Radiation-Induced Chromosome Aberrations in *Saccharomyces cerevisiae*: Influence of DNA Repair Pathways

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ABSTRACT

Radiation-induced chromosome aberrations, particularly exchange-type aberrations, are thought to result from misrepair of DNA double-strand breaks. The relationship between individual pathways of break repair and aberration formation is not clear. By electrophoretic karyotyping of single-cell clones derived from irradiated cells, we have analyzed the induction of stable aberrations in haploid yeast cells mutated for the *RAD52* gene, the *RAD54* gene, the *HDF1(=YKU70)* gene, or combinations thereof. We found low and comparable frequencies of aberrational events in wildtype and *hdf1* mutants, and assume that in these strains most of the survivors descended from cells that were in G_2 phase during irradiation and therefore able to repair breaks by homologous recombination between sister chromatids. In the *rad52* and the *rad54* strains, enhanced formation of aberrations, mostly exchange-type aberrations, was detected, demonstrating the misrepair activity of a rejoining mechanism other than homologous recombination. No aberration was found in the *rad52 hdf1* double mutant, and the frequency in the *rad54 hdf1* mutant was very low. Hence, misrepair resulting in exchange-type aberrations depends largely on the presence of Hdf1, a component of the nonhomologous end-joining pathway in yeast.

STRUCTURAL chromosomal aberrations are an important consequence of ionizing radiation in eukaryotic cells, and may cause mitotic cell death and neoplastic cell transformation. In the earliest days of radiation biology, a model was already established saying that the generation of exchange-type aberrations requires breakage of chromosomes and reunion of the ''wrong'' ends (for review see Savage 1993; Natarajan and Obe 1996). While at that time the conceptions about the mechanisms underlying the reunion of ends were rather mechanistic, it is now widely accepted that exchange-type aberrations are produced enzymatically during rejoining of broken DNA ends in a process termed misrepair (Savage 1993). Mammalian cell mutants with decreased capacity for rejoining/repair of DNA double-strand breaks (DSB) show enhanced formation of chromosomal aberrations after irradiation (Darroudi and Natarajan 1987; Natarajan *et al.* 1993; Kirchgessner *et al.* 1993). It is not clear whether this is caused by longer persistence of open breaks (enhancing the probability of the wrong ends to meet) or by the eventual employment of mechanisms that are particularly prone to result in misrepair.

Three different pathways of DSB repair/rejoining have been observed both in *Saccharomyces cerevisiae* and mammalian cells: (1) homologous recombination associated with gene conversion events (Szostak *et al.* 1983; in the following text, this mechanism is referred to as homologous recombination), (2) single-strand annealing (SSA) requiring extensive homology between interacting sequences and associated with deletion formation (Lin *et al.* 1984; Ivanov and Haber 1995), and (3) more or less direct end joining, possibly accompanied by limited end processing, which requires no or little sequence homologies and is therefore called illegitimate recombination or nonhomologous end joining (NHEJ; Kramer *et al.* 1994; Mezard and Nicolas 1994; Nicolás *et al.* 1995).

The similarity between end products of DSB repair/rejoining in *S. cerevisiae* and mammalian cells suggests an evolutionary conservation of DSB processing pathways. The relative importance and efficiency of these pathways, however, seem to differ in yeast and mammalian cells. In *S. cerevisiae*, homologous recombination is the predominant mechanism (reviewed by Petes *et al.* 1991), but SSA and illegitimate recombination can also be detected using specific assays. In mammalian cells, illegitimate recombination is very efficient (Roth and Wilson 1986; Thacker *et al.* 1992; Rouet *et al.* 1994), but the other mechanisms have been observed as well (Lin *et al.* 1984, 1990; Chakrabarti and Seidman 1986; Rouet *et al.* 1994; Choulika *et al.* 1995).

Probably all of these pathways have the potential of causing exchange-type aberrations: Ectopic homologous recombination results in exchange-type aberrations if associated with a crossover (Jinks-Robertson and Petes 1986; Fasullo and Davis 1988; Fasullo *et*

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al. 1994). Using suitable recombination substrates and site-specific DSB induction, Haber and Leung (1996) showed recently in yeast that SSA can occur between broken ends of different chromosomes, thus leading to translocations. The high yield of aberrations found in irradiated mammalian cells suggests that NHEJ can also occur between ends originating from different chromosomes.

Because knowledge about repair proteins and their roles in the various mechanisms of DSB processing in mammalian cells is still limited, here we started to analyze the relationship between individual pathways of DSB processing and induction of chromosomal aberrations in the yeast *S. cerevisiae.* In this yeast, classical cytological techniques cannot be used because of insufficient chromatin condensation (Guacci *et al.* 1994). Structural aberrations that result in chromosome length alterations, however, can be made visible by electrophoretic separation of the chromosomal molecules according to size using pulsed-field gel electrophoresis (PFGE; Fasullo and Davis 1988; Game *et al.* 1989; Fasullo *et al.* 1994). To obtain sufficient DNA for electrophoretic karyotyping, however, it is necessary to clonally expand individual cells, thus restricting the analysis to stable aberrations.

We have analyzed yeast strains mutated for the genes *RAD52*, *RAD54*, and *HDF1*. It is well established that *rad52* mutant cells cannot repair DSB via homologous recombination. SSA seems independent of *RAD52* within large arrays of tandemly repeated sequences (Ozenberger and Roeder 1991), but for recombination between shorter direct repeats, a role for *RAD52* has been proposed (Ozenberger and Roeder 1991; Fishman-Lobell *et al.* 1992). Nonhomologous end joining seems independent of *RAD52* (Kramer *et al.* 1994; Mezard and Nicolas 1994; Moore and Haber 1996). In *rad54* mutants, gene conversion events are strongly reduced, but, in contrast to *rad52* mutants, not completely abolished (Liefshitz *et al.* 1995). It has been proposed that the *RAD52* gene is essential for homologous recombination, whereas the *RAD54* (and *RAD51*, *RAD55* and *RAD57*) gene products are accessory factors facilitating access to certain chromatin regions (Sugawara *et al.* 1995). The *RAD54* gene is not required for SSA; on the contrary, increased levels of SSA events are observed in *rad54* mutants (Liefshitz *et al.* 1995; Ivanov *et al.* 1996). No role for Rad54 in illegitimate end joining has been found so far (Moore and Haber 1996). *HDF1* is the yeast homolog of the mammalian Ku70 gene (Feldmann and Winnacker 1993). Together with the yeast Ku80 homolog, the Hdf1 protein forms a heterodimer that binds with high affinity to double-stranded DNA ends (Feldmann *et al.* 1996; Milne *et al.* 1996). In earlier work, we observed that deletion of the *HDF1* gene confers enhanced radiosensitivity in cells impaired in homologous recombination, and we proposed a role for Hdf1 in an *RAD52*-independent pathway of DSB repair (Siede *et al.* 1996). In the meantime, experimental evidence for involvement of the yeast Ku protein homologs in illegitimate joining of linearized plasmids and endonuclease-mediated chromosomal breaks has been obtained (Boulton and Jackson 1996a,b; Milne *et al.* 1996; Tsukamoto *et al.* 1996; A. A. Friedl, O. Inbar and M. Kupiec, unpublished results).

Here, we show that electrophoretic karyotyping is a suitable means for detection of stable, untargeted, radiation-induced, chromosomal alterations. Analysis of the patterns of chromosomal bands in aberrant clones suggests that aberration types are similar to those obtained in irradiated mammalian cells. The yield of aberrations in haploid wild-type and *hdf1* mutant cells after irradiation in stationary growth phase is low, presumably because most survivors descended from cells that were in G_2 phase at the time of irradiation. In *rad52* and *rad54* mutants, the yield was strongly enhanced, demonstrating the misrepair capacity of DSB repair pathways other than homologous recombination. In *rad52 hdf1* and *rad54 hdf1* double mutants, the yield in alterations was very low, showing that the Hdf1-mediated NHEJ pathway is very prone to result in misrepair.

MATERIALS AND METHODS

Strain construction: The strains used in this study are listed in Table 1. Deletion/disruption of *HDF1* and *RAD52* to create strains SX46A *hdf1*Δ, SX46A *rad52*Δ, SX46A *rad52*Δ *hdf1*Δ, and the respective derivatives of strain WS8105-1C have been described (Siede *et al.* 1996).

To delete the entire *RAD54* open reading frame, we applied a PCR-based gene disruption method (Wach *et al.* 1994) using a *rad54*Δ*::kanMX* fragment kindly provided by W.-D. Heyer (J. Schmuckli-Mauer and W.-D. Heyer, unpublished results). With this method, transformants are selected by growth on YPD containing 200 mg/liter of geneticin (GIBCO BRL, Gaithersburg, MD). Because strain SX46A turned out resistant to geneticin at concentrations up to 800 mg/liter, we were forced to use strain WS8105-1C for constructing a *rad54* and a *rad54 hdf1* mutant. Correct transplacement was verified in each case by Southern hybridization and phenotypic tests (sensitivity towards ionizing irradiation and temperature sensitivity).

γ Irradiation: Cells were grown in liquid YPD medium for 4 days to stationary growth phase, washed, and resuspended in potassium phosphate buffer. The cell suspension was aerated with O_2 30 min before and during irradiation. Cells were irradiated on ice in a ^{60}Co source (Atomic Energy of Canada, Ltd., Kanata, Ontario, Canada). Appropriate dilutions of irradiated and control samples were plated on YPD and incubated for 4 days (*RAD52* strains) or 6 days (*rad52* strains) at 30°. For determination of radiosensitivity, three plates per dose point were counted.

Clonal expansion of surviving cells: Single colonies derived from cells surviving irradiation or controls were picked and restreaked on YPD in ordered arrays. After further incubation for 4 days, the clones were transferred to 10 ml liquid YPD medium and grown for 4 days under shaking. Two milliliters of the cell suspensions were then used to generate frozen

TABLE 1

Yeast strains

Strain	Genotype
SX46A	*MAT***a***RAD HDF ade2 his3-532 trp1-289 ura3-52*
SX46A *hdf1*Δ	*MAT***a***RAD hdf1*Δ*::URA3 ade2 his3-532 trp1-289 ura3-52*
SX46A *rad52*Δ	*MAT***a** *rad52::TRP1 HDF ade2 his3-532 trp1-289 ura3-52*
SX46A *rad52*Δ *hdf1*Δ	*MAT***a** *rad52::TRP1 hdf1*Δ*::URA3 ade2 his3-532 trp1-289 ura3-52*
WS8105-1C	*MAT*α *RAD HDF ade2 arg4-17 trp1-289 ura3-52*
WS8105-1C *hdf1*Δ	*MAT*α *RAD hdf1*Δ*::URA3 ade2 arg4-17 trp1-289 ura3-52*
WS8105-1C *rad52*Δ	*MAT*α *rad52::TRP1 HDF ade2 arg4-17 trp1-289 ura3-52*
WS8105-1C *rad52*Δ *hdf1*Δ	*MAT*α *rad52::TRP1 hdf1*Δ*::URA3 ade2 arg4-17 trp1-289 ura3-52*
WS8105-1C *rad54*Δ	*MAT*α *rad54*Δ*::kanMX HDF ade2 arg4-17 trp1-289 ura3-52*
WS8105-1C *rad54*Δ *hdf1*Δ	*MAT*α *rad54*Δ*::kanMX hdf1*Δ*::URA3 ade2 arg4-17 trp1-289 ura3-52*

stocks, and the remainders were used to prepare genomic DNA.

Preparation of genomic yeast DNA and electrophoretic separation in pulsed-field gels: Preparation of genomic DNA of individual clones by the agarose plug method and PFGE using the CHEF DRII system (Bio-Rad, Richmond, CA) were performed as described (Friedl *et al.* 1995a). With the electrophoresis regime applied, a good spatial separation of all yeast chromosomes except chromosome XII can be achieved. For visualization of length variations in chromosome XII, the ZIFE electrophoresis system (Q-Life Systems, Kingston, Ontario, Canada; Noolandi and Turmel 1992) was used.

Visualization of electrophoretic karyotypes and image analysis: After electrophoresis, gels were stained with ethidium bromide and recorded by a CCD camera as described (Friedl *et al.* 1995a). Measurement of fluorescence intensity distributions along the gel lanes was described in detail (Kraxenberger *et al.* 1994). The fluorescence intensity profiles thus obtained were used as input data in an evaluation program that was previously established for the quantitation of radiation-induced DSB in yeast (Friedl *et al.* 1993, 1995a; Kraxenberger *et al.* 1994). Briefly, this program compares the measured fluorescence intensity profiles with computed profiles that are calculated assuming that breaks in chromosomal molecules are localized randomly. The quality of the agreement between observed and calculated profiles in dependence of the breakage frequency assumed is determined by a least squares procedure.

Determination of chromosomal lengths: The lengths of the chromosomal molecules of the parental strains SX46A and WS8105-1C were determined by comparing their electrophoretic mobility in CHEF gels with that of λ-phage concatemeres and yeast strains YNN295 and BK0, as described (Friedl *et al.* 1993, 1995b). We determined the following lengths: 260 kb (I), 300 kb (VI), 360 kb (III), 450 kb (IX), 590 kb (V, VIII), 690 kb (XI), 790 kb (X), 830 kb (XIV), 855 kb (II), 1000 kb (XIII and XVI), and 1135 kb (VII and XV). For chromosomes IV and XII, lengths of 1600 and 2200 kb were assumed. After a typical CHEF run, interpolation in a plot of molecular length *vs.* migration distance gives a smooth curve in the range between 260 and 1600 kb (see for example Friedl *et al.* 1995a), which allows us to derive the lengths of unknown molecules ≤1600 kb. The lengths of longer molecules were estimated according to their migration behavior in ZIFE gels.

Southern hybridization with chromosome-specific probes: For the identification of altered chromosomes in case of ambiguity, Southern hybridizations were performed using centromere- or gene-specific probes. Chromosomal DNA separated by PFGE was UV nicked, denatured in 0.5 M NaOH/1.5 M NaCl (30 min), and transferred onto nylon membrane (Qiagen, Chatsworth, CA) by capillary transfer using the same buffer. Prehybridization and hybridization were carried out according to the membrane manufacturer's directions. DNA probes were generated by plasmid digestion with appropriate enzymes and gel purification of the desired fragments according to standard methods (Sambrook *et al.* 1989). The fragments were radiolabeled by the random prime method (Feinberg and Vogelstein 1983). Plasmids pDJ4, pDJ3, pLA433, pDJ1, pDP22, pDP18, and pDP20, kindly donated by P. Philippsen (Biozentrum, Basel, Switzerland), contain chromosome-specific centromeric regions from chromosomes III, IV, VI, XI, XIII, XV, and XVI, respectively. Plasmid p441, containing a 3.2-kb *Eco*RI-*Bam*HI fragment from the *ADH4* gene (chromosome VII), was a gift from C. Morawetz (GSF-Forschungszentrum, Oberscheissheim, Germany); plasmid pM43, containing the *LYS2* sequence (chromosome II), was kindly donated by M. Kupiec (University of Tel Aviv, Israel). Plasmid C1/8, containing the *RAD5* sequence (chromosome XII), was provided by F. Ahne (GSF-Forschungszentrum, Oberscheissheim, Germany). The *URA3* gene (chromosome V) was prepared from the commercially available vector YIp5.

RESULTS

Construction of strains: The aim of this work was to investigate the influence of mutational inactivation of various DSB repair pathways on the generation of radiation-induced chromosomal aberrations. Homologous recombination between sister chromatids or homologous chromosomes is a very efficient and largely error-free DSB repair mechanism in yeast. To be able to detect the influence of minor repair pathways and to obtain a reasonable quantity of aberrational misrepair events, we investigated the induction of aberrations in haploid cells irradiated in highly stationary growth phase (G_0 cells). Furthermore, pilot experiments confirmed the observation (Mortimer *et al.* 1981) of very frequent spontaneous and radiation-induced chromosome loss in diploid *rad52* mutant cells. The spontaneous chromosome loss can occur at any time during pre- and postirradiation cultivation, and it results in a reduction of the relative intensities of individual chromosomal bands,

thus precluding a reliable analysis of aberrational events.

Repair-competent haploid G_1 or G_0 phase cells are incapable of DSB repair by homologous recombination using a homologous chromosome or sister chromatid as donor of information, but they can use ectopically located donors of information or alternative pathways of DSB repair other than homologous recombination. Cells mutated for *RAD52* are not able to perform any kind of homologous recombination; furthermore, they show reduced ability for SSA. Inactivation of *HDF1* affects NHEJ. Hence, in a *rad52 hdf1* double mutant, all kinds of DSB repair/rejoining mechanisms known so far are completely or largely blocked. We observed a high degree of secondary DNA degradation during post-irradiation incubation in *rad52 hdf1* double mutants that is evident neither in the single mutants nor in a *rad54 hdf1* double mutant (unpublished results), suggesting that one of two proteins, Rad52 or Hdf1, has to be present to protect broken ends against degradation. To be able to differentiate between effects caused by inactivation of homologous recombination, and those caused by enhanced DNA degradation, we included a *rad54* mutant in our studies. The reduction of homologous recombination in *rad54* mutants is not as complete as in *rad52* mutants, but it is stronger than in any other mutant of the *RAD52* epistasis group. Furthermore, *rad54* mutants differ from *rad52* mutants in that they are able to perform SSA.

In a first set of experiments, wild-type strain SX46A and its *rad52*, *hdf1*, and *rad52 hdf1* mutant derivatives were investigated. Construction of these strains has been described in detail (Siede *et al.* 1996). When we tried to construct a *rad54* mutant derivative in this series of strains, it turned out that strain SX46A is resistant towards geneticin, and for disruption of the *RAD54* gene using geneticin resistance as selection marker, another genetic background had to be used. We chose WS8105-1C, because a *hdf1* derivative of this strain was already available (Siede *et al.* 1996), and we performed a second set of experiments with strain WS8105-1C and its *hdf1*, *rad54*, and *rad54 hdf1* mutant derivatives.

Radiosensitivity of the tested strains: The radiosensitivity of strain SX46A and its *rad52*, *hdf1*, and *rad52 hdf1* derivatives have been described (Siede *et al.* 1996). Therefore, we give here only the results obtained with strains of the WS8105-1C series (Figure 1), which confirm the data obtained with the SX46A series. Even in highly stationary cultures, a small proportion (~5%) of the cells bear large buds indicative of G_2 phase cells. In a wild-type strain, this subpopulation of cells, which is able to perform homologous recombination between sister chromatids, gives rise to the so-called radioresistant tail observed in dose-response curves at higher irradiation doses (Brunborg and Williamson 1978). Because inactivation of *RAD52* completely abolishes homologous recombination, dose-response curves of *rad52* mutants show no radioresistant tail. Inactivation of *HDF1* sensitizes haploid wild-type cells irradiated in highly stationary growth phase and *rad52* mutant cells, suggesting that in this case, some alternative DSB repair pathway was inactivated. *HDF1* seems to not be required for G_2 phase-specific repair because in wild-type and *hdf1* mutant cells, the tail at doses ≥200 Gy is equally pronounced. The *rad54* and *rad54 hdf1* mutants behave like *rad52* and *rad52 hdf1* mutants, respectively, only at doses up to ≤200 Gy; at higher doses, they are more resistant. This suggests that in *rad54* mutants, some residual G_2-specific repair takes place, albeit at a low level, and supports the notion that *RAD54* is not indispensable for some low level of homologous recombination. A higher resistance of the *rad54* mutant, as compared to the *rad52* mutant, would be expected over the whole dose range if the ability to perform SSA had a clear impact on radiation sensitivity. The data obtained here suggest that this is not the case.

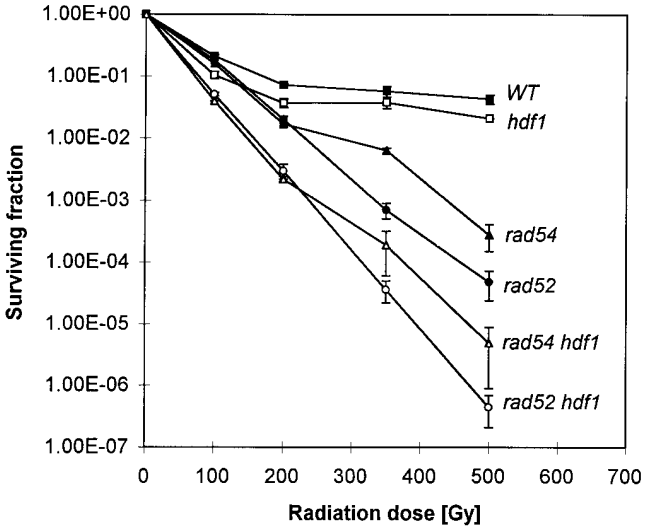

Figure 1.—Sensitivity to γ irradiation of haploid strain WS8105-1C and repair-deficient derivatives in highly stationary growth phase. Indicated are means and standard errors from two to five experiments per data point for wild-type WS8105-1C (■), WS8105-1C *hdf1*Δ (□), WS8105-1C *rad54*Δ (▲), WS8105-1C *rad52*Δ (●), WS8105-1C *rad54*Δ *hdf1*D (△), and WS8105-1C *rad52*Δ *hdf1*Δ (○).

Detection of altered karyotypes: Colonies arising from haploid cells that had survived irradiation and from unirradiated controls were picked randomly and expanded to obtain sufficient DNA for electrophoretic karyotyping by PFGE. Alterations in the localization of chromosomal bands or in relative band intensity are indicative of structural or numerical chromosomal aberrations. Examples of electrophoretic karyotypes in aberrant clones are shown in Figure 2. In these samples, which were all derived from strain SX46A *rad52*Δ, complete loss of chromosomal bands and appearance of new bands are clearly evident on the photographic image. Aberrational events that lead to alterations in the rela-

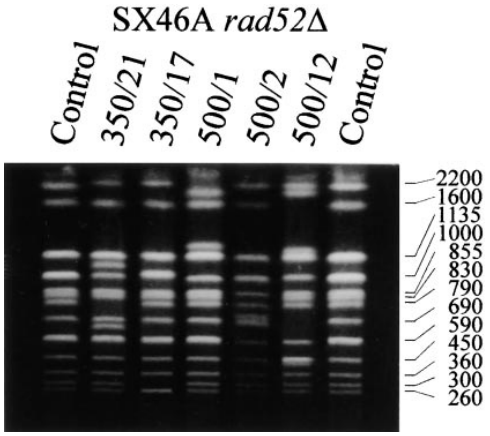

Figure 2.—Electrophoretic separation reveals karyotypic alterations. Chromosomal DNA from control clones (lanes 1 and 7) and aberrant derivatives (lanes 2–6) of strain SX46A *rad52*Δ was separated by PFGE. On the right side, the lengths of the chromosomal molecules in the respective bands of the control samples are indicated. The bands correspond to the following chromosomes: I (260 kb), II (855 kb), III (360 kb), IV (1600 kb), V (590 kb), VI (300 kb), VII (1135 kb), VIII (590 kb), IX (450 kb), X (790 kb), XI (690 kb), XII (2200 kb), XIII (1000 kb), XIV (830 kb), XV (1135 kb), and XVI (1000 kb). Note that the 590-, 1000-, and 1135-kb bands are doublets.

tive intensity of bands, however, are difficult to detect by eye because of the nonlinear response of photographic films (Ribeiro *et al.* 1989).

To unambiguously detect karyotypic alterations, we use a simulation procedure that was developed earlier for the measurement of the frequency of DSB in yeast cells (Friedl *et al.* 1993, 1995a; Kraxenberger *et al.* 1994). First, PFGE gels are stained with ethidium bromide under controlled conditions, and the distribution of fluorescence intensity along the gel lanes is measured by a CCD camera-based image analysis system (described in detail in Kraxenberger *et al.* 1994). These measured fluorescence intensity profiles represent distributions of DNA mass as a function of migration distance. We have shown earlier that these observed DNA profiles can be simulated if parameters such as length of intact chromosomal molecules, degree of, *e.g.*, preparation induced, DNA degradation, and migration behavior of the molecules in the gel are accounted for correctly. For the detection of karyotypic alterations, the observed DNA profiles of the individual gel lanes are then compared to simulated profiles that represent the expected outcome if a sample with normal karyotype had experienced the same degree of DNA degradation and the same electrophoretic resolution as the sample in question.

Examples for DNA profiles thus obtained are given in Figure 3A, showing the observed profiles of normal SX46A *rad52*Δ cells, as well as the aberrant derivatives SXrad52/350/21 and SXrad52/350/17 as solid lines. The respective simulated profiles are given as dashed lines. A good agreement between observed and computed profiles is seen for the control sample, whereas deviations are clearly evident in the samples of the aberrant clones. In clone SXrad52/350/21, the band corresponding to chromosome X (790 kb) is missing, and the double band corresponding to chromosomes XIII and XVI (1000 kb) exhibits a strongly decreased intensity. Simultaneously, two new bands whose localization correspond to the molecular lengths of 650 and 1050 kb, respectively, appear. In clone SXrad52/350/17, the band representing chromosome VI (300 kb) is absent, while the intensity of the 260-kb band is enhanced.

These examples show that by using the simulation approach, not only complete loss or gain of new bands are easy to detect, but increases or decreases in band intensity are also easy to detect. On the basis of the observed alterations, a guess about the karyotype, *i.e.*, lengths of individual chromosomal molecules and their relative frequencies, in the aberrant clone is made, and the validity of the karyotype is then checked by comparing the observed profiles to theoretical profiles that were computed on the basis of the new karyotype. Examples for derivatives SXrad52/350/21 and SXrad52/350/17 are shown in Figure 3B. For clone SXrad52/350/21, it was assumed that the parental chromosome X and one of the two 1000-kb chromosomes (XIII or XVI) were lost, leaving a single band at the position of the former 1000-kb double band, and that two aberrant chromosomes with lengths of 650 and 1050 kb, respectively, were generated. For clone SXrad52/350/17, it was assumed that the parental chromosome VI was lost, while a new aberrant chromosome comigrated with chromosome I, thus yielding a band of double intensity at the position corresponding to 260 kb. These assumptions led to a good agreement between observed (solid lines) and computed profiles (dashed lines).

Spontaneous karyotypic alterations: We analyzed the frequency of aberrant karyotypes after irradiation with various doses in three independent experiments for the strains SX46A, SX46A *rad52*Δ, SX46A *hdf1*Δ, and SX46A *rad52*Δ *hdf1*Δ, two experiments for WS8105-1C *hdf1*Δ, and one experiment each for WS8105-1C, WS8105-1C *rad54*Δ, and WS8105-1C *rad54*Δ *hdf1*Δ. In each irradiation experiment, we checked 10–20 clones derived from unirradiated control cells. In all strains tested, large variations (up to ~1000 kb) in the length of chromosome XII (normal length 2200 kb) in subclones derived from both irradiated and unirradiated cells were observed (data not shown). This chromosome bears a large rDNA cluster, which is known for extensive mitotic length alterations (Olson 1991). In strain SX46A *hdf1*Δ, chromosome VII (normal length 1135 kb) is ~50 kb longer than in the other strains of the SX46A series, and exhibits variations in length between individual clones in the range of ≥100 kb (data not shown). Since this band did not hybridize with a probe for 18S rDNA (data not shown), its instability seems to not be caused

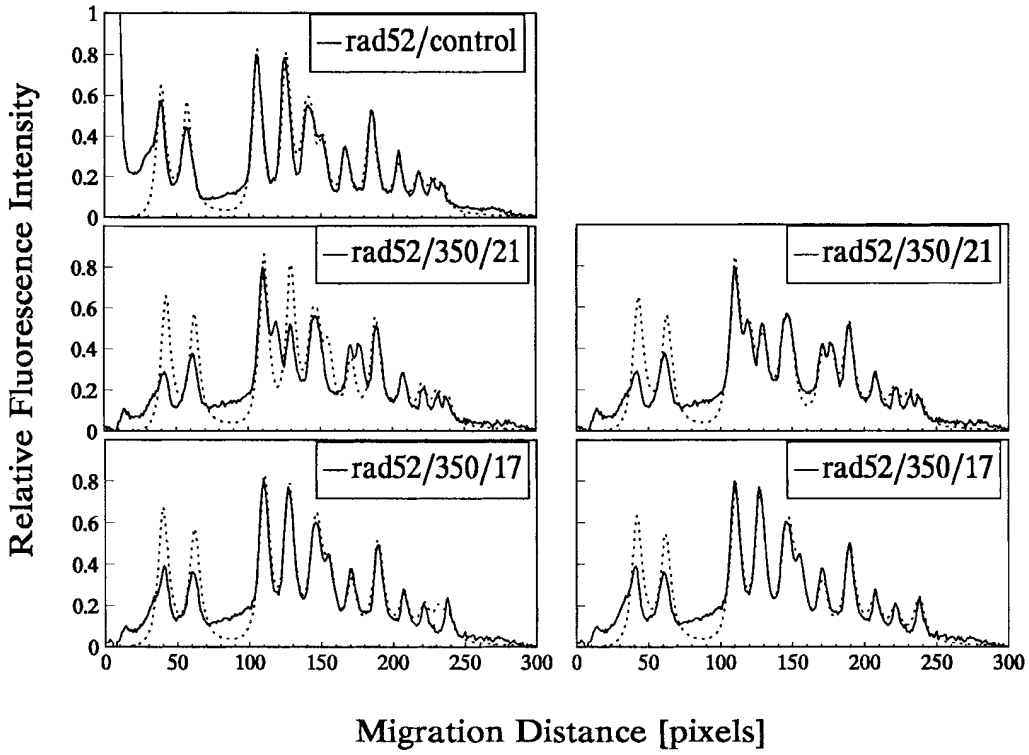

Figure 3.—Comparison between observed and calculated DNA profiles. (A) Observed DNA profiles (solid lines), as obtained from the gel depicted in Figure 1, and theoretical profiles calculated assuming a normal karyotype (dashed lines) for the control sample and two aberrants (SXrad52/350/21 and SXrad52/350/17) derived from strain SX46A *rad52*Δ. In the range corresponding to migration distances >90 pixels, the deviations between observed and calculated profiles indicate the presence of karyotypic alterations. Differences in the intensity of the peaks of the two largest chromosomes (migration distances <90 pixels) are not informative because of the self-trapping effect, which slows down molecules in this zone of the gel. (B) Comparison of the observed profiles (solid lines) from A to theoretical profiles that were computed assuming karyotypic alterations. For clone SXrad52/350/21, loss of the 790- and one 1000-kb band, as well as gain of a 650- and a 1060-kb band were assumed. For clone SXrad52/350/17, loss of the 300-kb band and gain of a 260-kb band were assumed. Under these assumptions, a satisfying agreement between observed and calculated profiles is obtained.

by a transmission of rDNA sequences. We decided that even extensive length alterations of these unstable chromosomes should not be regarded as induced chromosome aberrations unless they are accompanied by further karyotypic alterations.

In one of three independent experiments with strain SX46A *hdf1*Δ, the same type of alteration (lengthening of chromosomes XIV and XIII) was observed in one unirradiated subclone and two irradiated subclones (Table 2). These alterations were not regarded as radiation induced.

In the experiment with strain WS8105-1C *rad54*Δ, the same type of alteration (a putative translocation between VI and XI) was detected in three irradiated subclones, hinting at a spontaneous event that took place before irradiation, during cultivation of the cells. Again, these alterations were not regarded as radiation induced.

Frequency of radiation-induced karyotypic alterations: Colonies arising from cells that survived irradiation were picked without any bias. Therefore, the frequency of aberrant karyotypes detected reflects the radiation-induced frequency of aberrational events. The results are given in Table 2. In irradiated samples of wild-type strains, karyotypic alterations were detected at a low frequency (three out of 167 clones of irradiated cells analyzed in total in SX46A, and one out of 80 clones in WS8105-1C). In the *rad52* mutant (derived from SX46A) and the *rad54* mutant (derived from WS8105-1C), the frequency of aberrational clones was substantially increased (14 out of 181 and five out of 40, respectively). We assume that this difference in aberration yield reflects the difference in the use of repair pathways: In the wild type, the majority of clones surviving doses of ≥200 Gy probably originated from cells that were in the G_2 phase at the time of irradiation. These cells were able to repair DSB by homologous recombination between sister chromatids, a highly efficient process (Kadyk and Hartwell 1992) that should lead to visible alterations only in exceptional cases (unequal sister chromatid exchange). *rad52* and *rad54* mutants, however, are forced to use alternative pathways, which, because of the lower dependence on homology, may be more prone to misrejoining. Based on current knowledge, these alternative mechanisms could be SSA and NHEJ. We assumed that SSA plays only a minor

TABLE 2

Yield of aberrant clones

Strain	Aberrant clones per number of clones analyzed					Total yield of radiation-induced aberrants[a]
	0 Gy	100 Gy	200 Gy	350 Gy	500 Gy	
SX46A (WT)	0/49	1/20	1/64	0/24	1/59	3/167
WS8105-1C (WT)	0/20	—	—	—	1/80	1/80
SX46A *rad52*	0/50	2/13	2/66	2/28	8/74	14/181
WS8105-1C *rad54*	0/20	—	—	—	8/40[b]	5/40
SX46A *hdf1*	1/43	0/14	0/25	0/15	4/52[c]	2/106
WS8105-1C *hdf1*	0/40	—	—	—	1/124	1/124
SX46A *rad52 hdf1*	0/53	0/21	0/58	0/37	0/63	0/179
WS8105-1C *rad54 hdf1*	0/20	—	—	—	1/80	1/80

Yield of colonies exhibiting chromosomal alterations per number of colonies analyzed by electrophoretic karyotyping. Alterations in chromosomes exhibiting spontaneous length alterations (XII and VII; see text) were not included if not accompanied by other alterations. If several colonies derived from one irradiation experiment exhibited the same chromosomal alteration, these were not regarded as radiation induced. Data were pooled from three independent irradiation experiments for strains SX46A, SX46A *rad52*, SX46A *hdf1*, and SX46A *rad52 hdf1*, as well as two independent experiments for strain WS8105-1C h*df1*. Data for the other strains are from one experiment.

[a] Total yield of aberrant clones in irradiated samples, after subtraction of presumed preirradiation events.

[b] Three of these aberrants showed the same banding pattern, suggesting a spontaneous event in a progenitor cell.

[c] Two of these aberrants showed the same alteration as the aberrant in the unirradiated control, suggesting a spontaneous event in a progenitor cell.

role in repair of radiation-induced damage because of the relative paucity of repetitive sequences in yeast (Dujon 1996) and because *rad52* mutants, which are impaired in SSA except if the repeats are very long (Ozenberger and Roeder 1991), and *rad54* mutants, which are SSA proficient (Liefshitz *et al.* 1995; Ivanov *et al.* 1996), exhibit the same radiation sensitivity, except for the G_2 phase–specific component.

HDF1 is one of the few genes known so far to be involved in illegitimate end joining. The yield of karyotypic alterations in *hdf1* mutants (derived from SX46A and WS8105-1C) was low (two out of 106 and one out of 124, respectively) and comparable to the yield observed in wild-type cells.

Combination of the *hdf1* mutation with inactivation of *RAD52* or *RAD54* abrogates the increased formation of karyotypic alterations seen in the *rad52* and *rad54* single mutants, indicating that the generation of aberrations in these mutants depends largely on *HDF1*. In the *rad52 hdf1* double mutant (derived from SX46A), no aberrant clone was detected among a total of 179 analyzed clones; in the *rad54 hdf1* double mutant (derived from WS8105-1C), one out of 80 clones bore a karyotypic alteration.

Characterization of aberration events: To learn more about the formation of radiation-induced chromosomal aberrations in yeast, we started to characterize the radiation-induced aberrations found in samples with altered karyotypes. It was, however, not our intention to exactly elucidate the type of aberrational event in all clones because this would require detailed hybridization studies with a panel of probes for each chromosome.

In all aberrant clones, the chromosomal length and identity of altered bands was recorded. In case a chromosome that normally migrates in a double band with another chromosome was involved, the identity of the altered band was determined by hybridization with chromosome-specific DNA probes. The lengths of molecules migrating in newly arisen bands were determined using calibration curves for the relation between molecular length and migration distance, which were based on the positions of bands corresponding to chromosomes with known length. Descriptions of the karyotypes of all altered clones are given in Tables 3 and 4.

A typical stable outcome of misrepair events is a translocation. Translocations are expected to lead to loss of two (or more) parental bands and the simultaneous appearance of the same number of new bands. Furthermore, the sum of the lengths of the chromosomal molecules in the lost and new bands should be equal. We found a total of 14 aberrants that follow these criteria (Table 3). In 11 of these, two chromosomes were involved; in two aberrants (WS/500/64 and SXrad52/500/12), four chromosomes exhibited length alterations, and in one aberrant (SXrad52/500/77), three bands were altered. In most of the samples, the molecular lengths seem to not be exactly conserved, but the differences were on the order of a low percentage and may be explained by uncertainties in the determination of molecular lengths, especially when large chromo-

TABLE 3

***Bona fide* translocations**

Clone	Chromosomes involved (kb)	New bands (kb)[a]
SX/100/M5	VI (300), IV (1600)	205, 1730
WS/500/64	X (790), XIV (830), II (855), XVI (1000)	795, 810, 875, 995
SXrad52/100/M3	VI (300), XVI (1000)	**590**, 650
SXrad52/200/M1	V (590), VIII (590)	**450**, 690
SXrad52/350/21	X (790), XIII (1000)	650, 1050
SXrad52/500/1	XVI (1000), XII (2200)	1180, 2000
SXrad52/500/2	V (590), XIV (830)	**690**, 720
SXrad52/500/12	V (590), XI (690), XIII (1000), IV (1600)	420, **450**, 1210, 1800
SXrad52/500/54	VIII (590), II (855)	390, 1040
SXrad52/500/61	IV (1600), XII (2200)	1900, 1900
SXrad52/500/77	V (590), VIII (590), IV (1600)	300, 1220, 1220
WSrad54/500/19	X (790), XVI (1000)	620, **1135**
WSrad54/500/35	VII (1135), IV (1600)	**1000**, 1700
WSrad54/500/36	V (590), VIII (590)	330, 925

Bona fide translocations obtained after irradiation were defined by length alterations in two or more bands providing approximate conservation of the total genome size. In case one of the chromosomes migrating in double bands (V and VIII, X and XIII, or VII and XV) was involved, the identity of the altered chromosome was determined by Southern hybridization with chromosome-specific probes.

[a] Boldface indicates comigration of the aberrant band with an unaltered band.

somes were involved. As expected, in all cases further analyzed by hybridization with probes for lost bands, the signal was found at the position of one of the new bands (data not shown).

One major drawback of the system is caused by the mitotically unstable chromosomes (XII and VII) because it is difficult to prove their involvement in exchange-type aberrations on the basis of length alterations. We observed seven clones exhibiting a length alteration in one band only, which was not clearly com-

TABLE 4

Other types of alterations

Clone	Description[a]
SX/200/M4	Chromosome III (360 kb) circularized[b]
SX/500/18	Intensity of 855-kb band (II) doubled
SXrad52/100/M2	690-kb band (XI) lost; new band **450** kb
SXrad52/200/24	Intensity of 830-kb band (XIV) enhanced (1.5×); additional band (660 kb) with half of normal intensity
SXrad52/350/17	300-kb band (VI) lost; new band **260** kb
SXrad52/500/19	Putative dicentric by fusion of chromosomes II (855 kb) and XV (1135 kb)[c]
SXrad52/500/26	260-kb (I), 590-kb (V), 2200-kb (XII) bands lost; new bands 460 and **1135** kb
WSrad54/500/10	1600-kb band (IV) lost; new band 1200 kb
WSrad54/500/17	790-kb band (X) lost; new band 500 kb
SXhdf1/500/4	590-kb band (V) lost; new band 615 kb
SXhdf1/500/39	1000-kb band (XIII) lost; new band 1060 kb; chromatid type[d]
WShdf1/500/40	260-kb band (I) lost; new band **300** kb
WSrad54_hdf1/500/21	Chromosome III (360 kb) circularized[b]

Other types of chromosomal alterations not fulfilling the criteria for *bona fide* translocations. In case one of the chromosomes migrating in double bands (V and VIII, X and XIII, or VII and XV) was involved, the identity of the altered chromosome was determined by Southern hybridization with chromosome-specific probes. For further explanations, see footnotes and text.

[a] Boldface indicates comigration of the aberrant band with an unaltered band.

[b] Circularization experimentally proven by relinearization (see text).

[c] The aberrant chromosome (2000 kb) hybridizes with probes for II and XV.

[d] The intensity of the 1000-kb (double) band is 75% of the normal value; the intensity of the 1060-kb band is 50% of the value expected if stochiometric amounts of molecules were present.

pensated by a length alteration in chromosome XII (or the likewise unstable chromosome VII in strain SX46A *hdf1*Δ). These aberrants (included in Table 4) may represent either true deletions or insertions, or translocations involving the unstable chromosomes. Further hybridization studies will be required to elucidate the type of event in these clones.

Also listed in Table 4 are two clones (SX/200/M4 and WSrad54_hdf1/500/21) that lack the band representing chromosome III. Assuming that these clones might carry a circularized chromsome III (which cannot enter the gel), we γ-irradiated the cells of these clones to linearize the circle, prepared the genomic DNA directly after irradiation, and separated it by PFGE. Now, hybridization with a probe for *CENIII* gave a signal at a position corresponding to ~310 kb, agreeing well with the expected length if circularization occurred by fusion of the partially homologous regions *HML* and *HMR* (Klar *et al.* 1983; Game 1989).

In one case (clone SXrad52/500/19), an obviously dicentric chromosome was formed: in this clone, the bands corresponding to chromosomes II (855 kb) and XV (1135 kb) were missing, and one new band of ~2000 kb was observed. This band gave a signal after hybridization with both probes for chromosomes II and XV (data not shown).

Clone SXrad52/500/26 showed a puzzling phenotype: Bands of three chromosomes (I, V, and XII, representing a total of 3050 kb) were missing in the karyotype, and only two new bands (460 and 1135 kb) could be detected. If a three-way interchange had taken place in this clone, a further band of ~1450 kb would be expected, which was not present. It remains to be tested whether the missing genetic material is present in a circularized chromosome.

Doubling of the relative intensity of a band, presumably indicative for a disomy, was detected in clone SX/500/18 (855 kb). A special alteration is given by clone SXrad52/200/24: here, the intensity of the 830-kb band was increased by 50% only, but a band of 660 kb also arose, but this band had only half of the stochiometric intensity.

DISCUSSION

Fasullo and colleagues (Fasullo and Davis 1988; Fasullo *et al.* 1994) described the electrophoretic separation of yeast chromosomal molecules as a suitable method for the physical detection of directed ectopic recombination leading to translocations. Because their approach made the expected outcome of the recombination event known in advance, no elaborate method for the evaluation of events was necessary. Here, we demonstrate that electrophoretic karyotyping is also useful for detecting untargeted recombinational events. To avoid that some of the events escape notice, however, a more elaborate evaluation procedure is required. In theory, karyotypic alterations may be detected by comparing the fluorescence intensity profile of a sample under investigation to a profile obtained by a control sample run on the same gel. In practice, this approach may lead to evaluation errors if unequal amounts of DNA were loaded, or if the degree of preparation-induced DNA degradation varies between the samples. We therefore use a simulation procedure that can account for variable experimental parameters such as the amount of DNA, the degree of DNA degradation, and the actual relationship between molecular length and migration distance in the gel lane. Furthermore, the simulation procedure tests whether an observed banding pattern is compatible with assumptions made on the altered karyotype. Our observation that a high fraction (10 out of 44; see Tables 3 and 4) of recombinant or aberrant bands colocalized with bands of other normal chromosomes corroborates the need for a more scrutinizing evaluation procedure.

Electrophoretic karyotyping allows the detection of those structural aberrations that lead to altered chromosome lengths. We estimate the limit of detection of length alterations in CHEF gels to be ~10 kb, except for the two longest chromosomes, where the limit is higher. Translocations, deletions, and insertions that result in smaller length alterations will escape detection, as will inversions.

It should be emphasized that electrophoretic karyotyping requires clonal expansion of cells, thus only aberration types that do not prevent colony formation will be obtained. Hence, the distribution of observed aberration types will differ from that observed cytogenetically in irradiated mammalian cells, where aberrations are usually scored in the first metaphase after irradiation or in scarcely dividing lymphocytes. Although a thorough elucidation of aberration types was not intended here, the observed alterations in the banding pattern allow for some conclusions about the type of the underlying aberrational event.

The simplest type of a structural interchange aberration is an exchange between two chromosomes that results in a reciprocal translocation or a dicentric chromosome plus an acentric fragment (for a recent survey on aberration types see Sachs *et al.* 1997). *Bona fide* translocations (showing length alterations in two or more chromosome species with compensation of length) represent the largest class (14 out of 27) of event types observed in this study. While in 11 of the clones bearing translocations obviously two chromosomes interacted, rather we observed one clone where three chromosomes were altered, suggesting a so-called three-way interchange. Alterations in four chromosomes, as observed twice, could have been caused by two pairs of reciprocal translocation or by a four-way interchange. Analysis of radiation-induced aberrations in mammalian cells by multicolor fluorescence *in situ* hybridizations (FISH) gave the surprising result that complex aberra-

tions involving more than two chromosomes occur more often than anticipated (Lucas and Sachs 1993; Savage 1996). Our data suggest that complex aberrations occur also in yeast.

In mammalian cells, it has been observed repeatedly that the frequency with which certain chromosomes are involved in translocations is not proportional to their molecular lengths, *e.g.*, Knehr *et al.* (1994). It has been proposed that these deviations may be caused by the three-dimensional nuclear architecture (Cremer *et al.* 1996). Although the number of aberrants carrying translocations gathered in the present work is too low to allow general conclusions, it appears that certain chromosomes, *e.g.*, chromosomes V and VIII, are overrepresented, while, for example, chromosome XV, although being larger, has not been found to be involved in translocations. This effect may also be explained by conformational restraints; however, it cannot be excluded that certain translocations affect cell viability.

Structural interchange reactions can also produce dicentric chromosomes plus acentric fragments. Chromosomes with two functional centromeres as well as acentric fragments are considered unstable. In one clone (SXrad52/500/19), however, we found a putative dicentric. The apparent mitotic stability of this chromosome may be explained by mutational inactivation or loss of one of the centromeric sequences, as has already been described for yeast (Jäger and Philippsen 1989; Kramer *et al.* 1994).

Besides being associated with asymmetrical interchange reactions, acentric fragments can also result from intrachromosomal rearrangements (interstitial deletion) or simply from failure to rejoin a break (terminal deletion). Because of the remarkable compactness of the yeast genome (Goffeau *et al.* 1996), we expect that the loss of genetic information associated with loss of acentric fragments will not be tolerated by haploid cells in most cases. Therefore, those four aberrants that show considerable shortening of one chromosome species that is apparently uncompensated by enlargement of another chromosome presumably do not carry deletions, but rather as yet unproven translocations involving chromosome XII.

In a similar manner, an increase in length in one chromosome species can be explained by an interchange with chromosome XII (or chromosome VII in case of strain SX46A *hdf1*Δ). Alternatively, it may be explained by insertion of unknown genetic material (see below).

In mammalian cells, circularized chromosomes are often observed after DSB induction. They are considered mitotically unstable because sister chromatid exchange can interlock the chromatids and lead to proliferative cell death. In *S. cerevisiae*, however, spontaneous circularization of chromosome III by recombination between the *HML* and *HMR* loci has been described (Klar *et al.* 1983), and haploid cells carrying a circular chromosome III grow well (Haber *et al.* 1984). We observed circularization of chromosome III in two clones, and the length of the linearized chromosomes suggests that circularization may be caused by interaction between *HML* and *HMR*.

Besides structural chromosome aberrations, numerical aberrations have also been observed after irradiation in mammalian cells, showing that ionizing radiation has clastogenic as well as aneugenic activity (Kirsch-Volders *et al.* 1996; Hande *et al.* 1997). Our haploid system does not allow detection of chromosome loss, but we observed a banding pattern that suggests gain of chromosomes, presumably by nondisjunction, in clone SX/500/18. In clone SXrad52/200/24, the intensity of the band representing chromosome XIV was enhanced by a factor of 1.5, while an additional band appeared that had only half of the expected intensity and that represented molecules of an estimated size of 170 kb smaller than chromosome XIV. This pattern suggests that a combination of a chromatid-type aberration and a nondisjunction took place. A putative chromatid-type aberration was also observed in clone SXhdf1/500/39, where the newly arisen band had only half of the expected intensity while the intensity of the parental (double) band was reduced by ~25%.

In conclusion, the banding patterns observed in clones of irradiated yeast cells can easily be explained, assuming that aberration types are similar to those frequently observed in mammalian cells, suggesting that similar mechanisms took place.

Radiation-induced chromosome aberrations were rarely detected in wild-type cells. We assume that the majority of colonies formed after irradiation originated from cells that were in the G_2 phase during irradiation and thus able to repair breaks in an error-free manner by sister chromatid recombination. Although the low yield of aberrants obtained renders a statement on dose dependence difficult, no indication for an increase in the yield with dose is obvious. This suggests that the frequency of aberrants detected depends not only on the frequency of DSB, but also on the probability of G_0 *vs.* G_2 cells to survive irradiation. Out of four aberrants, we detected two *bona fide* translocations in wild-type cells. These may be explained by ectopic homologous recombination. Indeed, it has been shown with several kinds of model recombination substrates that induction of DSB leads to induced ectopic recombination (Fasullo *et al.* 1994; Liefshitz *et al.* 1995). For the largest class of naturally repetitive sequences in yeast, the Ty family of elements, however, damage induction of recombination is very low (Parket and Kupiec 1992; Kupiec and Steinlauf 1997), suggesting that at least this class of repetitive elements differs from nonrepetitive sequences in the regulation of recombinative events. Because other types of repetitive elements are quite scarce in yeast and because the translocations observed here do not involve known regions of duplications (Goffeau

et al. 1997), it is also possible that these translocations arose by use of other mechanisms for DSB repair, such as NHEJ.

In contrast to the wild type, in the *rad52* mutant, the yield of aberrants was substantially higher. The majority of events detected seems to have arisen by interchromosomal reactions. As interchromosomal homologous recombination depends strongly on *RAD52*, these aberrations must have been caused by an alternative mechanism. In contrast to earlier findings (Ho 1975; Resnick and Martin 1976), our data show that *rad52* mutants are not totally devoid of functions for the joining of radiation-induced chromosomal breaks. Indeed, analysis of the restitution of high molecular weight DNA using PFGE and the simulation procedure suggests that ~30% of the DSB are joined during postirradiation incubation in *rad52* mutant cells irradiated with 300 Gy (unpublished results). The frequency and types of aberrations in the *rad54* mutant are comparable to that observed in the *rad52* mutant, although another genetic background was used. The residual capacity of *rad54* mutants for G_2 phase-specific repair evident from the survival data probably is too low to exert a drastic effect on the frequency of aberrations. Furthermore, its ability to perform SSA seems not to influence aberration formation. From current models for SSA mechanisms, it follows that two breaks on different chromosomes must be present near to regions of homology if an exchange-type reaction is to occur. While experiments using model substrates and site-specific breakage showed that this can happen (Haber and Leung 1996), the probability that such a situation arises naturally is very low, especially if one considers the paucity of repetitive elements in the yeast genome (Dujon 1996). Hence, a good candidate for the mechanism leading to interchromosomal reactions in cells incapable of homologous recombination is NHEJ.

By use of systems depending on the circularization of plasmids linearized in regions lacking homology to the yeast genome, it has been shown that the yeast Ku protein homologs Hdf1 and yKu80 (Boulton and Jackson 1996a,b; Milne *et al.* 1996; Tsukamoto *et al.* 1996), Rad50 (Milne *et al.* 1996), and ligase IV (encoded by *DNL4*; Wilson *et al.* 1997) participate in NHEJ. It has also been shown that *HDF1* is required for the joining of site-specific chromosomal breaks in the absence of homologous recombination (Milne *et al.* 1996; A. A. Friedl, O. Inbar and M. Kupiec, unpublished results). Here we show that *HDF1* is also required for the enhanced formation of aberrations in *rad52* and *rad54* mutant cells after irradiation, as inferred from the fact that the frequency of aberrants among *rad52 hdf1* and *rad54 hdf1* double-mutant colonies was very low. We conclude that the pathway mediated by Hdf1 can join radiation-induced DSB and thus enhance the survival of cells depleted for other repair mechanisms. It should be noted that there is no reason to assume that every Hdf1-mediated rejoining leads to an aberration. Rather, we assume that NHEJ normally rejoins the ''right'' ends, leading to electrophoretically inconspicuous chromosomes.

In the *rad54 hdf1* double mutant, one aberration, a circularized chromosome III, was detected, which was probably caused by a recombination between the partially homologous regions *HML* and *HMR*. The occurrence of alterations in the two *hdf1* single mutant strains also shows that the *HDF1*-dependent pathway is not the only one leading to chromosomal alterations. Interestingly, all the aberrants obtained in unirradiated and irradiated samples of the two *hdf1* mutant strains show enlargement of one or two chromosome species without apparent length compensation. One of these aberrant clones (SXhdf1/500/4) was repeatedly subcultivated, and single-cell subclones were karyotyped to check the length stability of the altered chromosome. We found that the length varies between the subclones (data not shown). Unstable enlargement of one chromosome (VII) is also a general feature of our strain SX46A *hdf1*Δ. Additionally, in a recent attempt to construct a *hdf1*Δ derivative of SX46A with normal karyotype, we surprisingly observed the same type of alteration in one of four candidates tested by PFGE. Taken together, these observations suggest that inactivation of *HDF1* causes the insertion or amplification of (unstable) genetic material by some as yet unexplained mechanism. Therefore, we regard the chromosomal alterations found in the *hdf1* mutants as being qualitatively different from those observed in the wild-type strains.

To our knowledge, this work is the first description of a decrease in the frequency of aberration induction by inactivation of one type of DSB repair, showing that functional repair mechanisms can be a prerequisite for aberration formation. In particular, we find that the yeast Ku70 homolog Hdf1 plays an important role in the generation of misrepair events. The products of the *HDF1* and the *yKU80* gene form a heterodimer that constitutes the major DNA end-binding activity in yeast (Milne *et al.* 1996). The same biochemical properties of the Ku70/Ku80 complex have been observed in mammalian cells (Singleton *et al.* 1997; Gu *et al.* 1997), and it has been shown that Ku80 participates in NHEJ (Liang *et al.* 1996). A difference between yeast and mammalian cells is that in mammalian cells, the Ku proteins constitute the DNA-binding moiety of a larger complex, the DNA-dependent protein kinase (DNA-PK). The catalytic subunit of DNA-PK, called DNA-PKcs, is activated upon binding of the Ku heterodimer onto DNA ends (Gottlieb and Jackson 1993; Finnie *et al.* 1995). Mammalian cells deficient for the catalytic subunit, *e.g.*, murine scid cells, exhibit similar defects in DSB repair and V(D)J recombination as do Ku80-deficient cells (Blunt *et al.* 1995; Kirchgessner *et al.* 1995), suggesting that the catalytic subunit is required for the rejoining mechanism. Despite intensive search, how-

ever, no yeast homolog of DNA-PKcs has been detected so far (Boulton and Jackson 1996b). Hence, it is possible that NHEJ in yeast differs in certain regards from NHEJ in mammalian cells.

No aberration data on Ku70-deficient mammalian cells have been reported so far. If one assumes that mammalian cells deficient for Ku70, Ku80, or DNA-PKcs behave similarly with respect to misrepair of DSB, however, our results obtained in yeast seem to contradict results obtained in mammalian cells in certain regards. For the Ku80-deficient hamster cell lines xrs-5, xrs-6, and XR-V15B, and for DNA-PKcs-deficient murine scid cells, an increase in the frequency of chromosomal aberrations after treatment with DSB-inducing agents has been reported. This increase seems largely attributable to terminally unrepaired breaks, but with regard to the formation of exchange-type events, conflicting data have been reported: after irradiation, an increase in the yield of exchange-type events as compared to wild-type cells was found in xrs-5 and xrs-6 cells (Bryant *et al.* 1987; Natarajan *et al.* 1993). Increased formation of dicentrics was observed in irradiated scid murine cells (Kirchgessner *et al.* 1993; Evans *et al.* 1996). On the other hand, no enhanced levels in formation of exchange-type events were found in xrs-5 cells as compared to wild-type after electroporation of restriction enzymes (Bryant *et al.* 1987; Natarajan *et al.* 1993), and Helbig *et al.* (1995) did not find increased levels of dicentrics in XR-V15B after treatment with the radiomimetic drug neocarcinostatin. Further studies involving a clear differentiation between aberration types should help to clarify under which conditions induction of exchange-type aberrations is altered in Ku-deficient mammalian cells. If exchange-type events are increased in Ku-deficient mammalian mutants, their generation may be explained by use of other pathways, *e.g.*, by frequent ectopic homologous recombination between repetitive sequences. Alternatively, it is possible that the readiness to perform misrepair is reduced in cells impaired in NHEJ, but that the probability of the wrong ends meeting is enhanced because of longer persistence of open breaks, thus resulting in a net increase in exchange-type aberrations.

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