Homologous Recombinational Repair of Double-Strand Breaks in Yeast Is Enhanced by *MAT* Heterozygosity Through yKU-Dependent and -Independent Mechanisms

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Manuscript received August 7, 2000
Accepted for publication November 13, 2000

ABSTRACT

DNA double-strand breaks (DSBs) are repaired by homologous recombination (HR) and nonhomologous end-joining (NHEJ). NHEJ in yeast chromosomes has been observed only when HR is blocked, as in *rad52* mutants or in the absence of a homologous repair template. We detected yKu70p-dependent imprecise NHEJ at a frequency of ~0.1% in HR-competent Rad$^+$ haploid cells. Interestingly, *yku70* mutation increased DSB-induced HR between direct repeats by 1.3-fold in a haploid strain and by 1.5-fold in a *MAT* homozygous (**a**/**a**) diploid, but *yku70* had no effect on HR in a *MAT* heterozygous (**a**/α) diploid. *yku70* might increase HR because it eliminates the competing precise NHEJ (religation) pathway and/or because yKu70p interferes directly or indirectly with HR. Despite the *yku70*-dependent increase in **a**/**a** cells, HR remained 2-fold lower than in **a**/α cells. Cell survival was also lower in **a**/**a** cells and correlated with the reduction in HR. These results indicate that *MAT* heterozygosity enhances DSB-induced HR by yKu-dependent and -independent mechanisms, with the latter mechanism promoting cell survival. Surprisingly, *yku70* strains survived a DSB slightly better than wild type. We propose that this reflects enhanced HR, not by elimination of precise NHEJ since this pathway produces viable products, but by elimination of yKu-dependent interference of HR.

DNA double-strand breaks (DSBs) can be repaired by homologous recombination (HR) or nonhomologous end-joining (NHEJ). It is thought that HR is the dominant repair mode in the yeast *Saccharomyces cerevisiae*, while NHEJ plays a larger role in mammalian cells. There are several distinct modes of HR, including conservative processes such as gene conversion and crossing over, and the nonconservative process termed single-strand annealing (SSA) that operates between direct repeats. Gene conversion involves nonreciprocal transfer of continuous blocks of information from a donor to a recipient allele, termed a conversion tract. Conversion tract lengths reflect both heteroduplex DNA (hDNA) formation, resulting from strand invasion and branch migration of Holliday junctions, and mismatch repair of hDNA (Petes *et al.* 1991; Nickoloff and Hoekstra 1998; Weng and Nickoloff 1998; Nickoloff *et al.* 1999). NHEJ involves interactions between regions sharing little or no homology. NHEJ can be nonconservative and mutagenic since ends can be joined imprecisely via annealing between single-stranded ends sharing short (1–5 bp) homologies. DSBs with cohesive ends, such as those generated by endonucleases, can be repaired by conservative, precise NHEJ (religation).

In yeast, most DSB-induced HR requires *RAD52* and other members of the *RAD52* epistasis group (Paques and Haber 1999). NHEJ is Rad52p independent, but instead requires yKu70p and yKu80p (which forms the yKu heterodimer) and involves the Rad50p-Mre11p-Xrs2p complex, Lif1p, and ligase IV (Critchlow and Jackson 1998). yKu70p also serves a DNA end protection function since *yku70* mutants process DSBs to yield longer 3′ single-stranded tails than wild type (Lee *et al.* 1998). Recent studies have shown that NHEJ levels are influenced by mating-type status. Haploid cells, expressing either *MAT***a** or *MAT*α, and diploids homozygous at *MAT*, have levels of NHEJ 10-fold higher than those of cells expressing both **a** and α (*e.g.*, **a**/α diploids or haploid Sir$^-$ mutants; Astrom *et al.* 1999; Lee *et al.* 1999). Mating-type heterozygosity enhances DNA repair and HR (Friis and Roman 1968; Heude and Fabre 1993; Fasullo and Dave 1994; Fasullo *et al.* 1999; Lee *et al.* 1999), but it has not been clear how much of this effect was due to downregulation of the competing NHEJ pathway (yKu dependent) and how much was yKu independent.

Because of the high efficiency of DSB repair by *RAD52*-dependent HR, prior strategies for detecting NHEJ in yeast chromosomes employed *rad52* mutants (Kramer *et al.* 1994; Moore and Haber 1996b) or systems in which a broken molecule had no homologous repair template (Schiestl and Petes 1991; Schiestl *et al.* 1993; Manivasakam and Schiestl 1998). Although these studies clearly indicate that HR is much more

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efficient than NHEJ in yeast, they did not provide estimates of the relative rates of DSB repair via HR and NHEJ in strains fully competent to carry out HR (*i.e.*, in Rad$^+$ cells suffering a DSB in a duplicated region).

Here we report measures of the relative rates of repair of HO nuclease-induced DSBs by NHEJ and HR in Rad$^+$ HR-competent haploid and diploid yeast. We detected imprecise NHEJ in haploid cells at a frequency of ~0.1%. HR was increased by *yku70* mutation and by *MAT* heterozygosity. Part of the increase in HR seen with *MAT* heterozygosity was yKu dependent, but the majority was yKu independent, and the latter correlated with increased cell survival. We made the surprising finding that *yku70* mutation slightly increases cell survival following a DSB; this result is discussed in relation to possible mechanisms by which *yku70* mutation enhances HR.

MATERIALS AND METHODS

Plasmid DNA and yeast strains: Plasmid preparation and manipulation and yeast culture were described previously (Sambrook *et al.* 1989; Sweetser *et al.* 1994; Taghian and Nickoloff 1996; Cho *et al.* 1998). Strain JW3082, with *ura3* direct repeats flanking *LEU2* and pUC19, was described previously (Cho *et al.* 1998). The left (donor) *ura3* allele was inactivated by a +1 frameshift mutation (X764); the right (recipient) allele was inactivated by an HO site insertion into *Nco*I (HO432) and contained nine phenotypically silent restriction fragment length polymorphism (RFLP) mutations. JW3082 has a *MAT***a**-*inc* mutation to prevent HO cleavage of *MAT* and subsequent mating-type interconversion and diploidization (Sweetser *et al.* 1994). Strain DY3515-13 is a diploid with the same *ura3* alleles as JW3082 present in an allelic configuration (Nickoloff *et al.* 1999). These recombination substrates are diagrammed in Figure 1. JW3082 and DY3515-13 carry *GALHO* to allow delivery of DSBs to HO sites when cells are grown in medium with galactose. *yku70* mutant strains were constructed by transformation with *Xmn*I-digested plasmid pAF1 (Siede *et al.* 1996) (kindly provided by Anna Friedl). This replaces the endogenous *YKU70* locus with *TRP1*-disrupted *yku70*; mutant status was confirmed by Southern hybridization, growth defects at 37° (Siede *et al.* 1996), and reduced efficiencies of transformation with a linearized *HIS3/ARS1/CEN4* plasmid (data not shown).

Diploid strains constructed from *MAT***a**-*inc* and *MAT*α haploids were converted to *MAT***a**-*inc*/*MAT***a**-*inc* by 2-hr expression of *GALHO*. Cells were then plated for single colonies on YPD; ~50% were **a**-maters (either *MAT***a**-*inc*/*MAT***a**-*inc* or *MAT***a**-*inc*/*MAT***a**), and most had no changes in *ura3*. We confirmed that **a**-mating strains were *MAT***a**-*inc*/*MAT***a**-*inc* as they did not switch to nonmaters upon induction of *GALHO*. Genotypes of yeast strains are given in Table 1.

Recombination assays: DSB-induced and uninduced recombination frequencies were measured using nonselective assays (Cho *et al.* 1998). Briefly, 2-day-old colonies of parent strains were inoculated into 1.5 ml of YPGly medium and incubated for 24 hr. Cultures were divided, and cells were harvested by centrifugation, suspended in 1.5 ml of YPD (uninduced control) or YPGal (with 2% galactose; HO nuclease-induced), grown for 6 hr, and plated on YPD medium. JW3082 recombinants have one of four phenotypes (Figure 2). Ura$^+$ Leu$^+$ (gene conversion + unequal exchange), Ura$^+$ Leu$^-$ (deletion), and Ura$^-$ Leu$^-$ (deletion) products were identified by replica-plating to appropriate media. Ura$^-$ Leu$^+$ recombinants and parental cells have the same phenotypes, but these can be distinguished in a replica-plate assay involving reinduction of HO nuclease (Weng *et al.* 1996; Cho *et al.* 1998). In this assay, induction of HO stimulates HR in parental cells since these retain the HO site (producing many Ura$^+$ papillae in each colony transferred to uracil omission medium), whereas Ura$^-$ Leu$^+$ recombinants, which lack HO432 and are homozygous X764, do not yield Ura$^+$ papillae. Among Ura$^-$ Leu$^+$ recombinants, HO site loss reflects either long-tract gene conversion, which coconverts X764 (homozygous X764 and homozygous *Nco*I at position 432), or HO site inactivation by imprecise NHEJ yielding deletions or insertions (heterozygous at both X764 and *Nco*I). Primers complementary to a sequence downstream of *ura3* (5′-TGGAGTTCAATGCGTCCAT-3′) and the 3′ end of the *LEU2* fragment (5′-GGCACCACACAAAA AGTT-3′) were used to amplify a 1.3-kbp fragment containing the recipient *ura3* allele by PCR. Digestion of PCR products with *Nco*I identified gene conversions since these convert HO432 to *Nco*I. *Nco*I-resistant products were usually imprecise NHEJ products; some retained HO432 and presumably reflect inactivation of HO nuclease or the galactose regulatory system (*GALHO*$^-$). Ura$^-$ Leu$^-$ products could arise by HR (crossover, SSA, or unequal sister chromatid exchange) or by NHEJ, and these events were distinguished by Southern hybridization. Junctions formed by NHEJ were identified by DNA sequencing of rescued alleles as described (Cho *et al.* 1998) or by direct sequencing of PCR products.

DY3515-13 recombinants are Ura$^+$ or Ura$^-$, identified using uracil omission media and reinduction assays, respectively. NHEJ products of DY3515-13 were first sought among 130 Ura$^-$ products by using a PCR/*Nco*I screen as above, except that both copies of *ura3* were amplified. An additional 730 Ura$^-$ products were screened by using a pooling approach as follows. Ura$^-$ products were grown to stationary phase in 5 ml of YPD, and 73 pools were made by mixing 0.5-ml aliquots of each of 10 products. PCR was used to amplify both copies of *ura3* from genomic DNA isolated from each pool, and PCR products were analyzed by Southern hybridization using a ^{32}P-labeled probe specific to the wild-type *URA3* sequence opposite X764 (5′-TTTTGTTATCGGCTT-3′). This probe hybridizes to X764 heterozygotes (imprecise NHEJ or *GALHO*$^-$), but not to X764 homozygotes (gene conversion). A reconstruction experiment indicated that this strategy reliably detects a single heterozygote in a pool with nine X764 homozygotes. All products from each pool that had one or more X764 heterozygotes were retested individually by the PCR/Southern assay to identify X764 heterozygotes. X764$^-$ alleles were rescued as described (Nickoloff *et al.* 1999) and sequenced to distinguish imprecise NHEJ and *GALHO*$^-$ products. Complete product independence was guaranteed for putative NHEJ products since at most one candidate was characterized from each population of parent strains. Statistical analyses were performed with *t*-tests unless otherwise specified.

Measurement of DSB levels: DSBs were quantified essentially as described previously (Weng *et al.* 2000). Briefly, HO nuclease was induced for 4 or 6 hr and genomic DNA was prepared. For haploid strains, DSBs were detectable at 4- and 6-hr time points; we present the 4-hr data as this is least likely to be affected by repair. For diploid strains, DSBs were barely detectable at 4 hr, so only the 6-hr data are shown. For haploid strains, *Hin*dIII-digested genomic DNA was probed with a ^{32}P-labeled *ura3* fragment consisting of a 0.8-kbp sequence 3′ of HO432; this detects a 1.2-kbp donor fragment and a 6-kbp recipient fragment. Upon induction of HO nuclease, the 6-kbp fragment is cleaved into two fragments, but the probe detects only the smaller (0.8-kbp) fragment. DSB levels were calculated as the ratio of the signal from the 0.8-kbp fragment to the

TABLE 1

Yeast strains

Name	Genotype	Reference
JW3082	*MAT***a**-*inc ade2-101 his3-200 lys2-801*::pHSS*GALHO::LYS2 trp1*-Δ*1 leu2*-Δ*1 ura3*-X764-*LEU2*-*ura3*R-HO432	Cho *et al.* (1998)
GJK3465	Same as JW3082 except *yku70::TRP1*	This study
DY3515-13	*MAT***a**-*inc*/*MAT*α *ade2-101*/*ade2-101 lys2-801*::pHSS*GALHO::LYS2*/*lys2-801 his3-200*/*HIS3 trp1*-Δ*1*/*trp1*-Δ*1 leu2*-Δ*1*/*leu2*-Δ*1* RscRI-*ura3*R-HO432-*LEU2*/RscBam-*ura3*-X764-*LEU2*[a]	Nickoloff *et al.* (1999)
SB3466	*MAT*α *ade2-101 lys2-801 trp1*-Δ*1 leu2*-Δ*1*, RscBam-*ura3*-X764-*LEU2 yku70::TRP1*	This study
SB3467	*MAT***a**-*inc ade2-101 his3-200 lys2-801*::pHSS*GALHO::LYS2 trp1*-Δ*1 leu2*-Δ*1* RscRI-*ura3*R-HO432-*LEU2 yku70::TRP1*	This study
SB3468	Diploid product of SB3466 × SB3467; same as DY3515-13 except *yku70::TRP1*/*yku70::TRP1*	This study
SB3522	Same as DY3515-13 except *MAT***a**-*inc*/*MAT***a**-*inc*	This study
SB3523	Same as SB3522 except *yku70::TRP1*/*yku70::TRP1*	This study

[a] RscRI and RscBam replace *URA3* with pUC19-*ura3*-*LEU2*, carrying specific *ura3* alleles as indicated; see Nickoloff *et al.* (1999).

sum of the signals from all hybridizing fragments [quantified using a Molecular Dynamics (Sunnyvale, CA) Phosphorimager]. An analogous Southern strategy was used to measure DSB levels in diploid strains.

Cell survival and mating-type switching: Cell survival was assessed by measuring plating efficiency (PE) following 6 hr galactose induction or 6 hr growth in glucose as a control. PE was calculated as the ratio of YPD colonies to the number of cells plated. Cell numbers were determined using a Coulter Counter, and 350–1600 YPD colonies were scored per determination. Mating-type switching (from *MAT***a**-*inc*/*MAT*α to *MAT***a**-*inc*/*MAT***a**-*inc* or to *MAT***a**-*inc*/*MAT***a**) was stimulated by using standard *GALHO*-induction conditions described above. Cells were plated on YPD after 0, 2, 4, or 6 hr of growth in galactose medium and incubated for 2 days; colonies that had switched mating type were identified as those able to mate with a *MAT*α strain.

RESULTS

Experimental design: We examined relative rates of DSB repair by HR and NHEJ in Rad$^+$ haploid and diploid yeast strains with direct repeat and allelic recombination substrates (Figure 1). Because yKu70p plays a key role in NHEJ, we also examined DSB repair in isogenic *yku70* strains. Strain JW3082 (Cho *et al.* 1998) and its *yku70* derivative (GJK3465) carry *ura3* direct repeats flanking pUC19 and *LEU2*. One copy of *ura3* was inactivated by a +1 frameshift mutation (X764). The second copy was inactivated by an HO site insertion (HO432) and contained nine phenotypically silent RFLP mutations. Diploid strain DY3515-13 (Nickoloff *et al.* 1999) and its derivatives have these same *ura3* genes at allelic positions. All strains have a copy of *GALHO* integrated at *lys2*, providing a galactose-regulated source of HO nuclease to deliver DSBs to HO432. JW3082 has a *MAT***a**-*inc* mutation to prevent HO cleavage of *MAT* and subsequent mating-type interconversion and diploidization (Sweetser *et al.* 1994). The *MAT***a**-*inc* mutation is a single-base change that does not affect *MAT* coding potential; hence, in this report *MAT***a**-*inc* and **a** are equivalent.

HR in JW3082 can yield products with one of four phenotypes (Figure 2). Most Ura$^+$ Leu$^+$ products reflect short-tract gene conversion, which conserves the gross structure of the direct repeat; Ura$^+$ Leu$^+$ products may also result from unequal sister chromatid exchange, yielding three copies of *ura3* and two copies of *LEU2*, but these are rare in JW3082 and related strains (Cho *et al.* 1998; Nickoloff *et al.* 1989). Ura$^+$ Leu$^-$ and Ura$^-$ Leu$^-$ products ("popouts") reflect loss of pUC19, *LEU2*, and one copy of *ura3* by crossover, SSA, or unequal sister chromatid exchange (Ray *et al.* 1988; Nickoloff

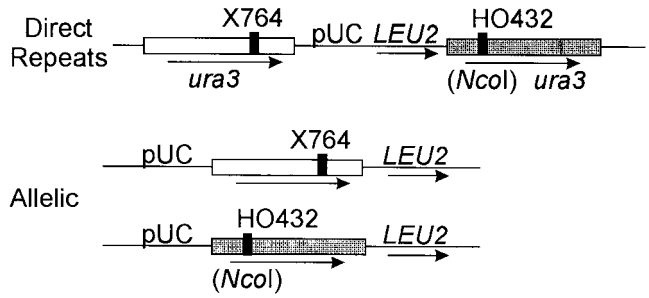

Figure 1.—Recombination substrates. (Top) *ura3* direct repeats separated by pUC19 and *LEU2* in JW3082 (Cho *et al.* 1998) and the *yku70* derivative GJK3465. The left copy is inactivated by X764 but is otherwise wild type, and the right copy is inactivated by insertion of an HO site at *Nco*I (HO432) and contains nine silent RFLP markers (shading); the RFLP markers were not scored in the present study. (Bottom) The same *ura3* genes present in allelic positions at the normal chromosome V position in DY3515-13 (Nickoloff *et al.* 1999) and its **a**/**a** and *yku70* derivatives. The flanking pUC19 and *LEU2* sequences were introduced during construction; the allelic substrates are not flanked by linked repeats.

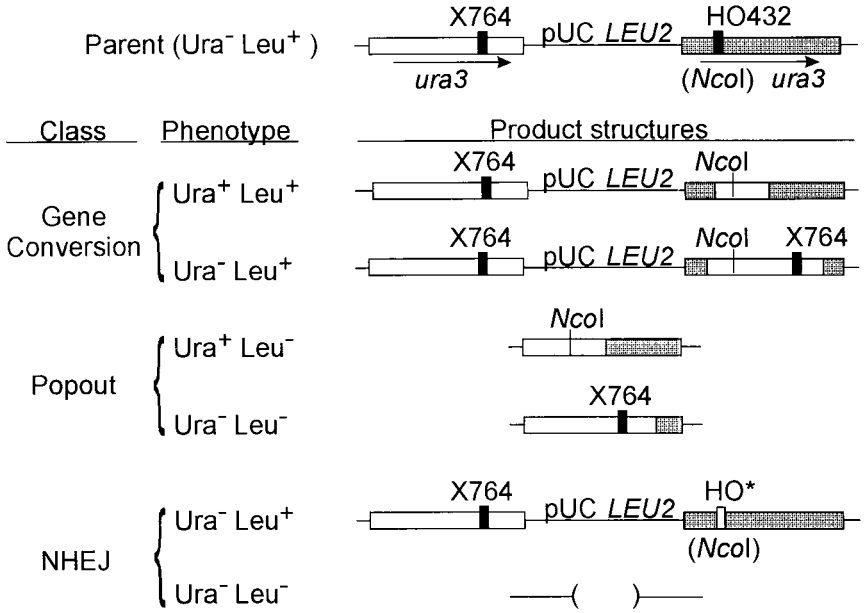

FIGURE 2.—Types of DSB repair products for *ura3* direct repeats. The parent structure is shown at the top. Three classes of events give rise to four phenotypes, distributed among six main product types. Short- and long-tract gene conversion yields heterozygous and homozygous X764, respectively. Triplications resulting from unequal sister chromatid exchange (Ura^+ Leu^+) are rare (not shown). All popouts are Leu^-. Imprecise NHEJ (Ura^- Leu^+) may delete some or all of HO432, indicated by HO*; larger deletions from NHEJ (Ura^- Leu^-) may remove some or all of the right *ura3* gene and some or all of *LEU2*, and may extend further (bottom product).

et al. 1989; FISHMAN-LOBELL *et al.* 1992). Most Ura^- Leu^+ products arise by long-tract gene conversion in which HO432 and X764 coconvert. Precise NHEJ restores the parental structure, but imprecise NHEJ can yield small deletions or insertions that inactivate the HO site (Ura^- Leu^+) or large deletions (>900 bp) extending from HO432 into the *LEU2* coding sequence (Ura^- Leu^-). With the allelic substrates in strain DY3515-13 and its derivatives, Ura^+ products reflect short-tract gene conversion, and Ura^- products reflect either long-tract conversion extending past X764 or imprecise NHEJ. We showed previously that *GALHO* induction in JW3082 and DY3515-13 increases HR by >100-fold (CHO *et al.* 1998; NICKOLOFF *et al.* 1999), and similar results were obtained in our study (data not shown). These induction levels ensure that essentially all products analyzed were DSB induced.

DSB repair by imprecise NHEJ in haploid, Rad^+, HR-competent yeast yields small deletions and insertions and requires *YKU70*: Imprecise NHEJ in yeast chromosomal DNA had previously been observed only in strains defective in HR, such as *rad52* mutants, or in the absence of a homologous repair template (SCHIESTL and PETES 1991; SCHIESTL *et al.* 1993; KRAMER *et al.* 1994; MOORE and HABER 1996b; MANIVASAKAM and SCHIESTL 1998). To detect imprecise NHEJ in haploid Rad^+ cells, we used a nonselective assay to identify Ura^- Leu^+ products of JW3082. Of 343 Ura^- Leu^+ products analyzed, 10 retained parental structures (intact HO432 sites); these presumably gained a mutation in HO nuclease or in the galactose regulatory network (*GALHO*$^-$) and were not analyzed further. Of the remaining 333 products, 319 arose by gene conversion (homozygous at X764), and 14 (4%) arose by imprecise NHEJ (Table 2). Of these, the most common product (6 of 14) had a 2-bp CA insertion, which likely resulted from partial pairing of the 4-base (5′-AACA) overhang followed by filling-in and religation. One product had a single nucleotide insertion within the overhang, and the rest had deletions of 1–17 bp. In all cases, the deletions could be explained as resulting from pairing between microhomologies ranging from 1 to 7 bp. In *rad52* mutants, DSB repair at *MAT* by imprecise NHEJ gives mostly small deletions and insertions, but 28% of deletions were >200 bp in length (KRAMER *et al.* 1994). In JW3082, large deletions extending into *LEU2* would give a Ura^- Leu^- phenotype, but among 100 Ura^- Leu^- products examined, none arose by NHEJ; large deletions in JW3082 may be inviable (see DISCUSSION). Ninety-eight were pop-out recombinants; two retained the parental direct repeat structure and may have sustained mutations in *LEU2*, perhaps as a consequence of DNA polymerase errors during repair synthesis templated from a sister chromatid (STRATHERN *et al.* 1995). Since Ura^- Leu^+ products comprise 2% of the total (Figure 3 and CHO *et al.* 1998), and 4% of these arise by imprecise NHEJ, ~0.1% of DSB repair leading to HO site loss/inactivation involves imprecise NHEJ in HR-competent Rad^+ haploid yeast.

yKu70p plays a key role in plasmid NHEJ in yeast (BOULTON and JACKSON 1996b; MILNE *et al.* 1996). To determine whether imprecise NHEJ of chromosomal DSBs detected in JW3082 was similarly yKu70p dependent, we characterized 127 Ura^- Leu^+ products from a *yku70* derivative of JW3082 (strain GJK3465). Nine products had intact HO432 sites (presumed *GALHO*$^-$) and the remainder were long-tract gene conversions. Thus, 0 of 118 DSB repair events in the *yku70* mutant involved imprecise NHEJ. This is a significant decrease compared to wild type ($P < 0.03$; Fisher exact test),

TABLE 2

Imprecise NHEJ in Rad$^+$, HR-competent yeast

Type	Sequence of NHEJ products[a]	± (bp)[b]	Microhomology (bp)[c]
HO432	AATTTCAGCTTTCCGC<u>AACA</u>GTATAAATTCCGCATGGAGGG	—	—
1	AATTTCAGCTTTCCGCA Δ CAGTATAAATTCCGCATGGAGGG	−1	1
2	AATTTCAGCTTTCCGCAA Δ GTATAAATTCCGCATGGAGGG	−2	1
3	AATTTCAGCTTTCCG Δ CAGTATAAATTCCGCATGGAGGG	−3	2
4	AATTTCAGCTTT Δ CAGTATAAATTCCGCATGGAGGG	−6	3
5	AATTTCAGCTTTCCGCAAC Δ AATTCCGCATGGAGGG	−6	2
6	AATTTCAGCTTTCC Δ GCATGGAGGG	−17	4
7	AATTTCAGCTTTCCGCA Δ TGGAGGG	−17	7
8	AATTTCAGCTTTCCGCAACcAGTATAAATTCCGCATGGAGGG	+1	1
9	AATTTCAGCTTTCCGCAACAcaGTATAAATTCCGCATGGAGGG	+2	1

[a] The sequence of HO432 is given in the top row; underlined bases designate the 3′ overhang produced by HO cleavage. Deletions are noted by Δ in sequences; inserted bases are lowercase. Each type was isolated once except type 9 was isolated six times.

[b] Length of deletion or insertion.

[c] Predicted length of microhomology overlap during end-joining.

confirming that imprecise NHEJ of chromosomal DSBs is yKu70p dependent.

We next sought NHEJ products among DSB-induced Ura$^-$ products in the diploid strain DY3515-13, which carries the same *ura3* genes as JW3082 at allelic positions (Figure 1). In this case, products are Ura$^+$ (short-tract gene conversion), Ura$^-$ (long-tract gene conversion or imprecise NHEJ), or sectored Ura$^{+/-}$ (independent G2 events or, less likely, segregation of X764). Of 860 Ura$^-$ products examined, only 2 lacked the *Nco*I site at position 432 and both had wild-type HO sites (presumed *GALHO*$^-$). These 860 Ura$^-$ products represent ~1100 products since Ura$^-$ products comprise ~80% of the total (Ura$^+$ + Ura$^-$). Thus, imprecise NHEJ in a Rad$^+$ diploid comprises <0.1% of total DSB repair.

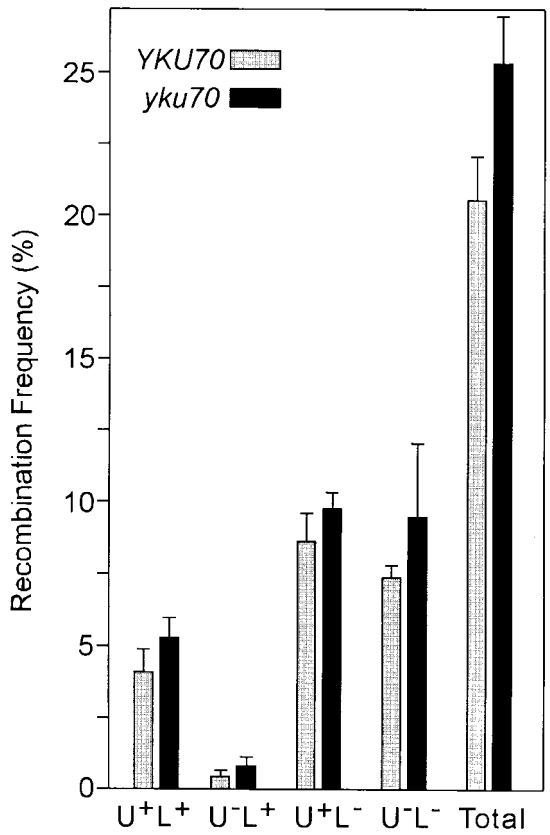

FIGURE 3.—DSB-induced direct repeat recombination. Frequencies of each of the four phenotypic classes, plus totals of all classes, are shown for JW3082 (*YKU70*) and GJK3465 (*yku70*). Data represent averages ± SDs for four determinations per strain; 1100–1200 colonies were scored per determination.

DSB-induced HR is increased in *yku70* mutants: It was reported that *yku70* mutation reduces spontaneous allelic HR by 10- to 40-fold (MAGES *et al.* 1996). We were surprised to find that DSB-induced HR in the haploid *yku70* mutant was 1.3-fold higher than the wild-type strain ($P < 0.006$; Figure 3). The level of gene conversion (Leu$^+$ recombinants) was also significantly increased by *yku70* mutation ($P = 0.05$). *yku70* also increased HR in an **a**/**a** background by 1.5-fold ($P < 0.0001$; Figure 4). These results can be explained by a model in which yKu70p mediates precise NHEJ of 20–30% of DSBs in wild-type cells that are instead processed by HR in *yku70* mutants. Alternatively, yKu70p may directly inhibit HR, although this is unlikely since *yku70* did not increase HR in the **a**/α background (Figure 4). Another possibility is that the increased HR in *yku70* reflects increased cleavage by HO nuclease, and we did find that DSB levels were slightly higher in *yku70* compared to wild type (Table 3). However, a similar correlation was not seen in the **a**/**a** background as *yku70* increased HR by 1.5-fold but did not increase DSB levels (Table 3). The slight increase in DSB levels in the haploid *yku70* mutant probably reflects reduced DSB repair by precise NHEJ.

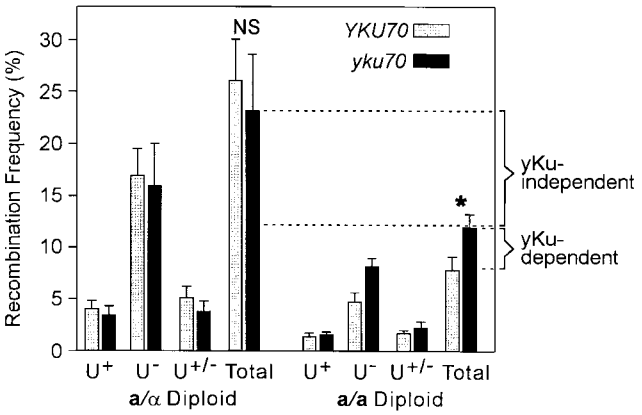

FIGURE 4.—DSB-induced allelic recombination. Frequencies of Ura^+, Ura^-, and $Ura^{+/-}$ sectored products plus totals are shown for **a**/α and **a**/**a** strains with wild-type or mutant *YKU70*. Data represent averages ± SDs for 8–13 determinations per strain, with an average of 1200–1500 colonies scored per determination. NS, not significantly different; *, a statistically significant difference.

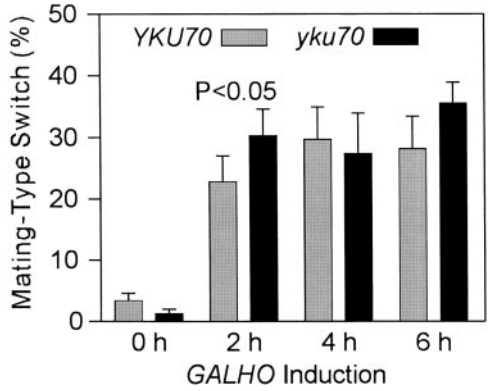

FIGURE 5.—Mating-type switching in *YKU70* and *yku70* strains. *GALHO*-induced mating-type switching was measured in two *MAT***a**-*inc*/*MAT*α strains (DY3515-13, *YKU70* and SB3468, *yku70*) as described in MATERIALS AND METHODS. The average percentage (± SD) of colonies that switched to α-maters are shown for four determinations per strain.

yku70* mutation does not reduce mating-type switching in a Rad$^+$ background:** *yku70* mutants reportedly have reduced levels of *GALHO*-induced mating-type switching (MAGES *et al.* 1996). We could not assay mating-type switching in our haploid cells because they are *MATa**-*inc*. Instead, we assayed *GALHO*-induced mating-type switching in *MAT***a**-*inc*/*MAT*α diploids. In agreement with our results at *ura3*, mating-type switching in the *yku70* mutant was significantly higher than wild type after a 2-hr induction ($P < 0.05$); at later times, switching reached similar levels in *yku70* and wild-type strains (Figure 5).

***MAT* heterozygosity enhances HR by yKu-dependent and -independent mechanisms:** The **a**/**a** diploid had a total HR frequency significantly lower than that of the **a**/α diploid (Figure 4). Only a fraction of this difference is yKu70p dependent since even in a *yku70* background, HR in the **a**/**a** diploid was ~2-fold lower than the **a**/α diploid ($P < 0.0001$). DSB levels were somewhat lower in **a**/α than **a**/**a**, and this was true in both *YKU70* and *yku70* backgrounds (Table 4), ruling out the possibility that reduced HR in **a**/**a** cells reflects fewer DSBs. Thus, HO-induced HR is reduced in **a**/**a** compared to **a**/α cells, and most of this difference is yKu70p independent, reflecting instead decreased HR in *MAT* homozygous strains. This decrease in HR closely correlates with decreased cell viability (see DISCUSSION).

A single DSB kills 10–20% of *MAT* homozygous cells, and killing is partially suppressed by *yku70* mutation: We compared cell viability following 6 hr of *GALHO* expression and repression in the three pairs of matched *yKU70* and *yku70* haploid (**a**) and diploid (**a**/α and **a**/**a**) strains. In diploid **a**/α cells, HO-dependent killing was only ~5%, whereas 10–20% killing was observed in **a** and **a**/**a** cells (Figure 6). Interestingly, **a**/**a** cells showed significantly less killing in the *yku70* mutant compared to wild type ($P < 0.05$). This trend was also apparent in the haploid and **a**/α diploid strains, although the differences were smaller and not statistically significant with these sample sizes ($P = 0.4$ and 0.08, respectively). We conclude that yKu70p has a small negative effect on cell survival following a single DSB in **a**/**a** Rad$^+$ cells.

Conversion tract lengths are not affected by *yKU70* or *MAT* status: The yKu70p/yKu80p heterodimer protects

TABLE 3

DSB and HR levels in *yku70* and *YKU70* backgrounds

	Relative levels: *yku70* divided by *YKU70*	
Strains	DSBs[a]	HR[b]
Haploids	1.1/1.2	1.3
a/α diploids	0.9/0.8	0.9
a/**a** diploids	1.1/1.0	1.5

[a] DSB levels were measured as described in MATERIALS AND METHODS. Values represent DSB levels in *yku70* strains divided by DSB levels in *YKU70* strains; values from two measurements are separated by slashes.

[b] HR levels in *yku70* strains divided by DSB levels in *YKU70* strains; data from Figures 3 and 4.

TABLE 4

DSB and HR levels in a/α and a/a diploids

	Relative levels: **a**/α divided by **a**/**a**	
YKU70 status	DSBs	HR
YKU70	0.9/0.8	3.3
yku70	0.7/0.6	1.9

Relative DSB and HR levels are given as described in Table 3.

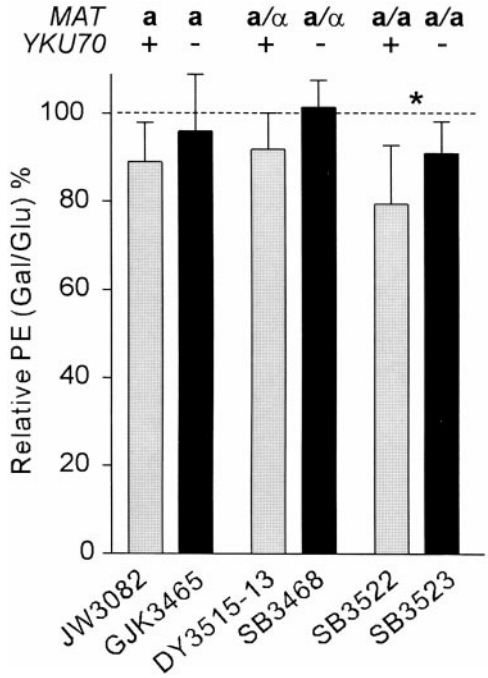

FIGURE 6.—Cell survival in *GALHO*-induced cultures. For each cell population, PEs were determined following 6 hr growth in glucose or galactose medium (see MATERIALS AND METHODS). The degree of HO nuclease-dependent cell killing was determined by dividing the galactose PE by the glucose PE for each determination. These ratios were converted to percentages and the averages ± SDs for four to eight determinations per strain were plotted. Values <100% are indicative of HO-dependent cell killing; * indicates a statistically significant difference.

ends from degradation (LEE *et al.* 1998). The longer single-stranded 3′ tails in *yku70* mutants may influence later steps in HR, such as strand invasion and pairing, and this could enhance hDNA formation and thereby increase gene conversion tract lengths. In our system, gene conversion initiated at HO432 produces Ura$^+$ or Ura$^-$ products, with the latter reflecting longer tracts that include X764. Thus, Ura$^+$:Ura$^-$ ratios provide an estimate of conversion tract lengths. By this measure, *yku70* did not increase tract lengths as Ura$^-$ products comprised ~80% of the total in *yku70* and *YKU70* strains (Figure 4). It is possible that *yku70* mutants show extensive 5′ end degradation only when HR is disabled (*i.e.*, in *rad52* or when no repair template is present; LEE *et al.* 1998).

It has been suggested that *MAT* heterozygosity enhances HR by enhancing pairing (FRIIS and ROMAN 1968; FASULLO and DAVE 1994; FASULLO *et al.* 1999; LEE *et al.* 1999), and this might be reflected in increased gene conversion tract lengths. However, we found that ~80% of products were Ura$^-$ in both **a**/α and **a**/**a** strains (Figure 4). If *MAT* heterozygosity enhances HR by enhancing pairing, this is not reflected in increased tract lengths. It is possible that tract lengths are primarily a reflection of branch migration of Holliday junctions and mismatch repair of hDNA, both of which are independent of end-processing and the efficiency of the initial pairing reaction.

DISCUSSION

Imprecise NHEJ is infrequent in the presence or absence of HR: In previous studies, the frequency of imprecise NHEJ was estimated by cell survival in *rad52* mutants or in the absence of a homologous repair template. Although an early study using an *HO swi1 rad52* strain suggested that imprecise NHEJ occurred at a frequency of 1% (WEIFFENBACH and HABER 1981), lower frequencies were seen in subsequent studies of *rad52* cells suffering DSBs in a dicentric chromosome (0.04% survival) or *rad52 MAT***a** cells expressing *GALHO* (0.01–0.04% survival; KRAMER *et al.* 1994). In Rad$^+$ cells lacking a homologous repair template, cell survival reflecting imprecise NHEJ was 0.22% (MOORE and HABER 1996a). In our study, we used a nonselective assay to estimate the frequency of imprecise NHEJ in Rad$^+$ yeast in the presence of a homologous repair template and found a comparable level of imprecise NHEJ (0.1%). Thus, imprecise NHEJ occurs at approximately the same low frequency in the presence or absence of the competing HR pathway.

We found that the rare imprecise NHEJ events in haploid Rad$^+$ cells resulted in small 1- to 17-bp deletions and small insertions and confirmed that these arose by a yKu70p-dependent mechanism. KRAMER *et al.* (1994) also found small insertions and some small deletions in *rad52 MAT***a** cells expressing *GALHO*, but 28% had deletions that ranged from 200 bp to >1 kbp. The formation of large deletions in haploid cells is limited by the proximity of essential genes to the DSB. Large deletions are possible at *MAT* because *MAT* is not essential. The closest essential gene to *ura3* is *TIM9*, present only 817 bp downstream of the DSB. In our direct repeat substrate, the 3′ end of the *LEU2* coding sequence is 950 bp upstream of the DSB. Therefore, symmetric deletions reaching *LEU2* would also delete part of *TIM9*, so it is not surprising that we did not detect large NHEJ-mediated deletions. Imprecise NHEJ was not detected in **a**/α diploid cells, consistent with the downregulation of NHEJ by *MAT* heterozygosity (ASTROM *et al.* 1999; LEE *et al.* 1999).

***yku70* mutation enhances nuclease-induced HR in Rad$^+$ yeast:** There are conflicting reports about *yku70* effects on HR and sensitivity to DNA damage. For example, two groups reported that *yku70* mutants are hypersensitive to methyl methanesulfonate (MMS) and bleomycin (MAGES *et al.* 1996; MILNE *et al.* 1996), but no effect was seen by a third group for MMS or ionizing radiation (SIEDE *et al.* 1996). MAGES *et al.* (1996) reported that *yku70* reduced spontaneous HR 10- to 40-fold. This result contrasts sharply with the lack of *yku70* effect on spontaneous and meiotic HR reported by TSU-

kamoto *et al.* (1996) and with the enhanced HR in *yku70* **a** and **a**/**a** cells that we observed (Figures 3 and 4). Mages *et al.* (1996) also reported that *yku70* reduced mating-type switching by 3-fold, but we found that *yku70* either had no effect or increased mating-type switching (Figure 5); these results may reflect differences in mating-type switching in haploid *vs.* diploid cells and/or differences in genetic background. Mages *et al.* (1996) used W303-derived strains that likely carried a cryptic *rad5* mutation (Fan *et al.* 1996; Astrom *et al.* 1999), whereas our strains, and those used by Lee *et al.* (1999) that were confirmed to be *RAD5*, were derived from S288C. Rad5p plays an important role in channeling repair from NHEJ to gene conversion (Ahne *et al.* 1997); however, recent results indicate that the W303 *rad5* mutation influences some but not all types of HR (L. Symington, personal communication). NHEJ assays in *RAD5* and *rad5* strains have also given conflicting results (Ahne *et al.* 1997; Hegde and Klein 2000).

Mating-type control of HR by yKu-dependent and -independent mechanisms: We assessed repair of a single chromosomal DSB per cell and found that *yku70* mutation increased HR by 1.3-fold in haploid yeast and by 1.5-fold in **a**/**a** cells, but there was no effect in **a**/α cells (Figure 4). *yku70* mutation increases end processing, resulting in longer 3′ single-stranded tails (Lee *et al.* 1998), and these may be better substrates for HR. However, *mre11* reduces end processing yet nuclease-induced HR in *mre11* occurs at essentially wild-type levels, albeit more slowly (Ivanov *et al.* 1994; Tsubouchi and Ogawa 1998), suggesting that the extent or rate of end processing does not strongly affect the efficiency of HR. This model also does not account for the lack of *yku70* effect on HR in **a**/α cells. Although NHEJ is downregulated in **a**/α cells, this is not due to decreased *YKU70* expression (Galitski *et al.* 1997; Astrom *et al.* 1999); thus one might expect similar alterations in end processing, and therefore enhanced HR regardless of *MAT* status, but this was not observed.

We present two alternative models for the enhanced HR in *yku70* haploid and **a**/**a** strains. The first model is based on the idea that NHEJ and HR compete for repair of DSBs. In this model, yKu70p mediates precise NHEJ of a fraction of HO nuclease-induced chromosomal DSBs in wild-type cells, but these DSBs are processed by HR in *yku70* mutants. This interpretation is consistent with the lack of *yku70* enhancement of HR (and the lack of imprecise NHEJ) in **a**/α cells since NHEJ is strongly downregulated in **a**/α cells (Astrom *et al.* 1999; Lee *et al.* 1999). Precise NHEJ has been directly detected in assays involving recircularization of linear plasmid DNA transformed into yeast; these events require yKu70p and yKu80p and are detected at lower levels in *lif4*, *lig4*, *rad50*, *mre11*, and *xrs2* mutants, but are *RAD52* independent (Mezard and Nicolas 1994; Boulton and Jackson 1996a,b, 1998; Herrmann *et al.* 1998; Lee *et al.* 1999). Recent studies of *Eco*RI expression in yeast provided evidence for precise NHEJ of chromosomal DSBs (Barnes and Rio 1997; Lewis *et al.* 1998, 1999). In mammalian cells, nuclease DSBs in transformed plasmid DNA and in chromosomal DNA were shown to be repaired by precise NHEJ (Roth and Wilson 1985; Lin *et al.* 1999). Additional support for the competition model comes from a study of HR in mammalian cells. NHEJ is a major DSB repair pathway in mammalian cells, requiring Ku70, Ku86, and the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs; Critchlow and Jackson 1998). We found that DSB-induced HR was threefold higher in Chinese hamster ovary cells with a defect in DNA-PKcs compared to derivatives carrying a complementing DNA-PKcs cDNA (C. Allen, A. Kurimasa, M. Brenneman, D. Chen and J. A. Nickoloff, unpublished results). Thus, elimination of NHEJ has a greater stimulatory effect in mammalian cells than in yeast, consistent with the idea that NHEJ is the dominant repair mode in mammalian cells (Liang *et al.* 1998). In contrast, wild-type and Ku80-defective hamster cells yielded similar levels of DSB-induced HR (Liang *et al.* 1996), although this result is questionable because the recombination substrate was present at different chromosomal locations and was therefore subject to position effects (Bollag *et al.* 1989; Taghian and Nickoloff 1997).

The second model suggests that yKu70p interferes with HR. Enhanced end processing in *yku70* mutants indicates that yKu70p has an end protection function, but it is important to note that this protection is not limited to the initial end but extends inward as 3′ tails are formed (Lee *et al.* 1998). Thus, the presence of yKu at processed ends might interfere with Rad51p function during the formation of nucleoprotein filaments or later during synapsis or strand exchange. In this view, Rad51p function would be enhanced in the absence of yKu70p, and this effect would likely be independent of the length of 3′ tails. Note that both models describe yKu-dependent mechanisms by which *MAT* heterozygosity might regulate HR, either by downregulating NHEJ (competition model) or by influencing yKu70p activity (interference model). The competition and interference models are not mutually exclusive; at present we cannot determine whether only one or both are operative, but our survival data suggest that increased HR in *yku70* mutants cannot be explained solely by the absence of precise NHEJ (see below).

Although HR is increased in *yku70* **a**/**a** cells compared to wild-type **a**/**a** cells, HR is still twofold lower than in **a**/α cells (regardless of *YKU70* status; Figure 4). Thus, the reduction of yKu70p-dependent competition or interference in **a**/α cells does not fully account for the difference in HR levels between **a**/α and **a**/**a** cells, indicating that *MAT* heterozygosity also enhances HR by a yKu70p-independent mechanism. Our data suggest that most of the difference in HR frequencies between **a**/**a** and **a**/α cells reflects cell killing. In *YKU70* strains, the

a/α HR frequency was 26%, compared to 8% in **a**/**a** cells. The difference of 18% correlates well with the ~20% cell killing in **a**/**a** cells (note that there is very little killing of **a**/α strains, regardless of *yKU70* status). A similar correlation is apparent in *yku70* strains: the **a**/α HR frequency was 23%, the **a**/**a** frequency was 12%, and the difference (11%) was similar to the 9% cell killing in **a**/**a** cells. These results suggest that the "missing" recombinants in **a**/**a** cells are in fact dead and that HR capacity in **a**/**a** cells is insufficient to confer full survival even with only one DSB per cell. In contrast, the higher capacity for HR in **a**/α cells is sufficient to confer nearly full survival. In this argument, we do not consider the survival value of NHEJ, but focus exclusively on HR. This is because *yku70* mutants do not display increased HO-dependent killing compared to wild type (this study and Milne *et al.* 1996) and Rad$^+$ *yku70* mutants are not more sensitive to killing by MMS and γ-rays than wild type (Siede *et al.* 1996). Our data indicate that the yKu-independent mechanism by which *MAT* heterozygosity regulates HR has a stronger effect than the yKu-dependent mechanism(s) (Figure 4).

Slight DSB survival advantage of *yku70* mutants: We found that in an **a**/**a** background, *yku70* conferred a slight, but significant increase in survival of a single DSB; this trend was also apparent in **a** and **a**/α cells (Figure 6). Although Milne *et al.* (1996) remarked that a *yku70* haploid strain showed wild-type survival following HO-induced cleavage at *MAT*, survival in *yku70* was actually 20% higher than wild type in their experiments. In an *mre11* background, *yku70* confers sixfold higher survival following exposure to 150 Gy of ionizing radiation (from 1 to 6%; Bressan *et al.* 1999). How can inactivation of the yKu70p-dependent DSB repair pathway lead to greater survival of DSB damage? It is doubtful that the observed cell killing reflects chromosome loss because we have previously shown that our diploid cells survive the loss of one copy of chromosome V (Nickoloff *et al.* 1999). Yeast cell survival of DSB damage correlates with HR efficiency, as shown here and by others (Mortimer 1958; Saeki *et al.* 1980; Kadyk and Hartwell 1992; Fasullo *et al.* 1994; Schild 1995). *yku70* mutation increased HR in both **a** and **a**/**a** backgrounds, and it is likely that the increased HR underlies increased survival. In **a**/α cells, *yku70* has minimal effect on survival and no effect on HR, which may be a reflection of near-maximum HR levels conferred by *MAT* heterozygosity. The increase in survival in *yku70* mutants cannot be explained solely on the basis of elimination of NHEJ because precise NHEJ produces viable products and imprecise NHEJ is extremely rare. Thus, it appears that *yku70*-dependent increase in survival is due to elimination of yKu interference with HR. Perhaps a small fraction of DNA ends are blocked from HR by yKu70p, yet fail to engage in a productive NHEJ reaction in a timely fashion, with cell death (or inability to form a colony) perhaps reflecting checkpoint activation. Lee *et al.* (1998) argued the opposite: that the increased single-stranded DNA in *yku70* mutants caused more efficient replication protein A-dependent checkpoint activation. However, in that study there was no possibility for HR because the cells lacked a homologous repair template. Thus, *yku70* may increase checkpoint activation only when HR is blocked. It should be possible to gain insight into the roles of checkpoint activation, end processing, and HR in *yku70*-enhanced cell survival by examining checkpoint mutants, and by using *mre11* mutants, which are competent for nuclease-induced HR (Ivanov *et al.* 1994; Tsubouchi and Ogawa 1998), but display reduced end processing even when combined with *yku70* (Lee *et al.* 1998). Also of interest will be studies with *lig4* mutants since these have a strong NHEJ defect (Boulton and Jackson 1998) but are not expected to display altered end processing characteristic of *yku70* mutants.

Helpful comments from Jim Haber, John Petrini, Mark Brenneman, Chris Allen, and Sean Palmer are greatly appreciated. We thank Anna Friedl for providing the *yku70* knock-out plasmid pAF1 and Kim Spitz for technical assistance. This research was supported by grant CA55302 from the National Cancer Institute of the National Institutes of Health.

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Communicating editor: L. S. Symington