisional cells of 1.00 and a DMF at 1% survival for budding cells of 0.57. (fig. 11) The shoulder observed might be expected to represent mainly budding cell resistance since the only normal class II gene present is XS6 which is responsible for the budding shoulder. To test this possibility the X-ray survival of individually isolated budding and interdivisional cells of the XS8 XS9/XS8 XS9 diploid homozygote was determined. Survival of budded cells exposed to 9.3 Kr is 47/50 or 0.94 which is close to wild type budding cell survival while that of interdivisional cells is only 16/50 or 0.32. After 56 Kr, the budded cell survival is 16/50 or 0.32, while that of interdivisional cells is 0.50. As a control, wildtype SC6 interdivisional cells similarly treated show a survival after 56 Kr of 0.30-0.50.

It should be noted that the observed budding cell survivals after 9.3 KR and 56 KR for the XS8 XS9/XS8 XS9 diploid are in good agreement with the macrocolony survival for this strain after these doses of radiation (see figure 11). Thus, it would appear that the interdivisional cells of this mutant are too sensitive to contribute to the low dose shoulder observed. Additional considerations of the survival kinetics of these strains may be found in the sections of the Discussion dealing with organization of pathways of radiation resistance.

Medium Stimulated Repair in Wildtype and Mutants

Medium stimulated repair, defined by Perper and Beam (69) as higher survival when cells are plated and irradiated on medium different than used for preirradiation growth, was studied by plating appropriate dilutions of a washed overnight YEPD broth culture of wild type, class I and class II mutants on YEPD agar and on minimal agar followed by irradiation at a series of doses. Medium stimulated repair was studied by taking the DMF at 1% survival from survival curves of cells irradiated on YEPD and on minimal medium for wild type and all mutants. The class II mutants respond to about the same extent as wild type, while the class I mutants show no detectable increase in survival (see figures 13 and 14).

Introduction of Non Lethal Damage in Wildtype and Mutant Strains

Perper (68) and Perper and Beam (69) utilizing the diploid Z2367 which is multiply marked on the right arm of chromosome V have shown that budding cell resistance to inactivation is accompanied by a resistance to several non lethal effects. It was considered pertinent to determine the effects of the mutations conferring sensitivity to inactivation on the susceptibility of budding cells of these strains to inducted recessive homozygosis at several loci. This non lethal endpoint was studied with synchronous preparations of interdivisional and singly budded cells of wildtype, the class I mutant

homozygote XS5/XS5, and the class II mutant homozygote XS8/XS8, exposed to an X-ray dose sufficient to give approximately 40% survival. The XS5 and XS8 mutants were employed since only these two mutants retain ability to sporulate when homozygous, and would most closely resemble wild type in genetic background.

The results given in tables 10 and 11 show that budding cells of the XS5 and XS8 homozygotes respond differently to radiation produced homozygosis. In the XS5 diploid, in which budding and interdivisional cells show the same sensitivity to inactivation, homozygosis is inducted at the same frequency in both types of cells. Therefore, budding confers resistance to neither inactivation nor homozygosis. In the case of XS8, the budding cells of which are substantially devoid of resistance to inactivation, the incidence of homozygosity in budding cells is that observed in wild type budding cells. Therefore, the budding cells in this strain are resistant to homozygosis, but not to inactivation.

To summarize, in the case of XS5, inactivation and homozygosis are both affected by the mutation. XS8 appears to affect inactivation only.

Discussion

1. General consideration of radiation resistance

Genetic control in yeast of interdivisional cell resistance to UV has been thoroughly explored by Cox and Parry (14) and others (3, 60, 71, 72). The investigations in the present study establish that X-ray resistance conferred by budding is also under genetic control, and has the following properties: Mutations conferring sensitivity may reduce budding cell resistance to the level of an interdivisional cell (class I), or may produce subnormal resistance (class II). These mutations may be regarded as resulting in a loss of some functions responsible for normal resistance. The presence of either a single class I mutation, or all three class II lesions in a single genome reduces resistance to the level of an interdivisional cell. With the exception of XS6, the more sensitive the class II haploid budding cells are, the more sensitive are the corresponding homozygous diploid interdivisional cells. Thus, genes responsible for sensitivity in budding cells are effective in diploid interdivisional cells. Presumably, these genetic lesions are similar to mutations conferring sensitivity in other organisms. With respect to survival kinetics after radiation, and the genetic control of resistance to inactivation, the following similarities in yeast and other fungi are found. Wright and

Pateman (88) foreshadowed our finding of two phenotypically different classes of sensitive budding cell mutants by their isolation of two such phenotypically different groups of uvs mutants in Aspergillus nidulans. One group shows exponential survival to ultraviolet light and nitrous acid, while survival curves of the second group show the wild type multihit character, but with subnormal resistance to these two agents. Genetic control of radiation resistance in other eukaryotes is exemplified by the isolation of sensitive mutants in Neurospora (75), Ustilago (37), and yeast (3, 15, 72, 79). Generally, these studies are consistent with the results of the present investigation. All show that the property of resistance is controlled by gene products. The mutant alleles of these genes are recessive, and these alleles complement when crossed one with the other to give a heterozygous diploid with wild type resistance.

Regarding class I mutants, two lines of evidence suggest that the class I mutant XS5 is different from the phenotypically similar X^s1 of Nakai and Matsumoto (61), and XS1 of Resnick (72). XS5 segregates independently from X^s1 when crossed with it. XS1 was not available for similar testing. The XS5/XS5 diploid, however, produces viable ascospores when sporulated, where XS1/XS1 shows no spore viability. Thus, these two genes are functionally separable. Consideration of

the roles of mutants and their functional organization will be presented in Section II.

It was argued in the Introduction, that repair and its control is the most plausible basis for budding cell resistance. One type of dark repair of X-ray damage, called liquid holding recovery, is well established in yeast. Patrick and Haynes (66, 67) have shown liquid holding recovery in diploid but not in haploid interdivisional cells after exposure to X-rays. Parry and Parry (65) have demonstrated differing responses to liquid holding recovery of the uvs mutants of Cox and Parry.

Other treatments, which delay the first postirradiation division, produce similar perhaps related effects. James and Werner (43) have shown that prolongation of the interval between X-irradiation and the first division by treatment of X-irradiated diploid interdivisional cells with beta-mercaptoethanal resulted in reduction of several kinds of damage (inactivation, doublet formation, abortive colony formation and lethal sectoring). Haploid interdivisional cells when similarly treated were unaffected. Repair by budding cells of lethal X-ray damage by plating of irradiated cells on agar medium other than that used for preirradiation growth has been observed by Perper (68). This effect was called medium stimulated repair and was observed for diploid and haploid budding cells and to a lesser extent for diploid, but not haploid

interdivisional cells. Averbeck et al (3) have similarly demonstrated that wild type yeast and their haploid r mutants which are sensitive to uv and X-rays are capable of post-irradiation recovery on agar from uv damage. It should be noted that this agar holding recovery and the medium stimulated repair reported by Perper (68) are restricted to the high dose range (survival 1% or lower) where survival of a non-synchronous population is due mainly to budding cells. These effects are not found after low doses of radiation which permit a higher fraction of interdivisional cell survival. These haploid r mutants like our class II XS mutants, show the same degree of repair as wild type cells. Additional evidence of repair by budding cells is the following: Langguth (53) has shown that synchronous populations of haploid yeast cells show liquid holding recovery after X-irradiation during the budding portion of the cell cycle. Interdivisional cells lack such recovery. Moustacchi and Enteric (58) have demonstrated that in a nonsynchronized population, budding cells of haploid wild type and the uvs 1-3 mutant which are more resistant to the immediate lethal effect of uv light have greater ability to repair lethal damage during dark liquid holding than non-budded cells. The interdivisional cells of the uvs1-3 mutant show a decrease in survival after dark holding, while the budding cells show repair. The presence of caffeine during

dark holding reduces primarily the dark recovery of haploid interdivisional cells. Caffeine only slightly affects dark holding recovery of lethal uv damage in budding cells, or in diploid interdivisional cells. These data strongly suggest that budding and haploid interdivisional cell resistance to uv are mediated by different cell processes, and are consistent with repair being the basis of budding cell resistance. These data are also consistent with the observed relationship between budding cell resistance, and the resistance of the diploid low dose shoulder to X-rays.

If repair is the basis of budding cell resistance, the question arises as to the nature of the repair, and of the defects which confer sensitivity to the mutant strains. One could suppose that recombination repair, excision repair, or both might be involved. Recombination repair may be excluded since XS5/XS5 and XS8/XS8 show normal meiotic recombination for the linked markers try 5 and leu 1. Also XS5/wild type diploids do not show enhanced recombination as a result of medium stimulated repair (68). If excision repair were important, then the defect conferring sensitivity would be due to defective excision of damaged DNA, defective DNA repair synthesis, or a defect in the rejoining of newly synthesized DNA to the old parental strand. A defect in the excision process, such that damaged regions of DNA are not excised

does seem implausible on the basis of considerable work with bacteria. McGrath and Williams (54) have demonstrated that X-rays produce single strand breaks in E. coli DNA. Kaplan (46) has shown also in E. coli that ionizing radiation produces single as well as double strand scissions. Presumably, then after ionizing radiation no endonuclease activity is required to introduce a nick into damaged DNA. With uv, on the other hand, Kaplan et al (47) and Grossman et al (29) have isolated from Michroccocus lysodeikticus, and characterized an endonuclease responsible for the introduction of single strand breaks into uv irradiated DNA as well as an exonuclease which excises photoproducts from DNA. Secondly, with respect to excision of damaged DNA, Fangman and Russl (26) have isolated an X-ray sensitive mutant of E. coli which shows excessive DNA degredation after X-irradiation, but which lacks repair DNA synthesis. Single strand breaks introduced into the DNA of this strain are not repaired. Trigocervic and Kucan (8) have demonstrated a correlation between radiation sensitivity of E. coli mutant strains, and DNA degradation after X-rays. In all cases high sensitivity to inactivation was positively correlated with a high degree of DNA breakdown. If the ultimate fate of an irradiated cell depends on whether the induction of damage is followed by excessive DNA degradation or repair, then the relative activities of the enzymes

responsible for mediating these processes would determine the sensitivity to radiation. DNA polymerase has been proposed by a number of investigators as responsible for the DNA repair resynthesis stages in the repair process (45, 48, 49, 55, 87). Monk et al (55) have studied the repair of lethal damage induced by uv in pol A1 mutants of E. coli, which lack DNA polymerase. The uv sensitivity of these strains is regarded as due to a reduction in excision repair, since the pol A1 uvr A6 double mutant is only slightly more sensitive than the excision defective uvr A6 mutant itself. Repair in these strains is mediated by the rec repair genes. Kato and Kondo (48) have shown that their polymerase deficient strains of E. coli designated res- are also sensitive to X-rays. After X-rays these strains show no repair of single strand breaks and show extensive DNA breakdown. The authors proposed that the res genes were necessary for DNA repair resynthesis. Kelly et al (49) have demonstrated that DNA polymerase is responsible for excision as well as repair synthesis in vitro in uv irradiated E. coli. Other models for the defect in polymerase deficient strains have been proposed. Kanner and Hanawalt (45) have observed that the polymerase deficient mutant of E. coli excises dimers, and produces a DNA of lower molecular weight than the pol+ parent during the period of growth following uv. Presumably, a step following repair synthesis is

lacking in these mutants. If a late step in excision repair involved the displacement of the repair polymeruse from the DNA by the ligase after repolymerization, then the pol A1 mutant could be sensitive for one of the following reasons: The altered DNA polymerase binds too tightly to the DNA to be displaced; it hinders recognition of single strand breaks, or prevents access of the ligase enzyme to the site. Witkin (87) has suggested that the normal uv induced mutability of pol A1 mutants despite the enhanced sensitivity to the lethal effect of uv may be due to a reduced efficiency in such a late step in excision repair.

If DNA polymerase levels and resistance to X-rays are related, one might expect yeast cells to show high levels of polymerase during the budding period of the cell cycle. Eckstein et al (20) have demonstrated in their yeast strains that extracts from synchronized yeast cells show oscillation of DNA polymerase activity in the cell cycle correlated with respect to DNA duplication. Activity is maximum at the onset of DNA synthesis, and decreases to a minimum during the most active period of DNA replication. X-irradiation dissociates DNA replication from the oscillation of the DNA polymerase. Under these conditions, DNA replication is inhibited, while the DNA polymerase activity continues to oscillate. Apparently, the appearance of DNA polymerase does not trigger DNA replication. Nor is the oscillation of polymerase activity controlled

by production of replicated DNA. The question remains whether this oscillation of a DNA polymerase activity may be correlated with budding cell radiation resistance and its oscillation.

Eckstein (20) indicated that in his yeast, DNA content is double during growth of the buds. Beam and Himes (6) have shown in the haploid SC7K used in our studies that DNA synthesis is complete before the onset of budding. Therefore, in SC7K, DNA synthesis must precede the onset of radiation resistance in the budding yeast cell. Williamson (85) and Hartwell (31) on the other hand, report that in their strains DNA synthesis commences with the onset of budding and is completed during the first quarter of the budding cycle while the buds are still quite small. If the period of DNA synthesis in the yeast cells used by Eckstein et al (20) also occupies only a brief interval of the budding cell cycle as observed by Williamson (85) and Hartwell (31), then the interval in the cell cycle where a correlation was observed between maximal polymerase activity and minimal DNA synthesis may also be correlated with most of the interval involved in bud formation. There is agreement nevertheless that DNA synthesis is complete while the budding cell is still quite small. One might therefore propose that the occurrence of radiation resistance during bud formation is correlated with the increased levels

of a DNA polymerase activity as observed by Eckstein et al (20), that budding cell resistance is due to a DNA polymerase, and that mutations conferring budding cell X-ray sensitivity cause deficiencies in a DNA polymerase. The mutants isolated in these studies would then be similar to the pol A1 radiation sensitive mutant strains of E. coli isolated by several investigators (19, 45, 48, 49) as discussed above.

X-ray sensitivity could also result from defects in the ligase responsible for joining the newly synthesized DNA strands (Okazaki pieces) to the old parental strand. Boyce and Tepper (8) have demonstrated rejoining of single strand breaks induced by X-rays in DNA of lambdaphage during post-irradiation incubation of superinfected bacteria. Tsuboi and Terasima (82) have demonstrated that rejoining of single strand breaks produced by X-rays is also possible in mammalian cells. Weiss and Richardson (84) have isolated a ligase which mediates this rejoining process. If a ligase is necessary for the repair of single strand breaks induced by X-rays, then a ligase deficient mutant should be X-ray sensitive. Dean and Pauling (18) have shown that a temperature sensitive ligase deficient mutant of E. coli TAU bar is sensitive to X-rays. Revertants of this mutant are identical to wild type with respect to radiation sensitivity and temperature sensitivity, suggesting control by a single revertible gene. McGrath and

Williams (54) have shown that single strand breaks induced by X-rays in the DNA of the highly resistant E. coli B/r and the sensitive E. coli Bs-1 are repaired presumably by ligase only in B/r during postirradiation incubation. The ligase activity responsible for rejoining of broken pieces of DNA is lacking in E. coli Bs-1; cell death in this strain after X-rays is due to the failure to rejoin broken DNA.

The nature of the defects in the mutants conferring X-ray sensitivity to the budding cell can now be considered in terms of the details of excision repair, defects in any part of which could result in sensitivity. The posttreatment responses to radiation provide a useful clue as to the nature of the sensitivity in XS mutant strains. Parry and Parry (65) have isolated four distinct categories of response to posttreatments after uv from representatives of each of 21 of the 22 complementing uvs mutants of Cox and Parry (15). For example, the third group comprising two mutants which are also sensitive to gamma rays, and which lack any repair was interpreted in terms of the accumulation of lethal damage resulting from excessive local DNA degradation after excision. If any of our mutants resembled these, one would predict postirradiation accumulation of low molecular weight DNA. If a repair polymerase is responsible for repair in the budding cell, then the class I mutants should lack all repair polymerase activity, and class

II mutants should possess subnormal repair polymerase activity and would show less repair of breaks than wild type. Also, since ionizing radiation produces strand breaks in DNA, then the sensitivity of our mutants might reflect an altered ability to rejoin single strand scissions in DNA comparable to the mutants of Dean and Pauling (18), and McGrath and Williams (54). If failure to rejoin broken DNA strands is related to defects in the repair process in the budding yeast cell, then the class I mutants could lack all ligase activity, and class II mutants would have subnormal ligase activity. Alternatively, a mutant gene could produce a polymerase so altered as to bind tightly to the DNA, and hinder the rejoining of newly synthesized DNA to the old parental DNA strand, which would also result in a lower level of repair, thus subnormal budding cell resistance.

The otherwise pecular behavior of the class II double homozygote of XS6 and XS9, in which only XS8 of the class II genes is represented by a normal allele, can be interpreted in terms of some known aspects of excision repair. Budding cells of this mutant are of essentially interdivisional cell sensitivity, a fact not readily predictable from the resistance observed in strains containing the component genes XS6 and XS9 singly in the haploid state or as the homozygous diploids. If the normal XS6 and XS9 pertained to repair polymerase and/or

ligase and the XS8 gene specified an exonuclease, then in the absence of normal XS6 and XS9 genes, extensive excision of bases from DNA, converting single strand breaks into double strand breaks could occur, leading to extensive unrepaired damage, and extreme sensitivity.

II. Genetic control of budding cell resistance

The genetic analysis data (Tables 3, 4, 5) and the radiation survival data collectively show that budding cell resistance is under genetic control. The genes responsible for budding cell resistance apparently also account for 0.75 of the difference between haploid and diploid interdivisional cells (see figure 12 for survival of XS6 XS8 XS9/XS6 XS8 XS9).

Several possible resistance mechanisms were considered in the introduction, and all but repair and its presumably regulated oscillation during the cell cycle were eliminated on consideration of available evidence. If, as was argued in section I of the Discussion, the exonuclease, polymerase, and ligase system responsible for excision repair is the basis for yeast budding cell behavior, our mutants must represent defects in enzyme synthesis originating in alterations of the structural genes involved, or in genes responsible for their regulation. The present study has not embraced the chemical analysis of "repaired" and "unrepaired" yeast DNA in mutant and wild type yeast. Such studies might be expected to yield direct evidence

with respect to this hypothesis. These present investigations are restricted to an analysis of the phenotypes (X-ray responses) of the mutants. These phenotypes - singly, heterozygously and in other combinations nevertheless provide a basis for the formal ordering of the genes in a pathway leading to the full expression of budding cell resistance, and to some speculation as to the roles of the individual genes.

Full repair capacity in budding cells can be regarded as requiring a full complement of class I and class II genes. Mutations at the various loci result either in no effective repair enzymes (class I), or in defective enzymes (class II). No repair - exponential inactivation equivalent to haploid interdivisional cells - is found in budding cells of both class I mutants and in the triple class II mutant, whether in haploid or diploid condition. The impaired resistance of all haploid or diploid single and double class II mutants can be regarded as indicating defects in different stages of repair.

There are many alternatives in the rational ordering of the genes in a pathway. The two class I genes though essential, could nevertheless function either in parallel or in sequence. If they operate in "parallel", they must act dependently, such that gene products of both are required for function, with the active product a dimer composed of products of each gene. This hypothesis entails predictions of the behavior of heterozygotes

that can be compared with our results. The XS5/+ heterozygote shows about 0.93 of wild type function (DMF = 0.93); the $X^S1/+$ in contrast possess 0.58 of wild type function. The double heterozygote $X^S1/XS5$ should show significant reduction of function, but its DMF of 0.55 indicates that it does not. This result resembles much more the consequences of simple gene dosage (with a normal XS5 gene approaching full dominance) in a sequential pathway in which the two genes have distinct steps, then it does their control of different components of a single active product. Zimmermann et al (91) and Zimmermann and Gundelach (90) studying the is-1 locus of Saccharomyces cerevisiae have shown that simple gene dosage relations are realized in only a few cases. The wide variation in observed enzyme activity of threonine dehydratase above and below the expected range of 50% in is-1 mutants x wild-type heterozygotes was attributed to the formation of inactive or active hybrid enzymes composed of monomers from wildtype and mutant genes in different combinations, in addition to the pure wildtype enzyme. The threonine dehydratase enzyme isolated from a number of single mutant heterozygotes was insensitive to the normal feedback inhibition (90), signifying a difference from the wildtype gene product. Such findings establish a precedent for the differences in dominance and complementation observed in our class I mutants, but provide no basis upon

which we can regard these genes as contributing to a single product. It is therefore suggested that these genes control distinct steps in a sequential pathway. No attempt is made to order these two genes. This sequential model also allows for other as yet undetected genes in the sequence (XS1 of Resnick, for example).

The class II mutants present a more complicated problem, firstly because by definition some budding cell function remains in each of them, secondly because their dysfunction is additive in double mutants, and thirdly because complementation is incomplete in double heterozygotes. Additivity of dysfunction has been used by some authors (9, 11, 16, 25, 37, 50) as evidence for the existence of more than one dark repair pathway in yeast, and other organisms. Unfortunately, this criterion would be valid only if all mutational blocks were complete, in which case all blocks in a given pathway would be equivalent in preventing the repair functions determined by the pathway. Residual repair in a mutant strain would result only from the function of the undamaged pathway (s), and complementation in multiple heterozygotes would reflect solely the degree of competence of singly represented functional genes (gene dosage) in these pathways. Mutation does not, however, necessarily impart total dysfunction to a locus; it can be "leaky", i.e., it apparently performs its normal function but

with reduced efficiency (15). A mutant strain having any single gene in a pathway altered in this way would show residual function, and a strain containing more than one such altered gene would be expected to show cumulative dysfunction. A sequential pathway model incorporating these features will be considered in detail later, during the course of attempts to construct a reasonable ordering or pathway for the class II genes.

The concept of hybrid enzyme formation (90, 91) is somewhat more difficult to apply to class II mutants than to those of class I. Because of the budding cell resistance that remains in each of the mutant strains, one must imagine that if the gene is not leaky as described above, this function must result either solely from the action of the other genes or from partial function of mutant gene products. Either case could involve combinations of two or more gene products to form polymeric enzyme hopefully with predictable consequences.

For instance, a class II mutant in the haploid condition might be supposed to produce a partially effective protein which together with the products of other unaltered resistance genes confers the observed resistance. In a diploid heterozygous for a single class II mutant gene, budding cell resistance is substantially augmented, but not restored to normal (see figures 3 - 7). If each class II gene coded for

an enzyme which was ultimately dimerized, the heterozygote might be expected to produce double wild type, hybrid and double mutant dimers in a ratio of 1:2:1. Since the pure mutant enzyme is itself partially effective (figures 10 and 11), the observed DMFs of 0.70 - 0.85 in budding cells of class II heterozygotes does not exclude this interpretation. Double heterozygotes among class II mutants all show incomplete complementation but little change in level of budding cell resistance over single heterozygotes; only when all three class II mutations are present together in a haploid, or in a homozygous diploid is budding cell resistance function lost.

A simple series in which each gene (or enzyme specified by it) passes its product on to the next, and in which any mutation represents a severing of the chain, is of course impossible because the class II mutations all show some degree of budding cell resistance. The serial ordering can be retained, however, if one supposes that this residual function results from reduced but not destroyed activity (leakiness) at the locus. In these terms, the DMF of a mutant strain, recorded as a decimal fraction of normal function, represents the residual function of the mutant gene. Double and triple mutant function would then represent products of these decimal fractions for two and three leaky genes respectively. The observed DMFs of the various strains, and the DMF values prediced on this

basis are tabulated below. Obvious discrepancies are indicated by *.

MODEL A

XS Mutant Genes	Nonmutated Genes	Leaky Character	Observed DMF	Predicted DMF
6	89	0.40	0.40	-
8	69	0.48	0.48	-
9	68	0.70	0.70	-
68	9		0.2	(0.4)(0.48)=0.19
69	8		0.17	(0.4)(0.7)=0.28 *
89	6		0.33	(0.48)(0.7)=0.34
689	0		0.12	(0.4)(0.48)(0.7)=0.13

It can be seen that except for the somewhat poor agreement between observed and predicted DMF for the 69 double mutant, the model seems acceptable, particularly if the sequence is XS8, XS9 and XS6. This sequence is in agreement with the proposed exonuclease function of XS8, and the polymerase and ligase functions of XS6 and XS9, proposed earlier (Discussion Section I). This sequence is also compatible with the proposed budding cell shoulder function of the normal XS6 gene. In addition, the predicted complete loss of resistance in the triple mutant closely agrees with the observed DMF.

One may also consider the consequences of a parallel functional ordering of class II mutant genes in which three independent pathways may be regarded as responsible for the

repair of different types of X-ray induced damage. In this case, the fact that the single mutants show some budding cell resistance, does not require the assumption of leakiness in any of the mutant alleles, though it by no means excludes it. In simplest terms, however, the residual function of the mutant strains can be considered to represent the activities of the unmutated pathways only. The function of each normal class II gene is then inferred from the DMF of the complementary haploid double mutant, and the behavior of single mutants (double functions) may be predicted from their sums, as shown below.

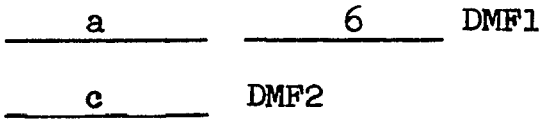
MODEL B

XS Mutant Gene	Nonmutated Gene	Observed DMF	Predicted DMF
6	89	0.40	0.17 + 0.20 = 0.37
8	69	0.48	0.33 + 0.20 = 0.52
9	68	0.70	0.33 + 0.17 = 0.50
68	9	0.20	
69	8	0.17	
89	6	0.33	
689	0	0.12	0.1 = 0.15
0	6, 8, 9	1.00	.33 + .17 + .2 + .12 = 0.82

As can be seen, the fit is neither very good nor very bad, and considering the errors inherent in the procedure, it could not be used either to accept or reject the model.

However, using the definition of residual function given above, and assuming that budding cell resistance would represent the sum of the DMFs of all complementary haploid double mutants, added to that of the triple mutant, which represents exclusive class I contribution to the phenotype; the sum of 0.82 seems considerably less than total budding cell function. This model has, however, the advantage that it allows for the special shoulder function of gene 6.

Another arrangement is shown below combining both sequential and parallel ordering.



This model requires leakiness for the gene in position a. This gene should have little or no effect when functional alone (when b and c are mutated). The DMF of the sequence a b (DMF1) and the single step c (DMF2) equal the DMFs for the complementary single and double mutant haploid respectively. The leakiness of the gene in position a is computed as follows:

Leakiness of gene a equals X. Therefore (DMF1) (X) = DMF (a c) double mutant. Four Models A, B, C, D, and their consequences are presented below. Obvious discrepancies are indicated by * .

Model	Leaky Gene	Gene Position		
		a	b	c
C	8	8	6	9
D	6	6	9	8
E	6	6	8	9
F	9	9	6	8

Model C Leakiness factor gene 8 = 0.47

XS Mutant Gene	Nonmutated Gene	Observed DMF	Predicted DMF
6	89	0.40	0.20 + 0 = 0.20 *
8	69	0.48	0.20 + 0.47(1) = 0.67 *
9	68	0.70	assumed = 0.70
68	9	0.20	assumed = 0.20
69	8	0.17	assumed = 0.1 *
89	6	0.33	(0.47)(1) = 0.47 *
689	0	0.12	0.1

Model D Leakiness factor gene 6 = 0.4

X Mutant Gene	Nonmutated Gene	Observed DMF	Predicted DMF
6	89	0.40	0.17 + (0.4)(1) = 0.57
8	69	0.48	0.48
9	68	0.70	0.17 + 0 = 0.17 *
68	9	0.20	0.4
69	8	0.17	0.17
89	6	0.33	0.1 *
689	0	0.12	0.1

Model E Leakiness factor gene 6 = 0.24

XS Mutant Gene	Nonmutated Gene	Observed DMF	Predicted DMF
6	89	0.40	0.2 + (0.24)(1) = 0.44
8	69	0.48	0.2 + 0 = 0.2 *
9	68	0.70	0.7
68	9	0.20	0.2
69	8	0.17	0. + (0.24)(1) = 0.24 *
89	6	0.33	0.1 *
689	0	0.12	0.1

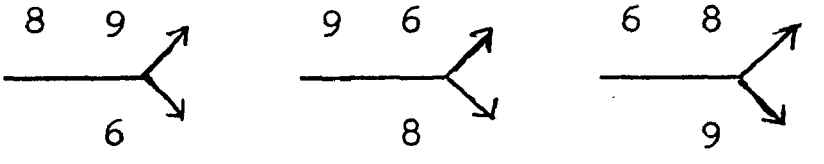
Model F Leakiness factor gene 9 = 0.7

XS Mutant Gene	Nonmutated Gene	Observed DMF	Predicted DMF
6	89	0.40	0 + 0.17 = 0.17 *
8	69	0.48	0.48
9	68	0.70	0.17 + (0.7)(1) = 0.87
68	9	0.20	0.1
69	8	0.17	0.17
89	6	0.33	0 + (0.24)(1) = 0.24 *
689	0	0.12	0.1

The predicted DMF values, computed for models C, D, E, and F, show such large disagreements with the observed values, that this combination of sequential and branching pathways is rejected.

Another branching pathway model is possible in which one class II gene is located just before the branch point, and the remaining two class II genes occupy positions on either arms of the fork. In this model, the gene at the branch point may be regarded as controlling an intermediate shared by the remaining genes, or specifying a function (for example, an exonuclease) which must precede those of the other genes.

Three variants are possible (Models G, H and I). Those models (G and H), in which gene 6 (the"shoulder" gene) is distal, may be preferable.



Model G Model H Model I

In all cases, leakiness must be proposed for the mutant gene at the branch point. The presumed leakiness of this gene can be taken from the DMFs of the single mutants, and are 0.40, 0.48 and 0.70 for XS6, XS8 and XS9, respectively. The fraction of normal function characteristically performed by each unmutated gene on an arm of the fork, is taken from the DMF of the complementary mutant strain, as previously described. For example, using Model G, the fraction of function of the normal 6, 8 sequence, equals 0.7, (the DMF of strain XS9),

while that of the normal 8, 9 sequence equals 0.40 (the DMF of strain XS6). The predicted consequences of the three models are presented below. Obvious discrepancies are indicated by *.

Model G Leakiness factor gene 8 = 0.48

XS Mutant Gene	Nonmutant Gene	Observed DMF	Predicted DMF
6	89	0.40	0.4
8	69	0.48	0.48
9	68	0.70	0.70
68	9	0.20	(.4)(0.48) = 0.19
69	8	0.17	0.1 *
89	6	0.33	(0.48)(0.7) = 0.34
689	0	0.12	0.1

Model H Leakiness factor gene 9 = 0.70

XS Mutant Gene	Nonmutant Gene	Observed DMF	Predicted DMF
6	89	0.40	0.40
8	69	0.48	0.48
9	68	0.70	0.70
68	9	0.20	0.1 *
69	8	0.17	(0.40)(0.70) = 0.28 *
89	6	0.33	(0.48)(0.70) = 0.34
689	0	0.12	0.1

Model I Leakiness factor gene 6 = 0.40

XS Mutant Gene	Nonmutated Gene	Observed DMF	Predicted DMF
6	89	0.40	0.40
8	69	0.48	0.48
9	68	0.70	0.70
68	9	0.20	(0.40)(0.48) = 0.19
69	8	0.17	(0.4)(0.7) = 0.28 *
89	6	0.33	0.1 *
689	0	0.12	0.1

Clearly, the best agreement between observed and predicted DMFs is found in Model G, where gene 8 is at the branch point, and final formation of the phenotype is shared by genes 6 and 9 in an approximate 30:70 ratio, respectively. This ratio is close to the observed DMFs of strains XS6 and XS9, of 0.4 and 0.7 respectively. The arrangement is even more persuasive when one considers that the XS6, XS9 haploid double mutant as well as the XS6 XS9/XS6 XS9 diploid, in both of which only XS8 is normal, show similar DMFs at 1% survival, and have virtually no budding cell resistance.

The XS8 gene may be regarded as responsible for the control of an intermediate step in repair of radiation damage, functioning as a bridge between the class I genes and the other class II genes. XS8 also might regulate DNA exonuclease activity, as described in Section I of the Discussion, in

which case either normal or leaky mutant XS8 gene would provide extensive DNA degradation; but with only mutant XS6 and XS9 alleles, repair synthesis could not follow.

In summation, the sequence model (Model A) in which resistance is due to residual leaky function of the mutants, and the branched model with XS8 before the fork (Model G), seem most clearly to account for possible genetic organization for class II genes. It is difficult at present to find a basis for choosing between them. The relatively poor agreement in Model A for strains containing mutated 6 and 9 genes, tends to favor the branched model.

III. Radiation Resistance in Relation to Meiosis, Meiotic Recombination and Sporulation

The relationship between radiation resistance and meiosis, meiotic recombination, and sporulation in fungi has been examined in a number of organisms (52, 72, 75, 88). Schroeder (75) observed that neither the uvs 3 nor uvs 5 mutants of Neurospora is fertile in the homozygous condition. Asci do not develop beyond the multinucleate ascogenous hypha stage. The uvs4/uvs4 homozygote shows reduced ascospore survival. The uvs1 mutant in Aspergillus of Lanier et al (52), when homozygous, yields dwarf sterile cleistothecia containing aborted asci. Wright and Pateman (88) have observed poor viability of ascospores in a uvs8/uvs8 Aspergillus nidulans

radiation sensitive mutant. In contrast to these results, the uvs4 mutant of Aspergillus rugulosus has no effect on ascus or ascospore development (83). Radiation sensitive mutants of yeast also do not show a close relationship between radiation sensitivity and defects in meiosis and sporulation. In yeast, meiosis occurs when diploid cells are inducted to sporulate and ends with the formation of an ascus containing four haploid ascospores (24). Of the 96 radiation sensitive mutants isolated by Cox and Parry (15), only uvs5, uvs6, uvs19, uvs20 and uvs21 when homozygous significantly reduce sporulation in diploid yeast. With respect to the radiation sensitive mutants of Resnick (72), only XS3/XS3 fails to sporulate. The phenotypically class I mutant XS1/XS1 sporulates, but produces inviable spores, and all other XS and uvs mutants show normal sporulation and spore viability when homozygous. None of the uvs mutants of Snow interferes with the meiotic divisions (79). Sporulation proceeded normally and the percentage of sporulated cells was about the same as observed in the controls. The investigations with our mutants suggest that mutations conferring budding cell sensitivity may in some cases also block meiosos (see table 6). The X^S1/X^S1, XS6/XS6, XS7/XS7, and XS9/XS9 diploid homozygotes fail to sporulate. However, the class I mutant XS5 and the class II mutant XS8 show good

sporulation when homozygous. Esposito et al (24) have attempted to define the functions unique to meiosis in yeast, and to determine their sequence of expression during sporulation. Three temperature sensitive mutants, Spo 1-1, Spo 2-1, and Spo 3-1 were characterized with respect to macromolecular synthesis and the nuclear divisions of meiosis. These sporulation deficient mutants do not form asci detectable on microscopic examination, and therefore may represent lesions throughout the sporulation cycle. The DNA synthesis which precedes meiosis is normal in Spo 2-1, but is about half that observed for wild type in Spo 1-1 and Spo 3-1. The mutants show normal RNA and protein synthesis, and protein turnover. Cytological evidence presented shows that all mutants initiate the first division of meiosis. However, only three to six percent of the Spo 2-1 and Spo 3-1 cells, respectively, and none of the Spo 1-1 cells proceed into the second meiotic division.

Sporulated cultures of mutants defective in the second meiotic division would be expected to contain mainly mononucleate and binucleate cells. Mutants affecting ascospore formation after meiosis would be expected to contain primarily tetranucleate cells. By this criterion the mutants of Esposito et al (24) as well as most of the radiation sensitive mutants of Neurospora (75) and Aspergillus (88) represent

defects before the completion of the meiotic divisions. In contrast, the uvs 2 mutant of Ustilage (37) when homozygous undergoes an abortive meiosis in which 2-4 nuclei are sometimes produced, but never basidiospores. This mutant may represent a defect in the spore formation after the meiotic divisions. Studies by Bresch et al (10) using Schizosaccharomyces pombe also indicate that sporulation depends on the completion of the meiotic divisions, since no evidence of spore development appears in mutants in which meiosis is affected. Nuclei from such sporulation negative mutants vary in number and size, presumably resulting from defects in the meiotic process.

Presumably our sporulation deficient mutants, as well as those of Cox and Parry (15) and others (52, 73, 88) represent defects either in the meiotic divisions, or in the processes leading to the formation of ascospores. No attempt has been made as yet to establish the nature of these deficiencies.

No clear correlation between radiation sensitivity and defects in meiotic recombination in radiation sensitive mutants has been found in fungi. Wright and Pateman (88) report no reduction in meiotic recombination frequency for the homozygous diploid uvs8/uvs8. The uvs4/uvs4 mutant of Aspergillus rugulosus shows normal meiotic recombination frequency for

y-isoleucine (83). Shanfield and Käfer (77) also report normal recombination frequencies for their uvsB2 homozygous diploid Aspergillus mutants. In addition, Chang and Tuveson (12) report that the uvs1 mutant of Neurospora which reduces ascospore viability to less than 1% does not affect recombination. Holliday (37) using Ustilage maydis has shown that the uvs1/uvs1 and uvs3/uvs3 mutants do not affect crossing over between ad1 and me-15, or conversion at the nar (nitrate reductase) locus. Snow (88) has also demonstrated that none of his uvs alleles when homozygous affects either meiotic intragenic recombination at the his1 locus or intergenic recombination between thr3 and arg6. Our investigations with XS mutants also reveal no clear correlation of sensitivity with meiotic recombination frequency for the interval between leu1 and try5 (table 7). To summarize, all these findings suggest that some, but by no means all mutations conferring radiation sensitivity, affect meiosis and sporulation. However, they do not influence meiotic genetic recombination in those strains in which meiosis and sporulation occur.

The relationship between induced mitotic recombination and radiation resistance will be considered in Section IV.

IV. Non-lethal radiation damage in wild type and mutants

One non-lethal endpoint examined in wildtype and mutant strains, was radiation induced recessive homozygosis. The

induction of nonlethal endpoints by X-rays has been previously studied by Perper (68), and Perper and Beam (69). James and Lee Whiting (42), and Hurst and Fogel (41) have shown that ultraviolet light induces recessive homozygosis at one or more loci in heterozygous vegetative cells of Saccharomyces cerevisiae. Mitotic recombination was considered to be the mechanism of homozygosis. This mechanism for homozygosis after uv light and ionizing radiation was later confirmed by Nakai and Mortimer (61). Homozygosis has also been studied in synchronous cultures of yeast by Parry and Cox (64) as well as by Esposito (23). Parry (62) has shown that mitotic recombinants are induced to the same extent by both ultraviolet light and the chemical mutagen, EMS, when measured at the same survival.

Sensitivity to these endpoints depends upon the stage in the cell cycle of the irradiated cells. Esposito (23), using synchronous cultures of yeast irradiated at different points during the mitotic cell cycle with nonlethal doses of uv or X-rays, has found that recombination induction can occur prior to DNA synthesis, and reaches a maximum frequency at the start of replication. It declines during replication and reaches a minimum just after replication is completed; cells undergoing budding are most refractory to induction of mitotic recombination. Perper (68) and Perper and Beam (69) have also observed that budding cells are resistant to homozygosis, and that

high recombination induction coincides with high sensitivity to inactivation of interdivisional cells.

Our finding that modification of the genetic control of budding cell resistance to inactivation may or may not be paralleled by altered frequencies of nonlethal endpoints, has been foreshadowed to some extent in the results of other investigators. Moustacchi (57) has studied uv induced homozygosis in her uvs, mutant which shows exponential survival with uv, but shows a normal budding cell component in gamma ray survival curves. Both interdivisional and budding cells show enhanced sensitivity to uv, but are of normal sensitivity to gamma rays. This mutant, when homozygous, confers a higher sensitivity to uv induced homozygosis in both interdivisional and budding cells than wild type, or the $+/uvs_2$ heterozygote. Moustacchi and Enteric (59) have found several uvs mutants of yeast sensitive to uv inactivation which are, however, of wild type sensitivity to petite induction. Since the studies were done with unsynchronized cultures and include high doses, it seems fair to conclude that the budding cells are sensitive to inactivation though of unaltered resistance to the nonlethal endpoint.

Only two of our mutants would sporulate when homozygous, but fortunately they represented both classes, I and II. These were XS5 and XS8. XS5 whose budding cells are of interdivi-

sional cell sensitivity to inactivation (namely no budding cell resistance) also shows no resistance to homozygosis. XS8 which when homozygosis shows very little remaining budding cell resistance to inactivation, but is fully normal in resistance to homozygosis. The fact, that XS5 and XS8 differ in their coupling of sensitivity to inactivation with sensitivity to homozygosis may be important. XS5, which in mutant form obliterates resistance to both inactivation and homozygosis may occupy a very early general role in DNA break closure. The fact that XS8, on the other hand, shows little resistance to inactivation, but full resistance to homozygosis, may reflect a diversity of repair pathways. The mechanism sufficient to prevent mitotic recombination does not prevent all lethal damage. A similar noncoupling of lethal and nonlethal effects is seen in the work of Parry and Cox (63) in which dark liquid holding of yeast cells following uv increases intragenic recombination, but decreases inactivation, mutation and intergenic recombination. Collectively, present knowledge indicates no systematic relation between lethal and nonlethal damage and their repair.

Abstract

Five X-ray sensitive mutants were selected from 10,000 colonies surviving treatment with ultraviolet light. These mutants were named XS5, XS6, XS7, XS8 and XS9. X^S1 was donated by Nakai. These mutants fall into two phenotypic classes: class I in which budding confers no resistance, and class II in which resistance is detectable but subnormal. These mutants when crossed with one another show a complex complementation pattern. The heterozygous diploids composed of X^S1 and any other class I or class II mutant show only 50% remaining wild type budding cell resistance function, while all other heterozygous diploids show at least 70% of normal function. The reduced function of X^S1 heterozygotes was attributed to a gene dosage effect at this locus. The subnormal function in all other heterozygotes was attributed to either hybrid enzyme formation which would lead to production of active as well as inactive enzyme aggregates, or to the presence of a leaky mutant. Incomplete dominance at the mutant loci was interpreted in the same manner.

The genetic and radiation survival data support the following conclusions. No direct correlation between the presence or absence of sporulation and radiation sensitivity may be made. Genes which influence budding cell resistance do not influence meiotic genetic recombination. Sensitivity to radiation induction of recessive homozygosis in mutants is complex.

Both coupling and noncoupling of resistance to lethal and nonlethal effects of radiation were observed. One mutant showing subnormal budding cell resistance is refractory to induction of the nonlethal alteration. One mutant which lacks any budding cell resistance is sensitive to induction of recessive homozygosis. The budding cell shoulder is under the control of a single gene. With the exception of the budding cell shoulder gene, there appears to be a direct correlation between the DMF (with reference to wild type of the budding cell survival and that of the diploid interdivisional cell survival.

Evidence was presented to show that the cellular processes conferring resistance to the lethal and nonlethal effects of ionizing radiation in yeast operate by means of repair of X-ray damage. Speculation concerning the genetic basis of the mechanisms involved in budding cell resistance were offered, in which sequential, parallel, and branching models for class I and class II gene organization were considered. It is proposed that the genes are organized either in a sequential pathway, or in a combination of sequential and branching pathways.

Bibliography

1. Adler, I, 1966 Genetic control of radiation sensitivity in microorganisms in O. G. Augenstein, R. Mason and M. Zello (eds)

2. Alper, T. 1959 Variability in the oxygen effect observed with microorganisms part I haploid yeast; single and budding cells. Int. J. Rad. Biel. 1:414-418

3. Averbeck, D. W. Laskowski, F. Eckardt and E. Lehmann-Brauns 1970 Four radiation sensitive mutants of Saccharomyces Molec. Gene Genetics 107: 117-127

4. Beam, C. A. 1955 The influence of ploidy and division stage on the anexic protection of Saccharomyces cerevisiae against X-ray inactivation Prec. Nat. Acad. Sci 48: 857-871

5. Beam, C. A. 1959 The influence of metabolism on radiation effects Rad. Res. Suppl. 1 372-390

6. Beam and Himes Unpublished

7. Beam, C. A. R. K. Mortimer, R. G. Wolfe and C. A. Tobias 1954 The relation of radioresistance to budding in Saccharomyces cerevisiae. Arch Biochem. Biophys. 49: 110-112

8. Boyce, R. P. and M. Tepper 1958 X-ray induced single strand breaks and joining of broken strands in superinfecting lambda DNA in Escherichia celi lysogenic for

lambda. Virology 34: 344-351.

9. Brendal, M. and N. Khan and R. H. Haynes 1970 Common steps in the repair of Alkylation and radiation damage in yeast Molec. Gene Genetics 106: 289-295.

10. Bresch, C. and G. Muller and R. Egel 1966 Genes involved in meiosis and sporulation of a yeast. Molec. Gen. Genet. 102: 301-306.

11. Brown, A. and B. Kilbey 1970 Hyper-uv sensitive yeast 1: Isolation and properties of two such mutants Molec. Gen. Genetics 108: 258-265.

12. Chang, L. and R. W. Tuvesen 1967 Ultraviolet sensitive mutants in Neurospora Cressa Genetics 56: 801-810.

13. Clark, A. J. and A. D. Margulies (1965) Isolation and characterization of recombination deficient mutants of E. coli K 12 Proc. Natl. Acad. Sc. U. S. 53: 451-459.

14. Corda-Olmedo and P. C. Hanawalt (1967) Repair of DNA damaged by N. Mythyl-N 1 nintro-N-mitrososguanidine in E. coli Mutation Res. 4: 369-371.

15. Cox, B. S. and J. M. Parry 1968 The isolation, genetics, and survival characteristics of ultraviolet light sensitive mutants in yeast Mutation Research 6: 37-55.

16. Davies, D. 1967 UV sensitive mutants of Chlamydomonas reinhardii Mutation Research 4: 765-770.

17. Davies, D. R. 1967 The control of dark repair mechanisms

in meiotic cells Molec. Gen. Genetics 100: 140-149.

18. Dean, C. and C. Pauling 1970 Properties of a DNA ligase mutant of Escherichia coli: X-ray sensitivity J. of Bacteriol 102 #2 588-589.

19. DeLucia, P. and J. Cairns 1969 Nature 224 11964

20. Eckstein, H. V. Paduch and H. Hils 1967. Synchronized yeast cells. 3. DNA synthesis and DNA polymerase after inhibition of cell division by X-rays. European J. Biochem. 3: 224-231.

21. Elkind, M. M. and C. A. Beam 1955 Variations in the biological effectiveness of X-rays and particles on haploid Saccharomyces cerevisiae Rad. Res. 3: 88-104.

22. Elkind, M. M. and H. Sutton 1959 The relationship between division and X-ray sensitivity, ultraviolet sensitivity and photoreactivation in yeast. Rad. Res. 10: 283-295.

23. Esposito, R. 1968 Genetic recombination in synchronized cultures of Saccharomyces cerevisiae Genetics 59: 191-210.

24. Esposito, M. R. Esposito M. Arnaud, H. Halversen 1970 Conditional mutants of meiosis in yeast. J. of Bacteriol 104: 202-210.

25. Fabre, F. 1971 A uv supersensitive mutant in the yeast Schizosaccharomyces pembe Molec. Gen. Genetics 110: 134-143.

26. Fangman, W. and M. Russel 1971 X-irradiation sensitivity

in Escherichia coli defective in DNA replication Molec. Gen. Genet. 4: 332-347.

27. Fogel, S. Personal communication.

28. Green, E. Personal communication.

29. Grossman, L. J. Kaplan, R. Kusher, and I. Mahler 1968 Enzymes involved in the early stages of repair of ultraviolet irradiated DNA Cold Spring Harbor Symposia on Quantitative Biology 23: 229-234.

30. Harm, W. and B. H. Hillebrandt (1960) A nonphotoreactivable mutant of E. coli B Photochem. 11. 271-272.

31. Hartwell, L. 1971 Genetic control of the cell division cycle in yeast II Genes controlling DNA replication and its enitration J. Mol. Biol. 59: 183-194.

32. Hawthorne, D. C. and R. K. Mortimer, 1969 Chromosome mapping in Saccharomyces: Centromere linked Genes Genetics 45: 1085-1110.

33. Haynes, R. H. (1964) Role of DNA repair mechanisms in microbial inactivation and recovery phenomena. Photochem and Photobiol. 3: 429-450.

34. Haynes, R. H. 1966 The interpretation of microbial inactivation and recovery phenomena. Radiation Research Suppl. 6: 1-29.

35. Hill, R. F. 1965 Ultraviolet induced lethality and reversion to phototrophy in Escherichia cell strains

with normal and reduced dark repair ability Photochem. Photobiol. 4: 563-568.

36. Holliday, 1961 Induced mitotic crossing over in Ustilage maydis Genet. Res. 2: 231-248.

37. Holliday, R. 1967 Altered recombination frequencies in radiation sensitive strains of Ustilage Mutation Research 4: 275-288.

38. Howard Flanders, P. 1969 DNA repair Ann. Rev. Biochem. 37: 175-200.

39. Howard Flanders, P. and L. Theriot 1966 Mutants of Escherichia coli K-12 defective in DNA repair and in genetic recombination Genetics 53: 1137-1150.

40. Howard Flanders, P. R. Boyce and L. Theriot (1966) Mutants of E. coli K-12 defective in DNA repair and in genetic recombination Genetics 53: 1137-1150.

41. Hurst, D. and S. Fogel 1964 Mitotic recombination and heteroallelic repair in Saccharomyces Cerevisiae Genetics 50: 435-458.

42. James, A. and B. Lee - Whiting 1955. Radiation induced genetic segregations in vegetative cells of diploid yeast Genetics 40: 826-831.

43. James, A. P. and M. M. Werner 1969 Beta mercaptoethanel induced recovery of X-irradiated yeast cells. Can. J. Genet. Cytol. II: 848-856.

44. Johnston, J. R. and R. K. Mortimer 1959 Use of snail digestive juice in isolation of yeast spore tetrads J. Bacteriol. 78: 292.

45. Kanner, L. and Hanawalt, P. 1970 Repair deficiency in a bacterial mutant defective in DNA polymerase Biochem. and Bi-phys. Res. Commun. 39 #1 149-155.

46. Kaplan, H. 1966 DNA strand scission and loss of viability after X-irradiation of normal and sensitized bacterial cells. Proc. Natl. Acad. Sci. US 55: 1442-1446.

47. Kaplan, J. S. Kushner, and L. Grossman 1971 Enzymatic repair of DNA III properties of the uv endonuclease and uv-exonuclease. Biochemistry 10: 3315-3324.

48. Kato, T. and S. Kondo 1967 Two types of X-ray sensitive mutants of Escherichia coli B. Their phenotypes characters compared with uv sensitive mutants. Mutation Research 4: 253-263.

49. Kelly, R., M. Atkinson, J. Huberman and A. Kernberg 1969 Excision of thymine dimers and other mismatched sequences by DNA polymerase of Escherichia coli Nature 224 495-501.

50. Khan, N., M. Brendel and R. Haynes 1970 Supersensitive double mutants in yeast. Molec. Gen. Genetics 107: 376-378.

51. Kohn, K. W., N. H. Steingbigal and C. L. Spears 1965 Crosslinking and repair of DNA in sensitive and resistant strains of E. coli treated with nitrogen mustard Proc.

Natl. Acad. Sci. U. S. 53: 1154-1161.

52. Lanier, W. B., R. W. Tuveson and J. E. Lennox 1968 A radiation sensitive mutant of Aspergillus nidulans Mutation Research 5: 25-31.

53. Langguth, E. Unpublished results.

54. McGrath, R. and R. Williams 1966 Renaturation in vivo of irradiated Escherichia coli deoxyribenucleic acid, the rejoining of broken pieces Nature 212: 534-535.

55. Monk, M. M., Peacy and J. Gross 1971 Repair of damage induced by ultraviolet light in DNA - polymerase defective Escherichia coli cells. J. Mol. Biol. 53: 623-630.

56. Mortimer, R. K. and D. C. Hawthorne 1965 Genetic mapping in Saccharomyces Genetics 53: 165-173.

57. Moustacchi, E. 1969 Cytoplasmic and nuclear genetic events induced by uv light in strains of Saccharomyces cerevisiae with different uv sensitivities Mutation Res. 7: 171-185.

58. Moustacchi, E. (1970) Differential liquid holding recovery for the lethal effect and cytoplasmic petite induction by uv light in Saccharomyces cerevisiae Molec. Gen. Genetics 109: 69-83.

59. Moustacchi, E. and S. Enteric 1970 Repair of cytoplasmic genetic damage in yeast. Proceedings of the IV Int. Congr. of Radiation Research Vol. I (Gordon and Breach eds).

60. Nakai, S. and S. Matsumoto (1967) Two types of radiation sensitive mutants in yeast Mutation Research 4: 129-136.

61. Nakai, S. and R. K. Mortimer 1969 Studies of the genetic mechanism of radiation induced mitotic segregation in yeast Molecular and General Genetics 103: 329-338.

62. Parry, J. 1969 Comparison of the effects of ultraviolet light and ethyl methanesulfonate upon the frequency of mitotic recombination in yeast Molec. Gen. Genetics 106: 66-72.

63. Parry, J. M. and B. S. Cox 1968 The effects of dark holding and photoreactivation on ultraviolet light induced mitotic recombination and survival in yeast. Genetic Res. 12: 187-198.

64. Parry, J. and B. Cox 1969 Mitotic recombination induced by uv light in synchronous cultures of yeast. Mutation Res. 5: 373-384.

65. Parry, J. M. and E. M. Parry 1969 The effects of uv light posttreatments on the survival characteristics of 21 UV sensitive mutants of Saccharomyces cerevisiae Mutation Research 8: 545-550.

66. Patrick, M. H. and R. H. Haynes 1964 Dark recovery phenomena in yeast II. Conditions that modify the recovery process. Radiation Research 23: 546-579.

67. Patrick, M. H. and H. Haynes and R. B. Uretz, 1964 Dark

recovery phenomena in Yeast I. Comparative effects with various inactivating agents. Radiation Research 21: 144-163.

68. Perper, T. 1968 Ph.D Thesis.

69. Perper, T. and C. A. Beam 1968. X-ray inactivation of budding yeast cells. Its mechanism and modification. Proceedings of the XII International Congress of Genetics Japan. Pa. 77.

70. Pettijohn, D. and P. Hanawalt (1964) Evidence for repair replication of uv damaged DNA in bacteria J. Mol. Biol. 9: 395-410.

71. Radman, M. L. Cordone, D. Kramanovic-Simic, M. Errera (1970) Complimentary action of recombination and excision in the repair of ultraviolet irradiation damage to DNA. J. Mol. Biol. 49: 203-212.

72. Resnick, M. Ph.D Thesis 1968 Genetic control of lethality and mutation in Saccharomyces cerevisiae.

73. Resnick, M. 1969 Genetic control of radiation sensitivity in Saccharomyces cerevisiae Genetics 62: 519-531.

74. Rupp, W. D. and P. Howard Flanders (1968) Discontinuities in the DNA synthesized in an excision defective strain of E. coli following ultraviolet radiation J. Mol. Biol. 31: 291-304.

75. Schroder, A. 1970 Ultraviolet sensitive mutants of Neurospora I. Genetic basis and effect on recombination 1970

Molecular and General Genetics 107: 291-304.

76. Setlow, J. K. 1966 The molecular basis of biological effects of ultraviolet radiation and photoreactivation in Ebert, M. and S. Howard (eds) Current Topics in Radiation Research Vol. 2 North Holland Publishing Co. Amsterdam, 197-248.

77. Shanfield, B. and E. Kafer 1969 UV sensitive mutants increasing mitotic crossing over in Aspergillus Nidulans Mutation Research 7: 485-487.

78. Smith, K. C. and D. H. C. Moun (1970) Repair of radiation induced damage in Escherichia coli I. Effect of rec. mutations on post replication repair of damage due to ultraviolet radiation. J. Mol. Biol. 51: 459-472.

79. Snow, R. 1967 Mutants of yeast sensitive to ultraviolet J of Bacteriol 94: 571-575.

80. Snow, R. 1968 Recombination in ultraviolet sensitive strains of Saccharomyces cerevisiae Mutation Research 6: 409-418.

81. Trgovcevic, Z. and Z. Kucan 1969 Correlation between the breakdown of deoxyribenucleic acid and radiosensitivity of Escherichia coli Radiation Research 37: 478-492.

82. Tsubei, A. and Terasimia, to 1970 Rejoining of single strand breaks of DNA induced by X-rays in mammalian cells. Effects of metabolic inhibitors. Molec. Gen.

Genet. 108: 117-128.

83. Tuveson, R. W and J. E. Lennex 1968 A uv sensitive mutant of Aspergillus Rugulosus unrelated to intergenic crossing over. Canadian Journal of Genetics and Cytology 10: 50-53.

84. Weiss, B. and Richardson, C. 1957 Enzymatic breakage and rejoining of DNA I. Repair of single strand breaks in DNA by an enzyme system from Escherichia coli infected with T4 bacteriophage Proc. Natl. Acad. Sci. U. S. 57: 1021-1028.

85. Williamsen, D. 1965 The timing of deoxyribenucleic acid synthesis in the cell cycle of Saccharomyces cerevisiae J. Coli Biol. 25: 517-523.

86. Witkin, E. M. (1966) Mutation and the repair of radiation damage in bacteria Radiation Research Suppl. 6: 30-53.

87. Witkin 1971 Ultraviolet mutagenesis in strains of Escherichia coli deficient in DNA polymerase Nature 229: 81-82.

88. Wright, P. and J. Pateman 1970 Ultraviolet light sensitive mutants of Aspergillus Nidulans. Mutation Research 9: 579-587.

89. Zimmermann, F. K. (1968) Sensitivity to methylmethane Sulfate and nitrous acid of ultraviolet light sensitive mutants in Saccharomyces cerevisiae Mol. Gen. Genetics

102: 247-256.

90. Zimmermann, F. K. and E. Gundelach 1969 Intragenic complementation, Hybrid enzyme formation and dominance in diploid cells of Saccharomyces cerevisiae Molec. Gen. Genetics 103: 348-362.

91. Zimmermann, F. K. I. Schmiedt and A. M. A. ten berge 1969 Dominance and recessiveness at the protein level in mutant X wildtype crosses in Saccharomyces cerevisiae. Molec. Gen. Genetics 104: 321-330.

Table I Yeast strains employed in this study

Source of XS mutants

Name			
SC7K			
SC7K - 4D	tryl	his2	

Centromers marked strains for genetic analysis

Name				
2181	a	adel	tryl	his2

Meiotic recombination and X-ray induced recessive homozygosis studies

Wild type	x/a	+/+	try5/+	leu1/+	ade6/+	met1/+	+/lys1
class I/class I	x/a	XS5/XS5	try5/+	le1/+	ade6/+	met1/+	+/lys1
class II/class II	x/a	XS8/XS8	try5/+	leu1/+	ade6/+	met1/+	+/lys1

Table 2

Sensitivity of single isolated interdivisional and budded cells of haploid yeast to a single dose of X-rays.

Strain	Interdivisional cell survival		Budding cell survival	
	4.7KR	56 KR	4.7 KR	56 KR
SC7K	0.12	-	0.64	0.50
X^S1	0.10	-	0.20	0
XS5	0.12	-	0.20	0
XS6	0.12	-	0.56	0.04
XS7	0.12	-	0.56	0.06
XS8	0.10	-	0.50	0
XS9	0.06	-	0.46	0.10

50 cells were micromanipulated on YEPD agar after exposure to X-rays. Unirradiated control cell give 100% survival.

Table 3

Number of PD, NPD and T Asci for radiation sensitive genes vs. Centromere linked genes

Mutant Strain	Mutant Class	Ad 1			his 2			tr 1		
		PD	NTD	T	PD	NPD	T	PD	NPD	T
X^S1	1	-	-	-	-	6	-	3	2	8
XS5	1	2	1	6	3	0	6	1	2	6
XS6	II	2	2	9	2	2	9	3	3	7
XS7	II	3	2	6	3	3	5	4	2	5
XS8	II	1	4	10	6	1	8	1	4	10
XS9	II	2	3	5	4	1	5	3	3	4

Table 4

Second division segregation frequency of sensitivity gene to centromere markers in mutant: wild type crosses

Mutant	Second division segregation frequency
X^s1	0.67 *
XS5	0.67
XS6	0.69
XS7	____ **
XS8	0.67
XS9	0.50

* From Nakai and Matsumoto

** Allelic to XS-6 (see tables 3 and 7)

Table 5

Mutant X mutant crosses

Cross	Class	Sporulation	PD	NPD	T
X^S1 . X^S1	1 . 1	0	-	-	-
X^S1 . XS5	1 . 1	SP	8	4	24
X^S1 . XS9	1 . II	SP	2	1	2
X^S1 . XS6	1 . II	SP	8	3	21
X^S1 . XS8	1 . II	SP	9	2	10
XS5 . XS5	I . I	SP	27	0	0
XS5 . XS6	I . II	SP	14	0	4
XS5 . XS8	I . II	SP	3	4	9
XS5 . XS9	I . II	SP	1	1	3
XS6 . XS6	II . II	0	-	-	-
XS6 . XS7	II . II	0	-	-	-
XS6 . XS8	II . II	SP	2	2	6
XS6 . XS9	II . II	SP	4	8	12
XS8 . XS9	II . II	SP	3	4	4
XS8 . XS8	II . II	SP	18	0	0
XS9 . XS9	II . II	0	-	-	-

XS5 and XS6 are linked

chi square for independence PD = NPD

at 5% level of significance

$X^2 = 14$

Table 6

Recombination frequencies for linked markers in a sensitive diploid strain

Cross	Interval		Ascal classes PD	NPD	T	Frequencies of recombination
Wild type	try5	leu1	23	0	8	0.13
XS5/XS5	try5	leu1	18	0	9	0.17
XS8/XS8	try5	leu1	14	0	5	0.13

Table 7

X-ray responses of diploids made from pairwise crosses of radiation sensitive mutants

	SC7K	X^S1	XS5	XS6	XS7	XS8	XS9
SC7K	+	+/-	+	+	+	+	+
X^S1	+/-	-	+/-	+/-	+/-	+/-	+/-
XS5	+	+/-	+	+	+	+	+
XS6	+	+/-	+	-	-	+	+
XS7	+	+/-	+	-	-	+	+
XS8	+	+/-	+	+	+	-	+
XS9	+	+/-	+	+	+	+	-

Key

\+ indicates growth and approximately wildtype resistance

\- indicates no growth and a sensitive phenotype

+/- indicates growth after 56 KR and no growth after 196 KR and incomplete complementation or incomplete dominance.

Table 8

X-ray response of single isolated budded cells of diploids heterozygous for sensitivity

Class	Source	Dose (KR)	Number of Cells Isolated	Microcolonies after 48 hours
SC - 6	C. A. Beam	0	100	100
		56	100	30
I/+	$X^S1/+$	0	100	100
		56	100	12
I/+	XS5/+	0	100	100
		56	100	28
1X1	X^S1/XS5	0	100	100
		56	100	13
1X11	XS5/XS8	0	100	100
		56	100	29
1X11	X^S1/XS8	0	100	100
		56	100	12
II/II	XS6/XS9	0	100	100
		56	100	32

$X^S1/+$, X^S1/XS5 and X^S1/XS8 are significantly more sensitive than wild type SC6, Chi square at 50% level of significance for one degree of freedom equals 8.

Table 9

Frequency of induced recessive homozygosis and petite in induction in wildtype, class I and class II diploids

A. interdivisional cells
B. budding cells

A. Strain	Dose	Percent Survival	Percent Auxotrophy	Number and frequency of auxotrophy ly	le	tr	ad	me
Wildtype	0 KR	100	(0/247) 0					
	56 KR	32	(46/135)34±8	5	0	4	18	19
XS5/XS5	0 KR	100	(8/1216)0.66±0.5	8				
	4.7 KR	44	(24/536)4.5±0.04	8			4	12
XS8/XS8	0 KR	100	(0/476)0					
	9.4 KR	40	(44/187) 24±6	16			8	20
B.								
Wildtype	0 KR	100	0					
	56 KR	42	(8/332)2.4±0.5	4			4	
XS5/XS5	0 KR	100	(4/759)0.53±0.04	2			2	
	4.7 KR	38	(14/286)4.9±0.5	2			5	7
XS8/XS8	0 KR	100	(0/512) 0					
	18.6 KR	32	(34/1428) 2.4±0.2	8			12	14

Recessive homozygosis indicated as percent auxotrophy plus or minus 2 standard error units. Recessive homozygosis indicated as percent auxotrophy plus or minus 2 standard error units.

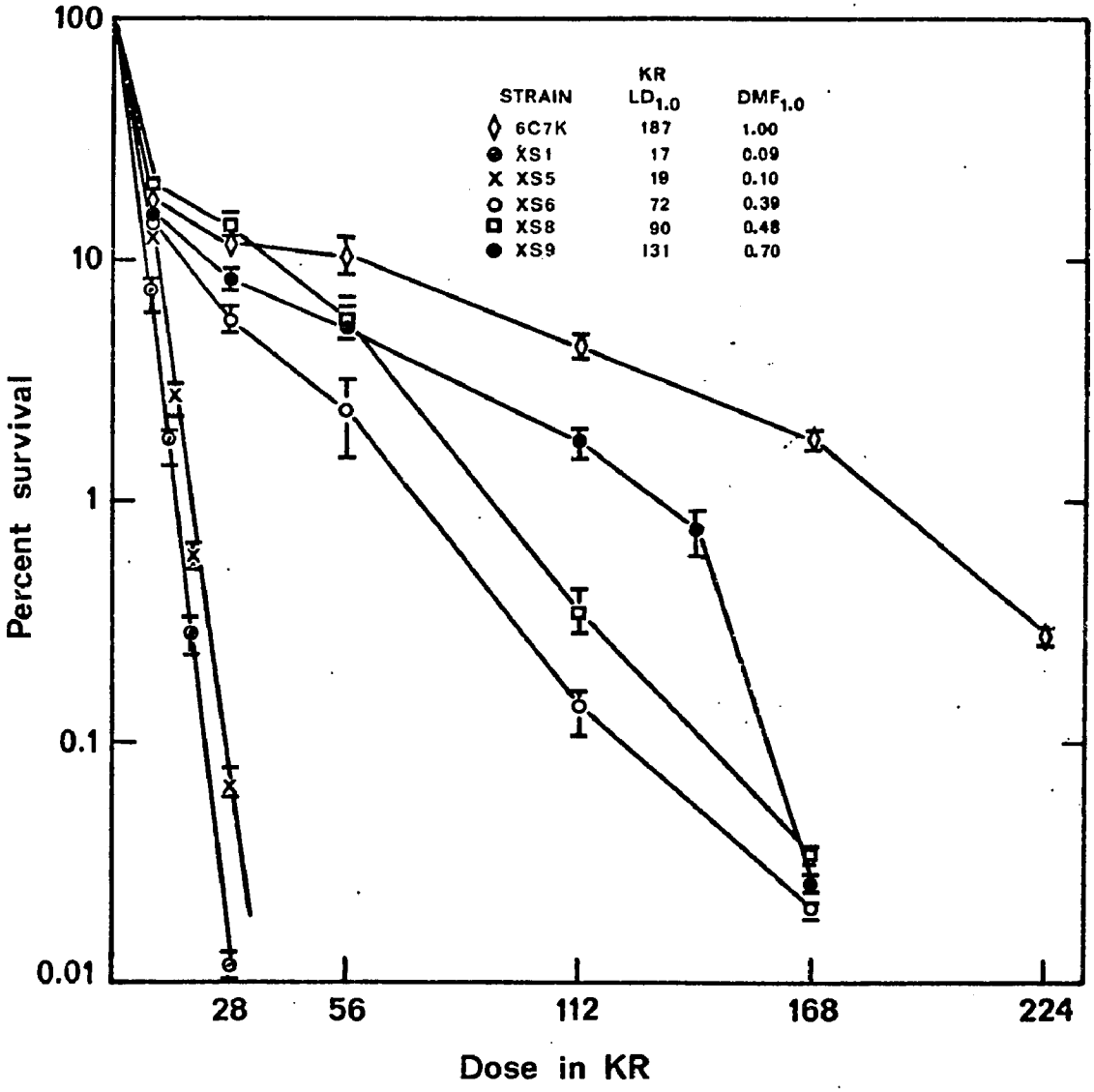


Figure 1. X-ray survival of haploids: SC7K, class I and class II mutants on YEPD.

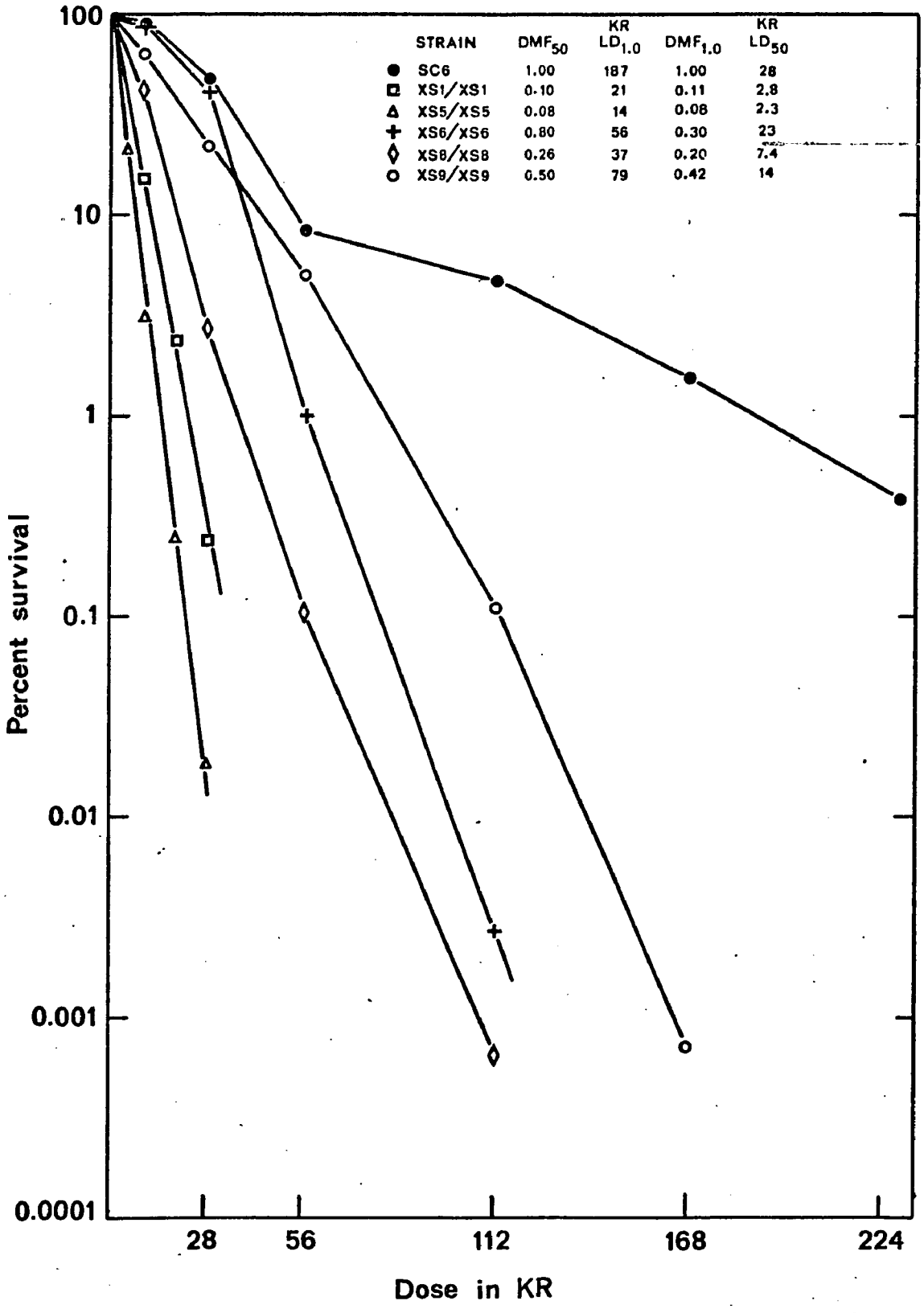


Figure 2. X-ray survival of diploids: SC-6 and homozygous XS/XS mutants on YEPD.

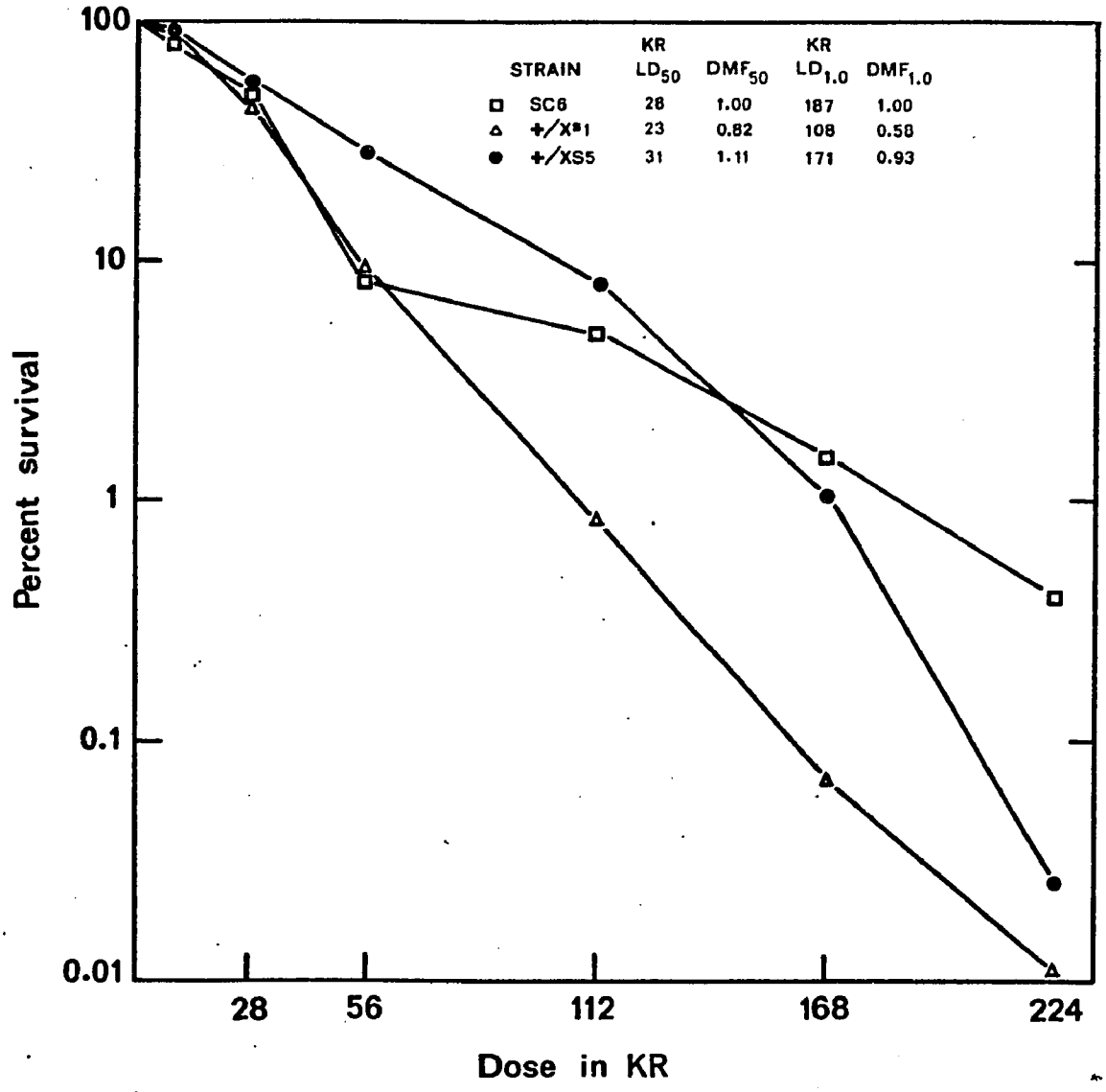


Figure 3. X-ray survival of wild type and class I heterozygous single mutants on YEPD.

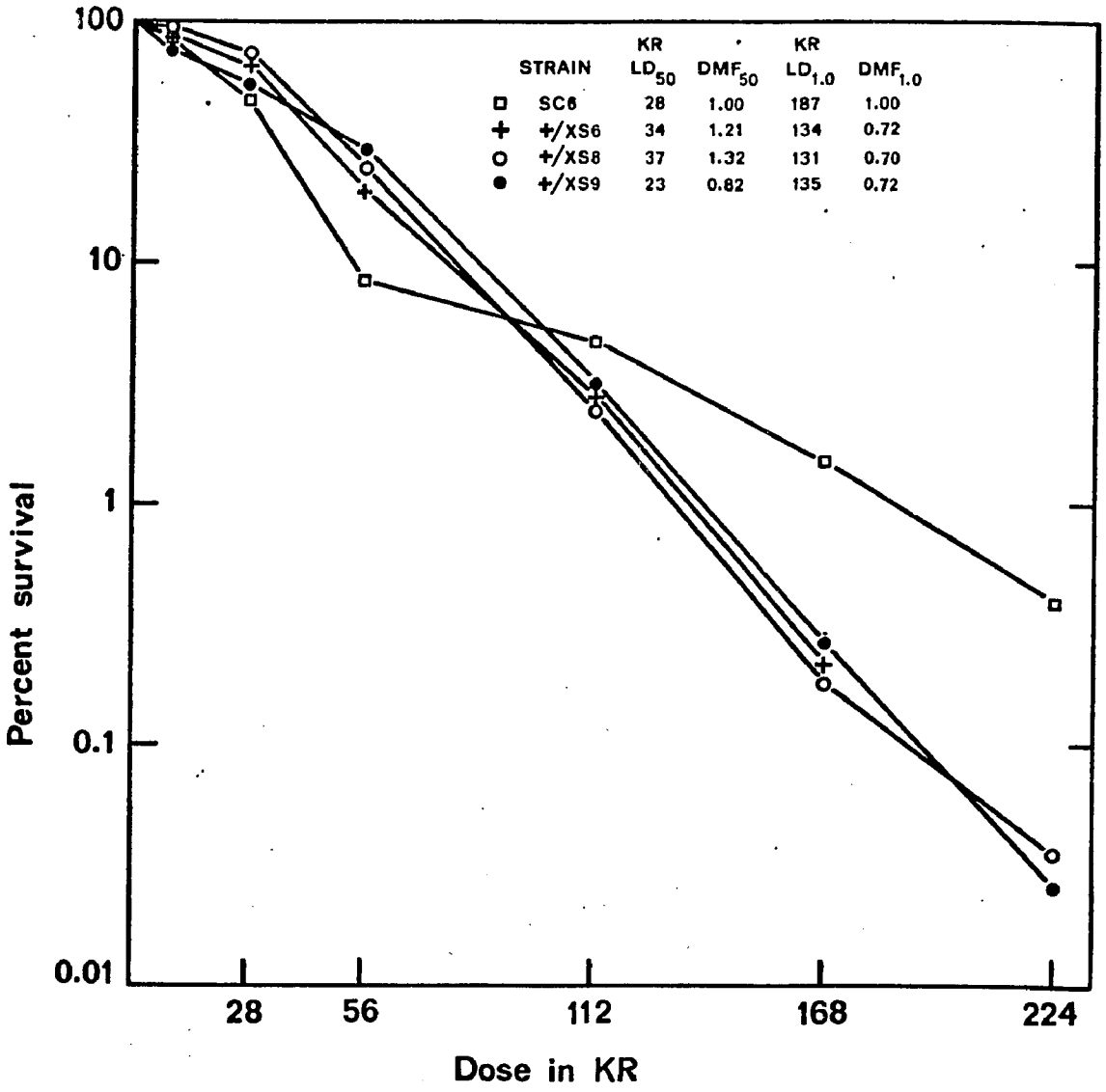


Figure 4. X-ray survival of wild type and heterozygous class II single mutants on YEPD.

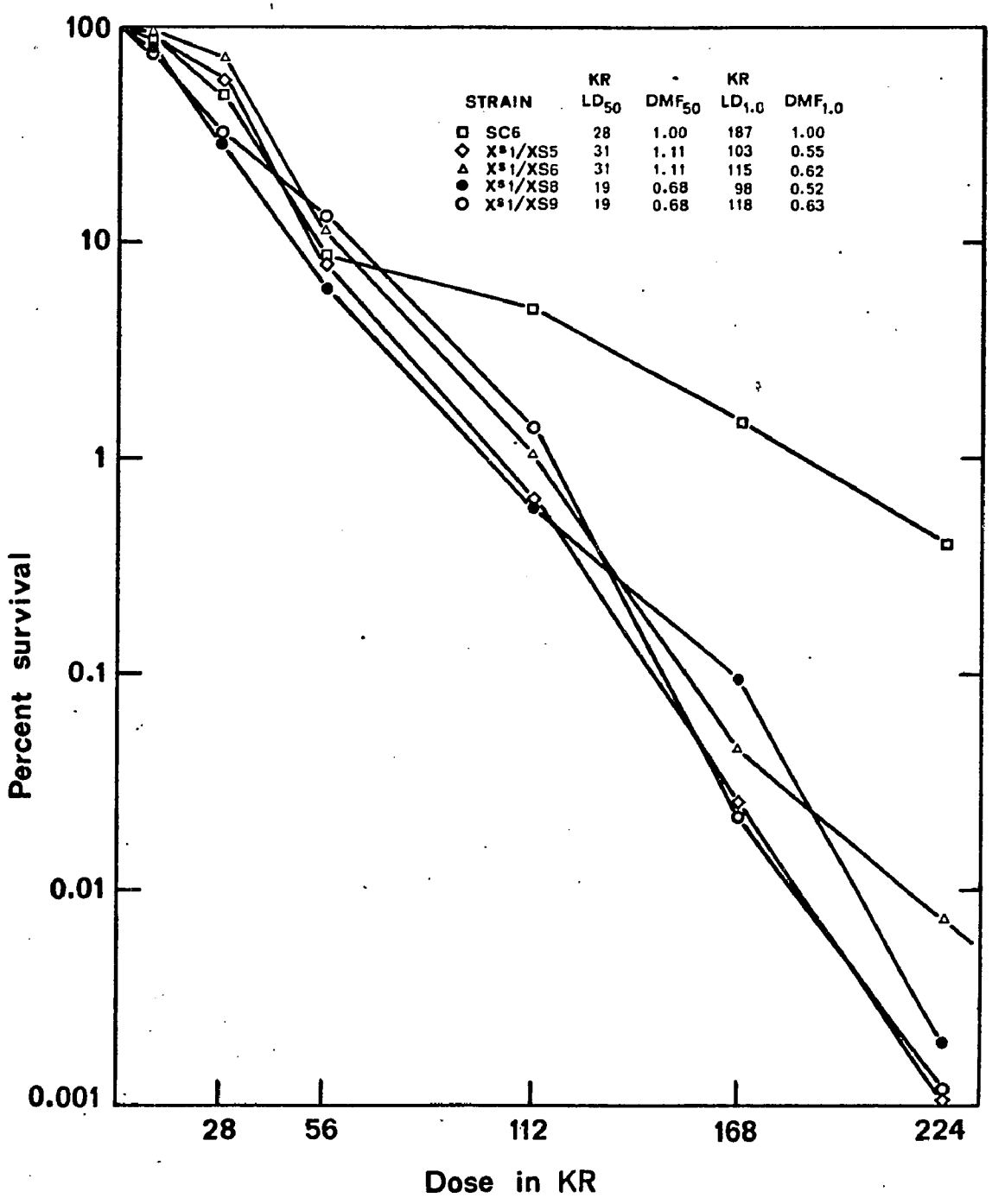


Figure 5. X-ray survival of: wild type, heterozygous class I, class I/ class II mutant diploids on YEPD.

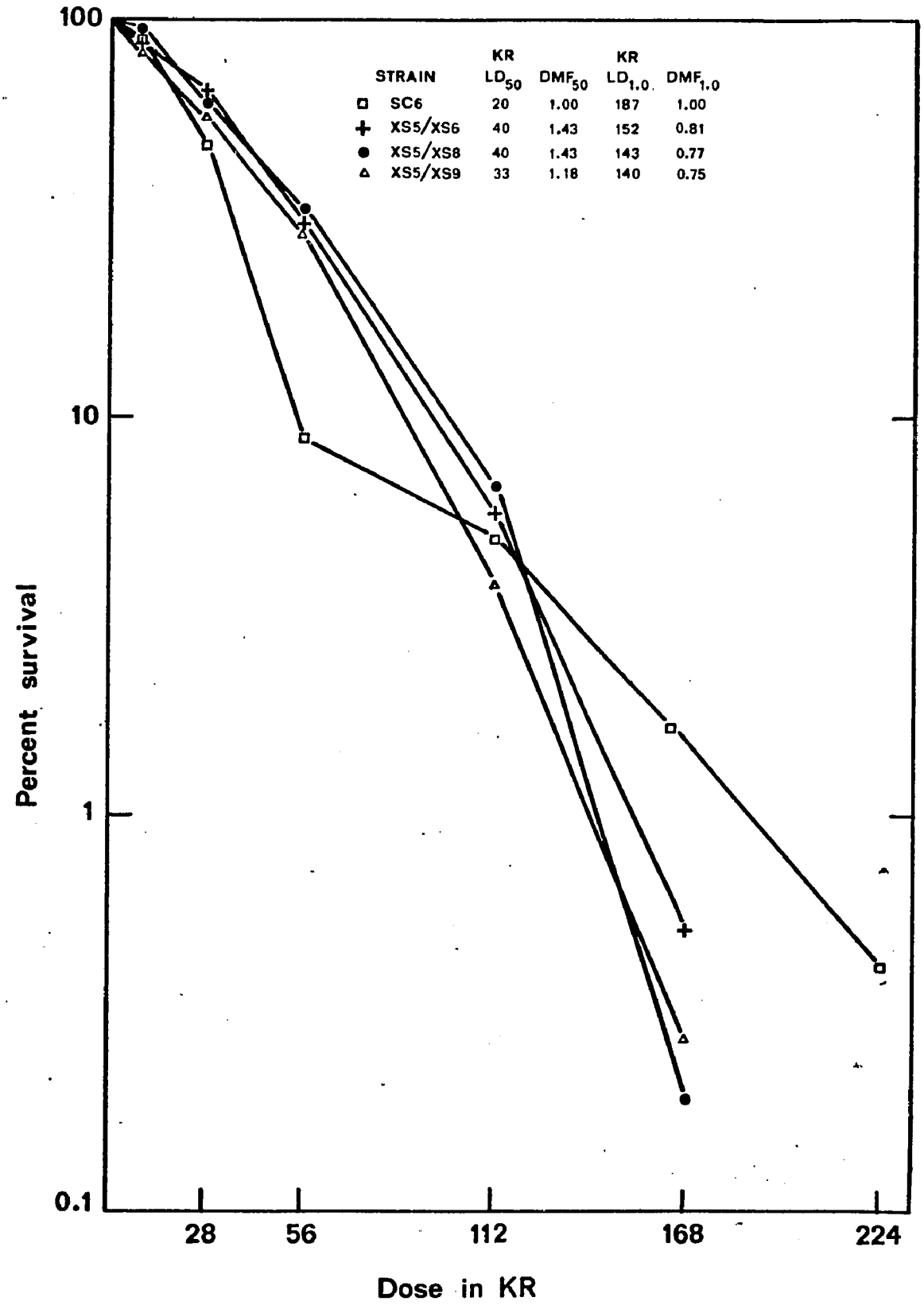


Figure 6. X-ray survival of: wild type, heterozygous class I/class II, class I/class II diploids on YEPD.

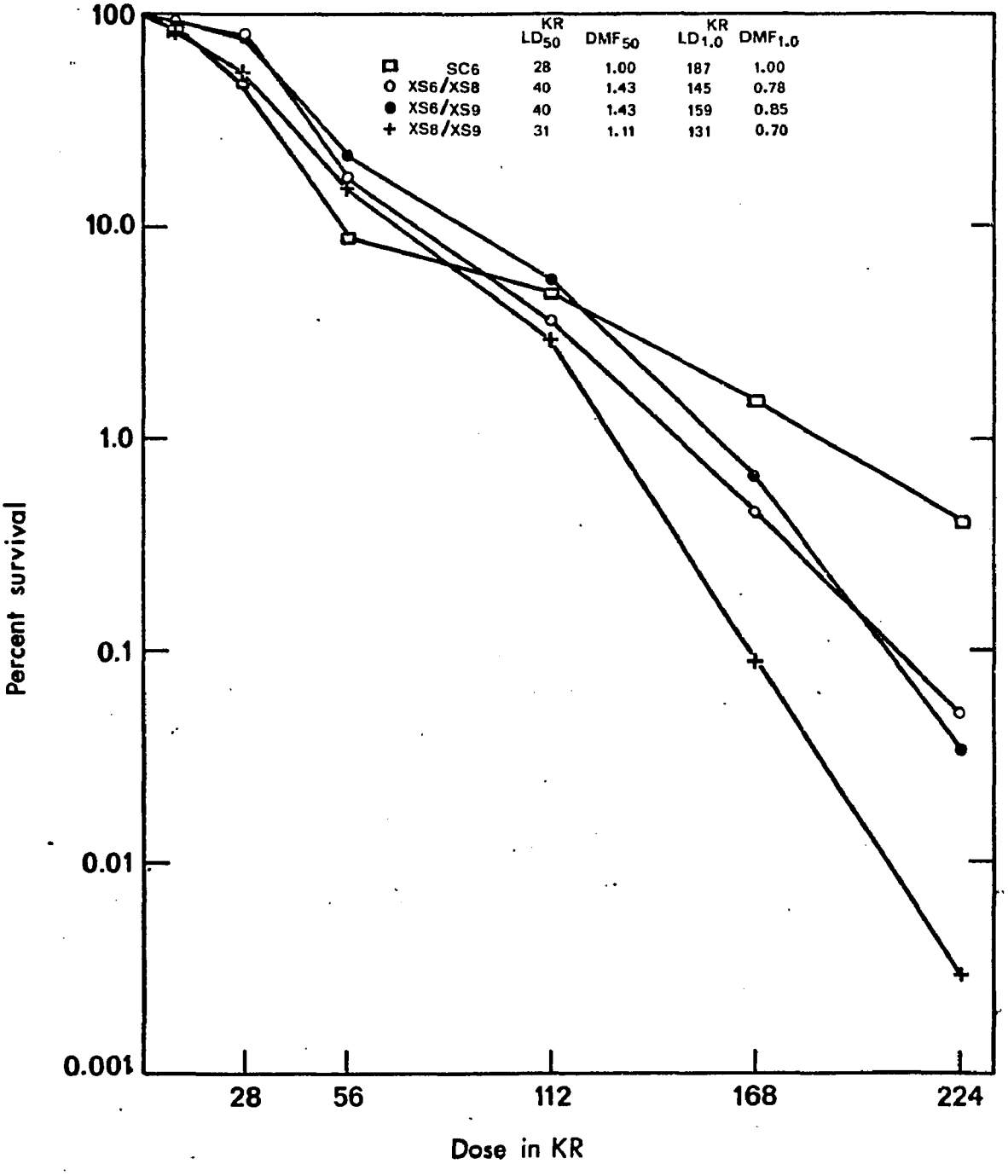


Figure 7. X-ray survival of heterozygous Class II · Class II mutant diploids on YEPD.

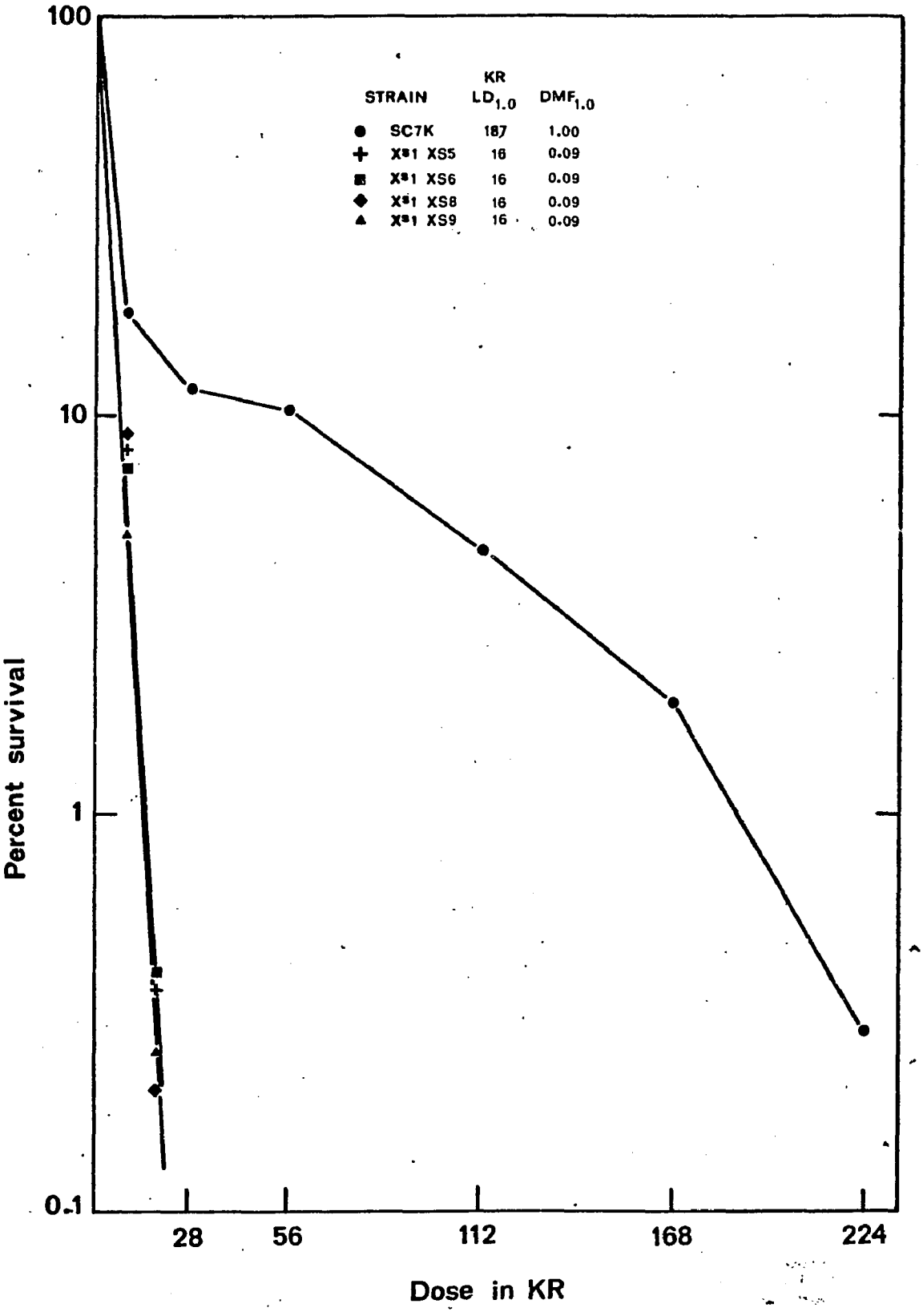


Figure 8. X-ray survival of wild type and haploid double mutants: X^{s1} XS5, X^{s1} XS6, X^{s1} XS8, X^{s1} XS9 on YEPD.

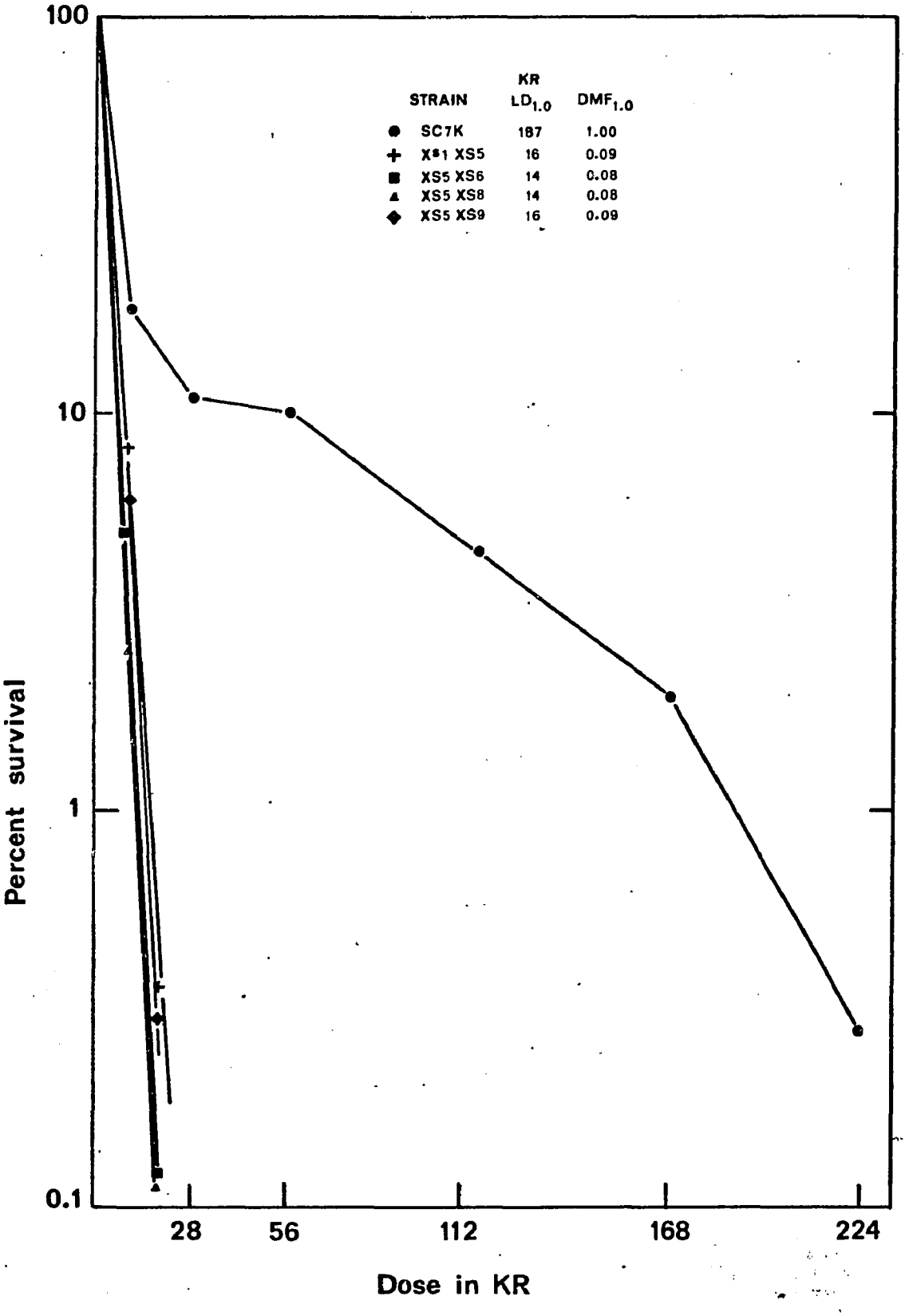


Figure 9. X-ray survival of wild type and haploid double mutants: X^{s1} XS5, X^{s5} XS6, X^{s5} XS8, X^{s5} XS9 on YEPD.

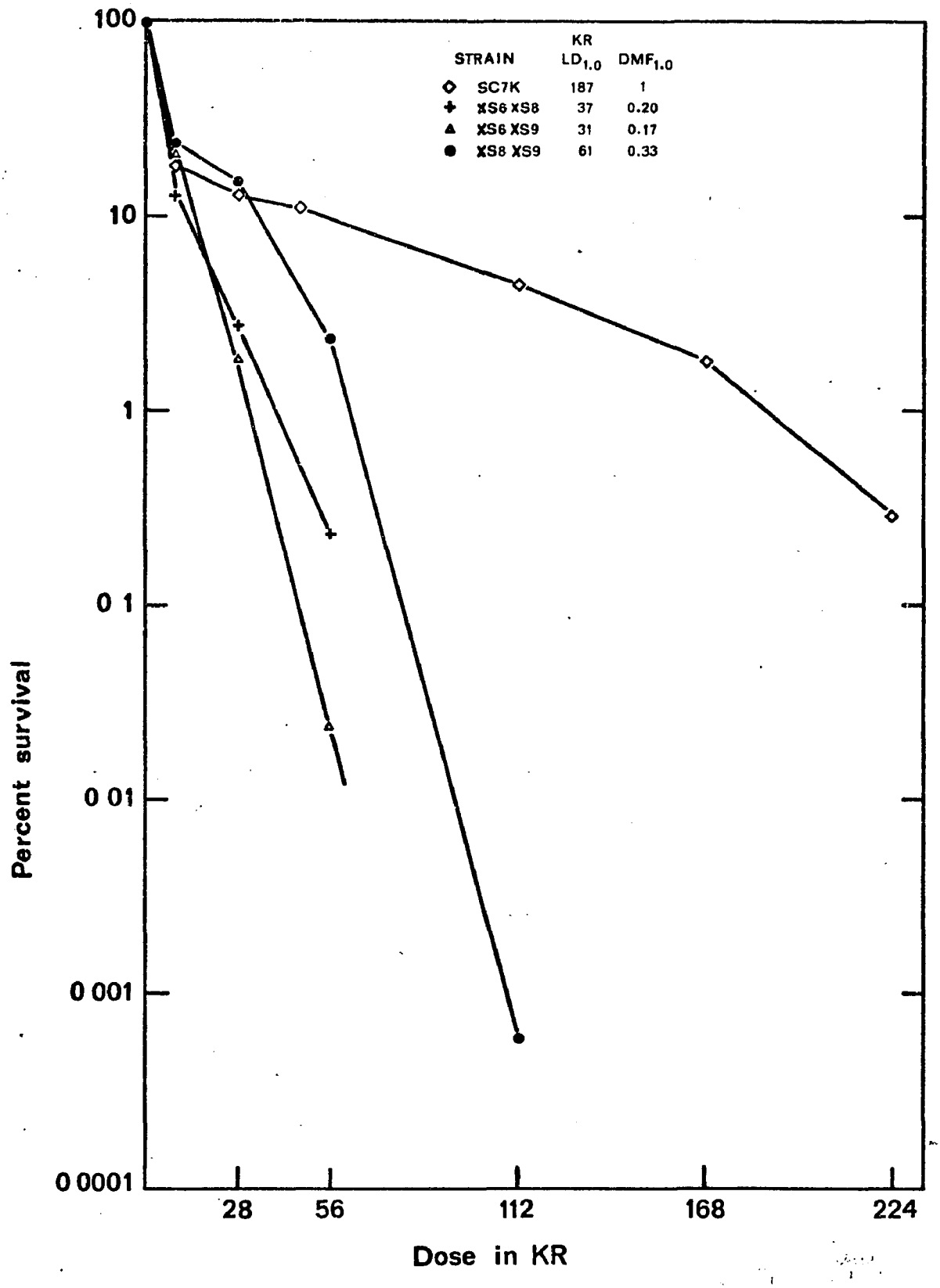


Figure 10. X-ray of wild type and haploid double mutants: XS6 XS8, XS6 XS9 and XS8 XS9 on YEPD.

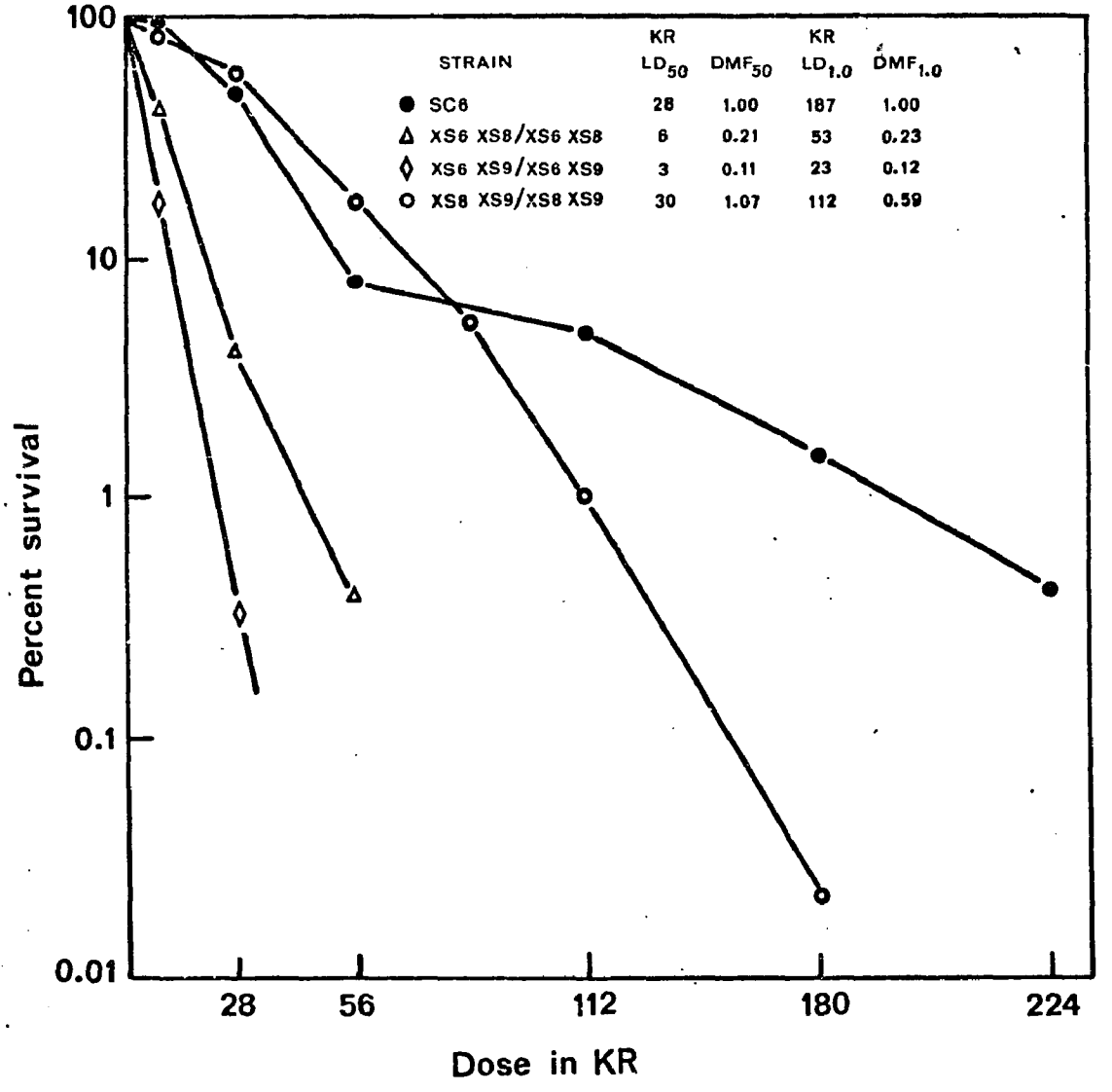


Figure 11. X-ray survival of wild type and class II/class II homozygous double mutant diploids on YEPD.

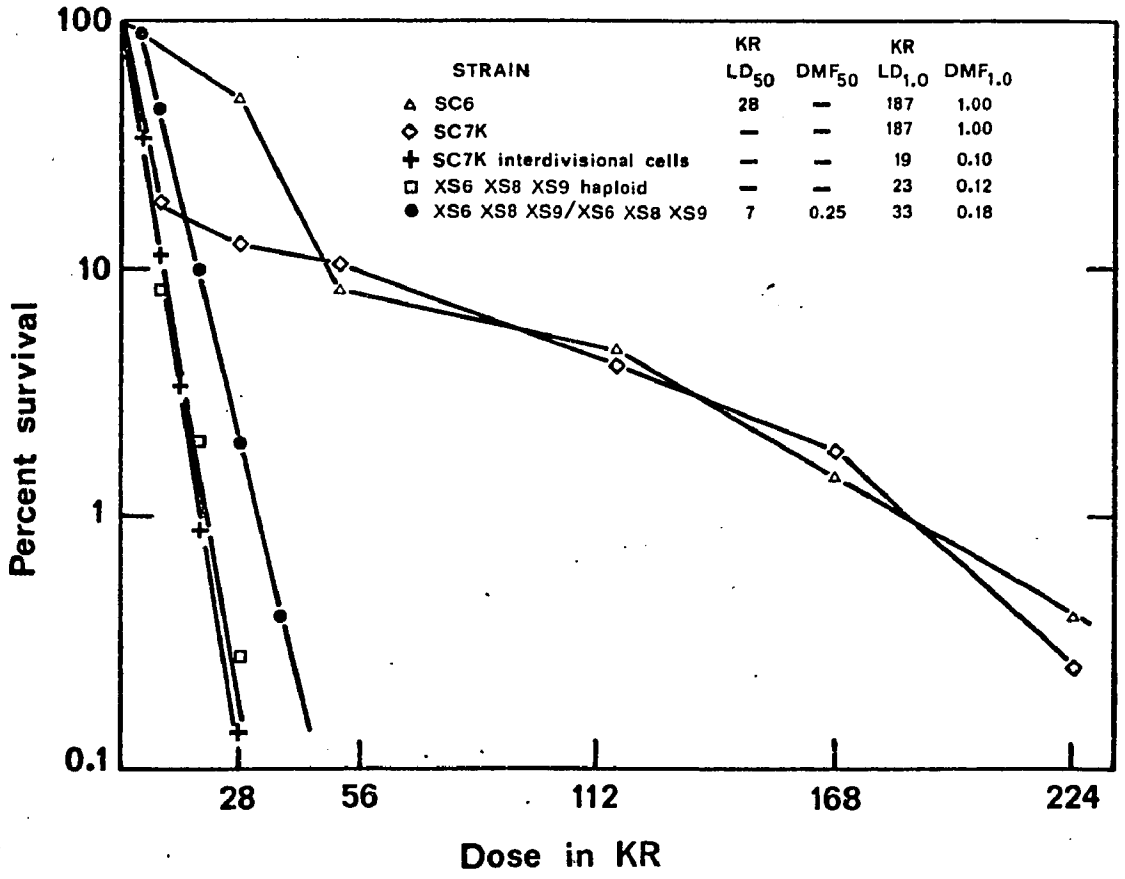


Figure 12. X-ray survival of: wild type diploid, wild type haploid, wild type haploid interdivisional cells, XS6 XS8 XS9 haploid and XS6 XS8 XS9/XS6 XS8 XS9 homozygous diploid on YEPD.

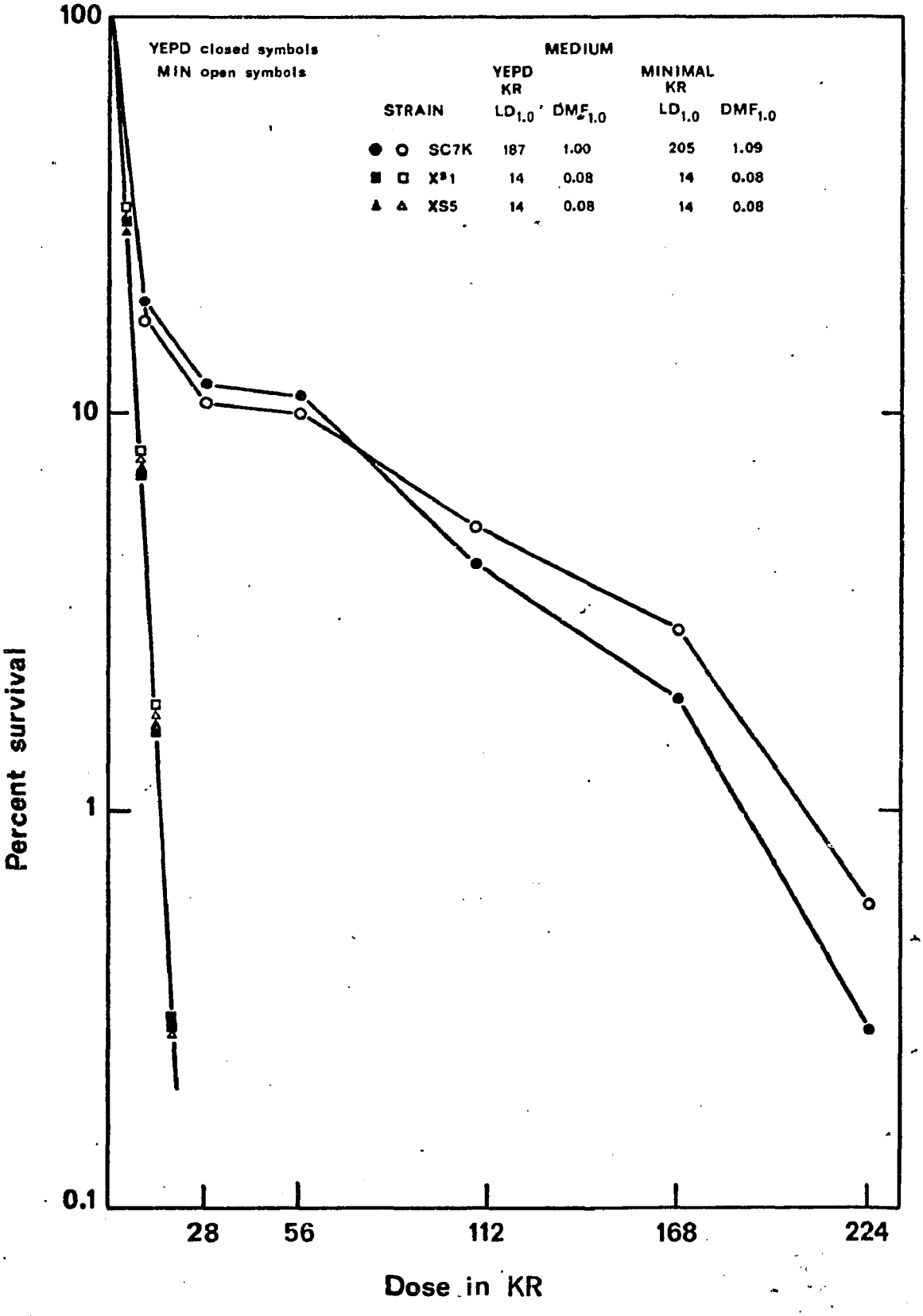


Figure 13. X-ray survival of wild type and class I mutants on YEPD and on minimal agar.

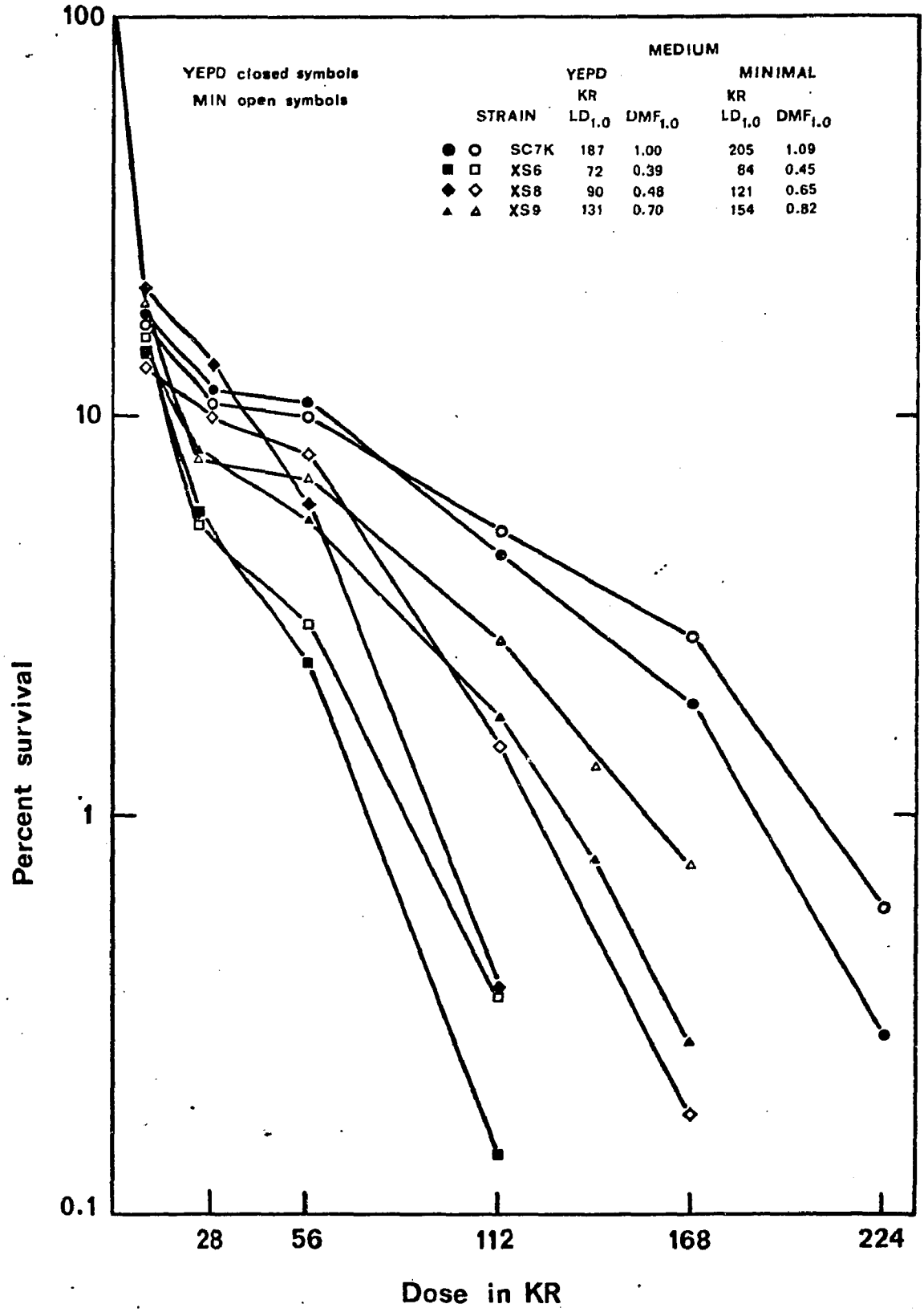


Figure 14. X-ray survival of wild type and class II mutants on YEPD and on minimal agar.