Characterization of Null Mutants of the *RAD55* Gene of *Saccharomyces cerevisiae*: Effects of Temperature, Osmotic Strength and Mating Type

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ABSTRACT

RAD55 belongs to a group of genes required for resistance to ionizing radiation, *RAD50-RAD57*, which are thought to define a pathway of recombinational repair. Since all four alleles of *RAD55* are temperature conditional (cold sensitive) for their radiation phenotype, we investigated the phenotype produced by null mutations in the *RAD55* gene, constructed *in vitro* and transplaced to the yeast chromosome. The X-ray sensitivity of these null mutant strains was surprisingly suppressed by increased temperature, osmotic strength of the growth medium and heterozygosity at the mating-type locus. These first two properties, temperature conditionality and osmotic remediability, are commonly associated with missense mutations; these *rad55* null mutants are unique in that they exhibit these properties although the mutant gene cannot be expressed. X-ray-induced mitotic recombination was also cold sensitive in *rad55* mutant diploids. Although mitotic growth was unaffected in these strains, meiosis was a lethal event at both high and low temperatures. Whereas the phenotype of *rad55* null mutants is consistent with a role of *RAD55* in recombination and recombinational repair, there is evidence for considerable *RAD55*-independent recombination, at least in mitotic cells, which is influenced by temperature and *MAT*. We discuss models for the role of *RAD55* in recombination to explain the unusual properties of *rad55* mutants.

RAD55 mutants of *Saccharomyces cerevisiae* have been isolated previously as mutants abnormally sensitive to the lethal effects of X-rays (GAME and MORTIMER 1974). Among genes affecting radiation repair, *RAD55* belongs to an epistatic group with the *RAD50, RAD51, RAD52, RAD54* and *RAD57* genes (BRENDEL and HAYNES 1973; GAME and MORTIMER 1974). Because these mutants affect survival primarily to agents that induce double-strand breaks into DNA and are defective in various recombinational processes as well, it is believed that these genes define a pathway of recombinational repair (LEMONTT 1980; HAYNES and KUNZ 1981; GAME 1983).

A novel property of *RAD55* is that all four alleles of *RAD55* are cold sensitive for radiation sensitivity. Cold-sensitive mutants are most commonly seen for proteins comprised of multiple subunits, most likely because protein-protein interactions are entropy driven and intrinsically cold sensitive (KAUZMANN 1959; SCHERAGA, NEMETHY and STEINBERG 1962). For example, large protein complexes such as microtubules are naturally cold labile. Some mutants can be obtained only as cold sensitive; attempts at isolating temperature-conditional mutants in ribosome assembly were unsuccessful until cold-sensitive alleles were sought (GUTHRIE, NASHIMOTO, and NOMURA 1969; TAI, KESSLER and INGRAHAM 1969). The fact that all alleles of *RAD55* are cold sensitive might indicate that *RAD55* is part of a multimeric complex, and possibly, since all *RAD55* alleles are conditional, that complete loss of *RAD55* function would be lethal to the cell.

By constructing null alleles *in vitro* and transplacing these to the yeast chromosome we have investigated the consequences of complete loss of *RAD55* function on various processes such as mitotic and meiotic viability, radiation repair and recombination. These *rad55* null mutations are unique in that some of their mutant phenotypes are temperature conditional (cold sensitive) and osmotically remedial, properties previously considered to be diagnostic of missense mutations. In addition, we find that the *MAT* genotype can modify some of these *rad55* mutant phenotypes. We have formulated several models of *RAD55* function in recombination to explain these unusual properties. In addition to their interest as a genetic novelty, these results raise questions about the complexity of genetic control of recombination in yeast.

MATERIALS AND METHODS

Strains, plasmids and media: The strains and plasmids used in this study are presented in Table 1; those we have constructed are described in further detail below. The media used are as described in SHERMAN, FINK and HICKS

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TABLE 1

Strains and plasmids

A. Strains	Genotype	Source
CG378	**a** *can1 ade5 trp1 leu2 ura3*	C. GIROUX
CG279	*α his7 ade5 trp1 leu2 ura3*	C. GIROUX
STL20-1	*α his7 ade5 trp1 leu2 ura3 rad55-5::LEU2*	This study
STL21-1	*α his7 ade5 trp1 leu2 ura3 rad55-6Δ::URA3*	This study
STL22-1B	**a** *ade5 trp1 leu2 ura3 rad55-6Δ::URA3*	This study
STL27-3C	**a** *can1 his1-1 trp2 leu2*	This study
STL27-4B	**a** *can1 his1-1 trp2 leu2 rad55-5::LEU2*	This study
STL28-9B	*α ura3 hom3 his1-7 leu2 ade2 rad55-5::LEU2*	This study
STL38-3D	*α ura3 hom3 his1-7 ade2*	This study
B. Plasmids	**Description**	**Source**
YCp50	*ARS* centomere vector	STINCHCOMB, MANN and DAVIS (1982)
YEp13	2 μ vector	BROACH, STRATHERN and HICKS (1979)
YEp13-*RAD55*-13C	Original *RAD55* clone in YEp13	CALDERON, CONTOPOULOU and MORTIMER (1983)
pJM3	*MAT***a** *Hin*dIII fragment in YCp50	J. MARGOLSKEE
pJM9	*MATα Hin*dIII fragment in YCp50	J. MARGOLSKEE
pSTL4	*RAD55 Hin*dIII fragment in YCp50	This study
pSTL11	*rad55-5::LEU2* in pSTL4	This study
pSTL29	*rad55-6Δ::URA3* in YEp13-*RAD55*-13C	This study

(1982). YEPD (supplemented with 30 μg/ml adenine sulfate for *ade* auxotrophs) was used routinely for the germination of spores and X-ray survival assays. Supplemented synthetic media were used for the selection of diploids, marker scoring and in X-ray-induced recombination experiments. Sporulation medium was 1% potassium acetate with necessary base and amino acid supplements.

DNA techniques: Plasmid DNA was purified by the alkaline SDS procedure and DNA fragments by electroelution (MANIATIS, FRITSCH and SAMBROOK 1982). Yeast was transformed by the LiCl method (ITO *et al.* 1983).

Construction of *rad55* mutants: The *RAD55* gene was derived from plasmid YEp13-*RAD55*-13C (CALDERON, CONTOPOULOU and MORTIMER 1983). A 1.8-kb *Hin*dIII fragment was cloned into centromere vector YCp50 (STINCHCOMB, MANN and DAVIS 1982) and the resulting plasmid, pSTL4, was shown to complement *rad55-3* and subsequent *rad55* null mutations by X-ray survival assays. Sequence analysis of this fragment (S. T. LOVETT and R. K. MORTIMER, unpublished data) yields an open reading frame extending from base 297 to 1306 as depicted in Figure 1. The *rad55-5::LEU2* mutation was constructed by inserting the *Xho*I/*Sal*I fragment of *LEU2*, derived from plasmid YEp13 (BROACH, STRATHERN and HICKS 1979) into the chromosomal *Sal*I site of pSTL4, creating plasmid pSTL11. The *rad55-6Δ::URA3* mutation was constructed in plasmid YEp13-*RAD55*-13C by deleting the 1.8-kb *RAD55* fragment by partial *Hin*dIII digests and inserting in its place the *URA3* *Hin*dIII fragment of YEp24 (BOTSTEIN *et al.* 1978). This plasmid, pSTL29, and pSTL11 failed to complement *rad55* mutations in X-ray survival tests. The *Hin*dIII fragment of pSTL11 (carrying *rad55::LEU2*) and the *Bam*HI/*Eco*RI fragment of pSTL29 (carrying *rad55Δ::URA3*) were transformed into CG379, yielding strains STL20-1 and STL21-1, respectively. STL22-1B was a segregant from the sporulation of diploid CG378/CG379 transformed with the *rad55Δ::URA3* construction. The *rad55* mutations in these strains segregate 2:2 in crosses, with *LEU2* or *URA3* cosegregating with the cold-sensitive *rad* mutation, and fail to complement the X-ray sensitivity of *rad55-3* in diploids. The mutant constructions were also confirmed by Southern blot analysis of DNA from these strains, probed with *RAD55* plasmid DNA. (It was also noted that no additional homologies to *RAD55* are present in these strains.)

X-ray survival assays: The X-ray source and dose rate were as described in GAME and MORTIMER (1974). For plate tests, patches were grown on YEPD at 23°, 30°, or 36°, replica plated to several plates which were then irradiated with 0, 25, 50, or 75 krad. Plates were scored after 2–4 days incubation at the appropriate temperature. For survival curves, strains were grown at the appropriate temperature to 1–2 × 10^7 cells/ml, sonicated to dissociate clumped cells, diluted in water, plated to YEPD, and immediately irradiated. Each dose was delivered to duplicate plates of the same dilution. For the osmotic remediability experiments, cells were grown to 5 × 10^6 cells/ml in YEPD. The culture was then split and spun down: one-half was returned to YEPD and the other resuspended in YEPD + 1 M KCl, NaCl or ethylene glycol. These cultures were incubated for 2 hr, then diluted and plated to YEPD or YEPD + 1 M KCl, NaCl, or ethylene glycol and irradiated as above. We found that pregrowth in high osmotic strength media was not necessary to observe a suppressive effect but did augment the response somewhat.

X-ray-induced mitotic recombination: The strains used in these experiments were constructed by at least two crosses with g833-2D (*α hom3-10 his1-7 ade2*) or g833-2B (**a** *can1 his1-1 trp2 leu2*) provided by JOHN GAME, starting with STL20-1. Diploids (*RAD*$^+$ and *rad55* mutant) were freshly selected, subcloned and grown in parallel in YEPD to 1–2 × 10^7 cells/ml. They were then washed in water, sonicated, diluted in water and plated to both synthetic complete medium (for total viable cell count) and to complete minus histidine (for recombinant count). Plates were irradiated immediately with duplicates for each dose and dilution. Data illustrated in the test are representative of 2–3 determinations, giving virtually identical results.

RESULTS

The *rad55::LEU2* disruption allele (Figure 1) was introduced by transformation into diploid yeast. The resulting strains were sporulated and the spores were dissected. Viability of these spores was essentially

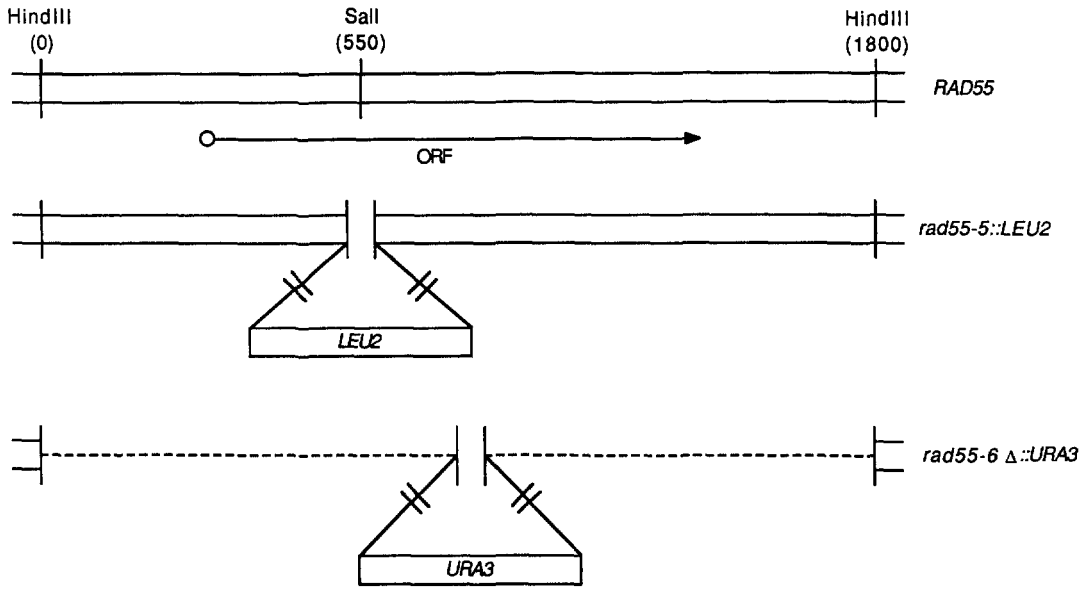

FIGURE 1.—Construction of *rad55* null alleles.

100%, with *LEU*$^+$ segregating 2:2; therefore an intact *RAD55* gene is not essential for mitotic growth. When the resulting spore clones were assayed for X-ray survival, we found to our surprise that the *RAD55*::*LEU2* segregants appeared very sensitive when grown and assayed at 23° (at levels close to the most X-ray sensitive *rad* mutants such as *rad52*) but quite resistant at 36°. Since it was possible that these constructions might lead to a partially functional *RAD55* product by allowing a truncated or fused protein to be expressed, we constructed a deletion which spanned the entire *RAD55* gene (Figure 1), transformed this into yeast and assayed the resulting strains for X-ray sensitivity. We found that even the complete deletion of *RAD55* led to a cold-dependent X-ray-sensitive phenotype, virtually identical to that seen for the disruption allele. Figure 2, A and B, illustrates the dramatic increase in X-ray survival with increased temperature for these *rad55* null mutant strains. *rad55* null mutants are therefore behaving like classic missense mutants, exhibiting a strongly temperature-conditional phenotype.

Another property which has been considered to be diagnostic of missense mutations is osmotic remediability of the mutant phenotype. This phenomenon, common in both bacteria and yeast (KOHNO and ROTH 1979; HAWTHORNE and FRIIS 1964), is not completely understood, although in some cases, mutant enzymes have been shown to be rescued by increased ionic strength *in vivo* and *in vitro*. It is generally assumed that increasing the osmotic strength of the growth media leads to perturbations of ionic components with the cell resulting in a protein conformation more enzymatically active or less susceptible to proteolysis. We tested the X-ray survival of *rad55* mutants when grown in various high osmotic strength media. When grown in high concentrations of KCl, the X-ray sensitivity of *rad55* deletion mutants was substantially suppressed (Figure 2C). Very similar results were obtained when NaCl or ethylene glycol were substituted for KCl (data not shown).

We have found one other factor which, like increased temperature and osmotic strength, suppresses the X-ray sensitive phenotype of *rad55* null mutants—heterozygosity at the mating type locus. Introduction of a centromere plasmid carrying *MAT***a** into a *MAT*α *rad55* disruption strain improves survival substantially compared to the same strain transformed with an analogous plasmid carrying *MAT*α (Figure 2D). The suppressive effects of temperature and *MAT* heterozygosity are additive; the X-ray resistance of these *MAT***a**/*MAT*α *rad55* mutant strains is still improved with increased temperature. In agreement with this observation we have isolated mutations in the *SIR3* and *SIR4* genes as partial suppressors of *rad55* null mutations (S. T. LOVETT and R. K. MORTIMER, unpublished results); these mutations allow both *MAT* alleles to be expressed by eliminating repression of normally silent loci, *HML* and *HMR*. *MAT* heterozygosity also suppresses the X-ray phenotype seen in *rad55* mutant diploids; whereas α/α *rad55* diploids show a sensitivity roughly equivalent to *rad55* haploid strains, the corresponding **a**/α *rad55* diploids are more resistant at each temperature (data not shown).

MAT effects on X-ray survival and recombination in wild-type strains have been noted previously: **a**/α diploids are slightly more X-ray resistant than their **a**/**a** or α/α counterparts (MORTIMER 1958; LASKOWSKI 1960). **a**/α strains are also severalfold more competent for certain types of spontaneous and UV-induced recombination events (FRIIS and ROMAN 1968, ESPOSITO *et al.* 1982). These results have been interpreted

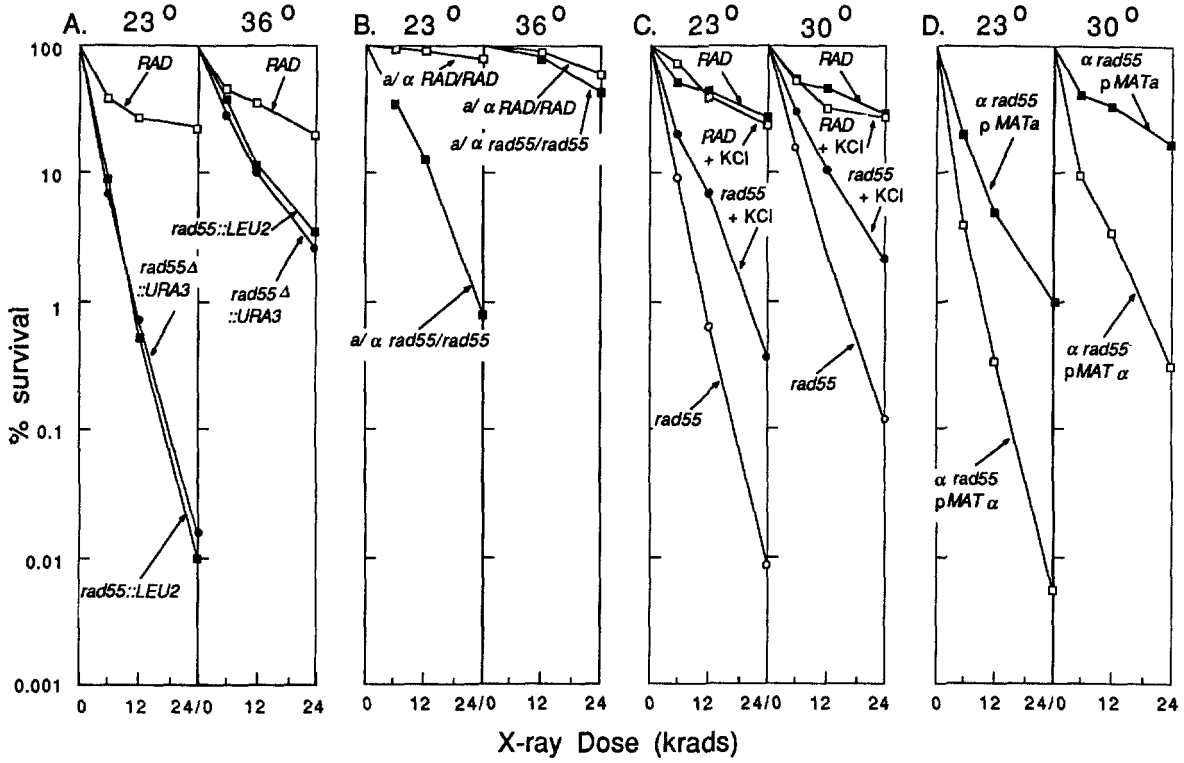

FIGURE 2.—X-ray survival curves. A, Temperature effects on X-ray survival of isogenic haploids CG379 (*RAD*$^+$), STL20-1 (*rad55*::*LEU2*) and STL21-1 (*rad55*Δ::*URA3*). B, Temperature effects on survival of **a**/α diploids CG378/CG379 (*RAD*$^+$/*RAD*$^+$) and STL21-1/STL22-1B (*rad55*Δ::*URA3*/*rad55*Δ::*URA3*). C, Osmotic effects on survival of haploids CG379 (*RAD*$^+$) and STL21-1 (*rad55*Δ::*URA3*). D, *MAT*-effects on survival of haploids STL20-1 (*rad55*::*LEU2*).

by supposing that either some functions that are limiting for recombination and recombinational repair are under *MAT*-regulation or that chromosome structure or the progression through the cell cycle is more advantageous for recombination in heterozygous *MAT* strains. The more extreme effect we see of *MAT* heterozygosity in *rad55* strains may be a manifestation of the phenomenon seen with wild-type strains and reflect an increased capacity to undergo recombinational repair, especially with *RAD55* gene product is not present.

Since *rad55* mutants are extremely sensitive to X-rays (at least at 23°) and other agents known to induce double-strand breaks in DNA, we might expect that these mutants would be defective in some aspect of double-strand break repair mediated by recombination. We were therefore interested in determining if our mutants were defective in recombinational events induced by ionizing radiation. Previous studies have conflicted on this matter (STRIKE 1978; SAEKI, MACHIDA and NAKAI 1978). With **a**/α diploids carrying the *his1-1* allele on one chromosome and *his1-7* on the other we assayed the ability of *HIS*$^+$ recombinants (presumably gene convertants) to be produced after X-irradiation at both 23° and 36°. We found that X-ray-induced recombination at *HIS1* was less efficient in *rad55* mutants than in the *RAD*$^+$ control at 23°; at 36°, recombination in *rad55* mutants approached that of the *RAD*$^+$ strain (Figure 3). Virtually identical results were obtained for analogous strains carrying the *rad55*Δ::*URA3* allele (data not shown). A low level of induction of recombination was seen in *rad55* mutants even at 23°; this may be due to partial suppression of *rad55* effects by *MAT* heterozygosity in these strains. Nevertheless, the recombinants which were obtained in the *rad55* strain at 23° appeared only after prolonged incubation (7–9 days *vs.* 4 days for *RAD*$^+$). In these same strains spontaneous recombination at *HIS1* was not noticeably reduced even at 23°, among several determinations.

Mutations in the *RAD50-57* epistasis group lead to meiotic inviability (spores are produced but they fail to germinate) presumably because they are defective in some aspect of recombination which is necessary to insure proper chromosome segregation at the first meiotic (reductional) division. *rad55* mutations have previously been shown to be meiotically lethal (GAME and MORTIMER 1974) but we wished to confirm this observation and see if the lethality would be influenced by temperature in our *rad55* null mutants. The diploid *rad55* strain STL27-4B/STL28-9B was sporulated at 23° and 34° and asci dissected. The sporulation efficiency was approximately 70% at both temperatures. Spore viability was 0/40 at 23° and 0/48 at 34°C. When the spores were examined microscopically, not a single division had occurred. Spore via-

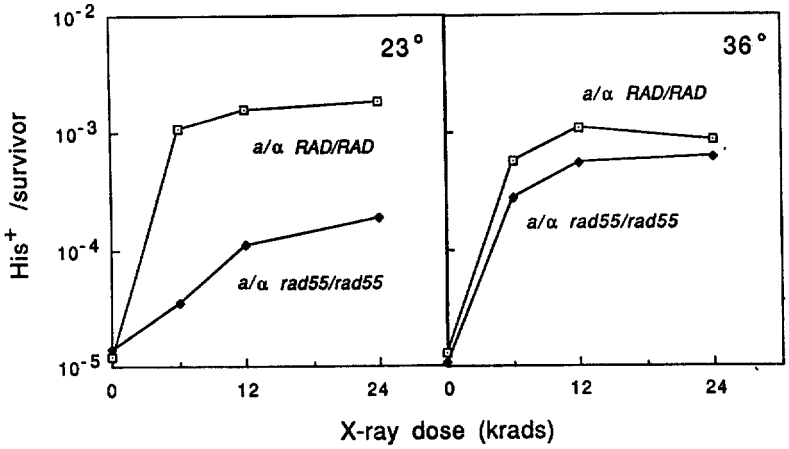

FIGURE 3. X-ray induced recombination of *his1-1/his1-7* at 23°, 36°, for a/α diploids STL27-4B/STL28-9B (*rad55::LEU2/rad55::LEU2*) and STL27-3C/STL38-3D (*RAD$^+$/RAD$^+$*).

bility of the RAD$^+$ diploid STL27-3C/STL38-3D was 90–100%. The temperature conditionality seen for radiation survival in *rad55* mutants is not seen for meiotic viability, although meiotic survival in these mutants may be influenced by temperature but at levels below our detection. Also note that since these are **a**/α diploids, expression of both *MAT* alleles, which is capable of largely suppressing the radiation phenotypes of *rad55* mutants, does not promote successful meiosis. Therefore, the *rad55*-independent process which promotes almost normal X-ray survival in these mutants at high temperatures or in heterozygous *MAT* strains is not operative at sufficient levels to insure normal meiotic survival. The requirement of efficient recombination may be more stringent in meiosis than for mitotic repair processes, or alternatively, a *RAD55*-independent process may operate in mitotic but not in meiotic recombination.

DISCUSSION

The *RAD55* gene is a member of a group of genes, *RAD50-57*, which are believed to be involved in recombination and recombinational repair [reviewed in LEMONTT (1980), HAYNES and KUNZ (1981) and GAME (1983)]. Mutants in these genes are sensitive primarily to agents (such as ionizing radiation) known to induce double-strand breaks into DNA which must be repaired by the interaction of homologous chromosomes. Moreover, mutants in these genes also have broad effects on various mitotic recombinational processes. All these mutants are meiotically lethal; formation of crossovers is required to align and segregate chromosomes at the first meiotic division (BAKER *et al.* 1976). Some mutants have been shown by various means to meiotically Rec$^-$ as well.

We have characterized null mutations in the *RAD55* gene which lead remarkably to a cold-sensitive and osmotically remedial X-ray survival phenotype. Mutations which produce temperature conditional and osmotically modified phenotypes have been assumed to result from the conditional function of the mutated gene. In this case, deletion mutations in *RAD55* which disallow any expression of the gene produce conditional phenotypes; the conditional phenotype must therefore be due to the conditional function of other processes which are revealed when the *RAD55* gene is absent. It is important to note that X-ray survival and X-ray induced recombination are not intrinsically cold sensitive and that the temperature dependence of these processes appears only when the *RAD55* gene is mutant.

We have also seen that heterozygosity at the *MAT* locus (specifically the coexpression of the *MAT***a***1* and *MATα2* genes—LOVETT and R. K. MORTIMER, unpublished data) dramatically increases the "permissivity" of *rad55* mutations with respect to radiation survival. We believe that this is a reflection of a similar phenomenon seen in wild-type strains where heterozygosity at *MAT* increases somewhat the competence of cells for radiation repair and recombination.

Similar to this increased competence for repair and recombination, competence for induction of meiosis and sporulation requires heterozygosity at *MAT*. The *MAT* regulation of sporulation appears to be mediated by the RME gene, since mutations in *rme* alleviate the requirement for *MAT* heterozygosity in sporulation (HOPPER and HALL 1975; KASSIR and SIMCHEN 1976; RINE, SPRAGUE and HERSKOWITZ 1981). Disruption mutations in *rme*, however, do not increase X-ray survival in strains homozygous for *MAT*, either *RAD$^+$* (A. MITCHELL and I. HERSKOWITZ, personal communication) or *rad55* (S. T. LOVETT and R. K. MORTIMER, unpublished data). It is likely therefore that *MAT* effects on recombination and repair are mediated differently than those on meiosis and sporulation. However, sporulation is less efficient and much slower in *rme*-suppressed homozygous *MAT* strains than in wild-type *MAT* **a**/α diploids and we can not rule out the possibility that *rme* mutations have a weak effect on recombination and repair which is indetectable in our assays or that the two phenomena share an additional common regulatory gene which has yet to be isolated.

These unusual properties of *RAD55* are shared by another gene which belongs in its epistasis group, *RAD57*. Disruption mutations of *RAD57* are cold-sensitive for radiation survival; this sensitivity is relieved, to the same degree as that of *rad55* mutants, by high osmotic strength and heterozygosity at *MAT* (D. SCHILD, S. T. LOVETT and R. K. MORTIMER, unpublished data). In addition, *RAD55* and *RAD57* are very similar in their effects on recombination and meiosis. The double *rad55 rad57* mutant is identical in phenotype to either single mutant (S. T. LOVETT and R. K. MORTIMER, unpublished data). We think that it is extremely likely that these two genes share the same function.

We have shown that *rad55* mutations reduce the recovery of recombinants induced by X-rays and, like the sensitivity of *rad55* mutant strains to the lethal effects of X-irradiation, this defect in recombination is alleviated at high temperatures. Meiosis in our *rad55* mutant diploids is a lethal event at both high and low temperatures. These phenotypes and *RAD55*'s epistatic interaction with other genes such as *RAD52* point to a role of *RAD55* in some aspect of mitotic and meiotic recombination. However, there is substantial *RAD55*-independent repair and recombination, at least mitotically, and these processes may be influenced by temperature, ionic strength and the *MAT* locus. Because meiotic viability in *rad55* mutants is low even in heterozygous *MAT* strains at elevated temperatures we must presume that these *RAD55*-independent events are either not operative in meiosis or are at insufficient levels or of inappropriate character to allow meiosis to proceed productively.

We can envision three possible scenarios to explain the unusual properties of *rad55* and *rad57* mutants. First, *RAD55* and *RAD57* may be positive regulators of other recombination genes. These genes must then have the capacity to be induced independently by heat or osmotic shock or by *MAT*-heterozygosity. We have failed to find any effect of *rad55* mutations on the expression of *lacZ* fusions to two genes involved in recombinational repair, *RAD52* and *RAD54* (S. T. LOVETT and R. K. MORTIMER, unpublished data). Of course the possibility remains that *RAD55* and *RAD57* regulate the activity of the *RAD52* and *RAD54* gene products in a way not detected by these assays or that they regulate some other gene we have yet to test.

The second scenario presumes that *RAD55* and *RAD57* play a part in recombination as accessory proteins in a complex of recombination proteins. In their absence, the complex would still be functional but would tend to be unstable, especially at low temperatures where protein-protein interactions are less favorable. *MAT* heterozygosity must either allow limited amounts of this complex to be more efficient in recombination or perhaps increase the amount of the proteins making up the complex such that the overall concentration of active complex in the cell is greater. To account for the meiotic lethality of *rad55* and *rad57* mutations, we would have to assume that this level would be insufficient to mediate recombination adequately in meiosis to ensure normal disjunction for all of 16 chromosomes at the first meiotic division.

Our third scenario would suppose that *RAD55* and *RAD57* participate in a process in recombination for which a substitute function exists. This alternate process may be naturally temperature-dependent and perhaps regulated by *MAT* in mitotic cells. This process would then have to be somewhat inefficient and unable to compensate for *RAD55* and *RAD57* loss in meiotic recombination. Because the distribution and characteristics of various exchanges differ among spontaneous mitotic, UV or X-ray induced mitotic, and meiotic recombination (reviewed by ESPOSITO and WAGSTAFF 1981) it is likely that multiple modes of recombination exist, dependent perhaps on the cell cycle (G_1 *vs.* G_2, mitotic *vs.* meiotic), cell mating type, and type of DNA substrate used (nicked, gapped, or broken, replicating or nonreplicating). For instance, as noted by ESPOSITO, mutations in enzymes involved in the processing of HOLLIDAY junctions would be expected to yield very different effects depending on the type of recombinational event which is monitored. Because spontaneous mitotic recombination did not appear to be affected in our mutants at low temperatures whereas X-ray induced events were reduced, the idea of genetically distinct recombination "pathways" may be warranted.

Although it is not possible at this time to discern which of these scenarios is correct, the complex phenotypes shown by *rad55* and *rad57* mutants raise important questions about recombination in *S. cerevisiae*. What aspects of recombinational repair are subject to mating type control and how is this mediated? Are there multiple pathways of recombination in yeast, as is evident in *Escherichia coli* (CLARK *et al.* 1985) and how do they differ with respect to their regulation, genetic requirements, and the kinds of genetic exchanges they catalyze? We are pursuing two approaches to elucidate the roles of *RAD55* and *RAD57* in recombination. First, we plan to investigate in more detail the types of exchanges affected by these mutations. Second, we have isolated a suppressor mutation to *RAD55* and *RAD57* whose effects are not mediated by *MAT* heterozygosity and we hope that the future characterization of this gene, *SPX1*, will explain the role of *RAD55* and *RAD57* in recombination.

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