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Hdf1, a yeast Ku-protein homologue, is involved in illegitimate recombination, but not in homologous recombination

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ABSTRACT

Hdf1 is the yeast homologue of the mammalian 70 kDa subunit of Ku-protein, which has DNA end-binding activity and is involved in DNA double-strand break repair and V(D)J recombination. To examine whether Hdf1 is involved in illegitimate recombination, we have measured the rate of deletion mutation caused by illegitimate recombination on a plasmid in an *hdf1* disruptant. The *hdf1* mutation reduced the rate of deletion formation by 20-fold, while it did not affect mitotic and meiotic homologous recombinations between two heteroalleles or homologous recombination between direct repeats. Hence Hdf1 participates in illegitimate recombination, but not in homologous recombination, in contrast to Rad52, Rad50, Mre11 and Xrs2, which are involved in both homologous and illegitimate recombination. The illegitimate recombination in the *hdf1* disruptant took place between recombination sites that shared short regions of homology (1–4 bp), as was observed in the wild-type. Based on the DNA end-binding activity of Hdf1, we discuss models in which Hdf1 plays an important role in the late step of illegitimate recombination.

INTRODUCTION

Chromosome rearrangements are often caused by illegitimate recombination, which occurs between non-homologous DNA sequences or very short regions of homology. Whether Rad proteins, which are involved in DNA double-strand break repair and homologous recombination, are also involved in illegitimate recombination was investigated in *Saccharomyces cerevisiae.* Schiestl *et al.* showed that the frequency of illegitimate recombination during integration of a DNA fragment was reduced by *rad50*, *51*, *52* and *57* mutations (1). We have also indicated that the rate of deletion formation caused by illegitimate recombination was reduced by *rad50*, *52*, *mre11* and *xrs2* mutations, but not by *rad51*, *54*, *55* and *57* mutations (2). Some *rad* mutations exhibited different effects in integration and deletion analyses, implying different mechanisms for the two recombination events and/or different states of DNA molecules in chromosomes and plasmids.

One of the interesting mammalian factors involved in DNA double-strand break repair and V(D)J recombination during rearrangement of immunoglobulin genes is the Ku-protein. The Ku-protein, which consists of a 70 kDa subunit (Ku70) and an 80 kDa subunit (Ku80), binds to double-stranded DNA ends and has DNA helicase activity (3–5). The Ku-protein is a component of a DNA-dependent protein kinase (DNA-PK), a serine/threonine protein kinase whose targets are p53, c-Myc, Sp1, simian virus 40 T-antigen, RNA polymerase II and Ku-protein itself (6–10). It is also known that the *XRCC5*, *XRCC6* and *XRCC7* mutants, which lack Ku80, Ku70 and the catalytic subunit of DNA-PK, respectively, are defective in DNA double-strand break repair and V(D)J recombination (11–19).

In yeast, a Ku-protein homologue, designated Hdf (high affinity DNA binding factor), was purified by its DNA binding activity (20). Hdf consists of 70 and 85 kDa subunits and has binding activity to the end of double-stranded DNA. The *HDF1* gene, which codes for the 70 kDa subunit, was cloned and sequenced. The amino acid sequence of Hdf1 shows a limited but significant homology with that of mammalian Ku-protein. Hdf1 and the 85 kDa subunit form a complex that has DNA end-binding activity. These properties are the same as that of the mammalian Ku-protein, but it has not been shown whether Hdf1 functions as a DNA helicase or in an interaction with a catalytic subunit of DNA-PK, as was observed for mammalian Ku-protein. Recently, Siede *et al.* showed that, though an *hdf1* single mutant did not exhibit any radiation sensitivity, it exhibited additional radiation sensitivity in a *rad52* background, indicating that Hdf1 is also responsible, at least partially, for repair of DNA damage (21).

To determine whether Hdf1, which has DNA end-binding activity, plays a role in recombination, we have examined the effect of a *hdf1* mutation on illegitimate recombination using the plasmid system for quantitative analysis of deletion formation. The rate of illegitimate recombination is shown to be reduced in the *hdf1* disruptant. We also show that the *hdf1* mutation does not affect mitotic and meiotic homologous recombination. These

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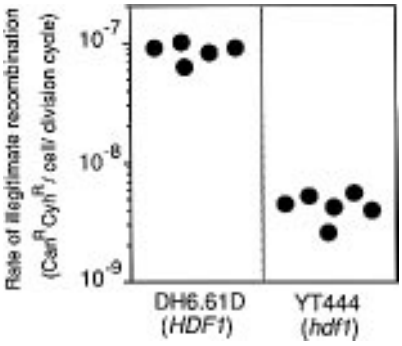

Figure 1. The rate of illegitimate recombination in an *hdf1* mutant. Recombination rate was determined by fluctuation analysis and the data were analysed by the method of the median (26,27). The average rates of plasmid deletions ± SD are as follows: *HDF1* strain, $8.5 \times 10^{-8} \pm 1.3 \times 10^{-8}$/cell/division cycle; *hdf1* mutant, $4.4 \times 10^{-9} \pm 1.0 \times 10^{-9}$/cell/division cycle. The result for the *HDF1* strain is from our previous work (2).

results indicate that Hdf1 is involved in illegitimate recombination, but not in homologous recombination.

MATERIALS AND METHODS

Bacterial and yeast strains

Escherichia coli recA strain DH10B was used for rescue of plasmid DNA isolated from *S.cerevisiae* (22). The yeast strains used are listed in Table 1. The *hdf1* mutants were constructed by the one-step gene replacement method using a *Sac*I–*Hin*dIII fragment containing *hdf1*::*LEU2* of the plasmid pGEM4ZS-H/LEU (20,23). MR93-28C and MR966 were kindly supplied by A. Sugino (Osaka University). MR93-28C, MR966 and their derivatives bear the same genetic background as SK-1 and sporulate rapidly and efficiently to produce dyad spores (24,25).

Table 1. Yeast strains used in this work

Strain	Genotype	Source
DH6.61D	MAT**a** trp1 his3 leu2 ura3 can1 cyh2	J. W. Szostak
YT101	As DH6.61D, except with rad52::TRP1	Y. Tsukamoto (2)
YT444	As DH6.61D, except with hdf1::LEU2	This study
MR93–28C	MAT**a** trp1-286 leu2-3,112 ura3-52 his1-1	M. A. Resnick (25)
YT461	As MR93–28C, except with hdf1::LEU2	This study
MR966	MATα trp1-286 leu2-3,112 ura3-52 his1-7	M. A. Resnick (25)
YT471	As MR966, except with hdf1::LEU2	This study
YT511	Diploid of MR93-28C and MR966	This study
YT521	Diploid of YT461 and YT471	This study

Recombination assay

Determination of the rate of illegitimate recombination and structural analysis of recombinants were carried out as described previously (2). Briefly, YCpL2, which carries two negative selection markers, the *CAN1* and *CYH2* genes, and three positive selection markers, the *URA3*, *TRP1* and *LEU2* genes, on a YCp plasmid was introduced into a haploid *can1 cyh2* strain. Because the wild-type *CAN1* and *CYH2* genes are dominant to the *can1* and *cyh2* mutations, a deletion mutation which simultaneously inactivates the *CAN1* and *CYH2* genes on the plasmid makes the transformant resistant to both canavanine (Can) and cycloheximide (Cyh). Our previous work showed that there were deletion mutations by illegitimate recombination on plasmids obtained from the Can^R Cyh^R cells (2). The rate of deletion mutation was determined by fluctuation analysis (26,27).

Mitotic and meiotic homologous recombinations between two *his1* heteroalleles were measured before and after shifting from YPA medium (1% yeast extract, 2% polypeptone, 2% potassium acetate) to sporulation medium (1% potassium acetate, 0.02% raffinose). Cells were grown in liquid YPA medium to a concentration of ~1×10^7 cells/ml. Then the cells were washed twice in sterile distilled water and resuspended in 2 ml sporulation medium. The cells were grown with shaking and plated onto SD and SD minus histidine plates at 24 h after the shift to sporulation medium.

The rate of recombination between direct repeats on the YCpD2 plasmid was determined using fluctuation analysis (26,27). YCpD2 carries a negative selection marker, the *CYH2* gene, flanked by the *CAN1* gene repeats on the YCp plasmid, which has three positive selection maker, the *URA3*, *TRP1* and *LEU2* genes. YCpD2 is an intermediary product in the course of construction of YCpL2, which was described in a previous report (2). The haploid *cyh2* strain, which carries YCpD2, is sensitive to cycloheximide. When a deletion between the *CAN1* repeats occurs, the cell becomes resistant to cycloheximide. The rate of deletion can be measured by plating YCpD2 transformants onto SD medium containing cycloheximide (10 μg/ml).

RESULTS

Effect of the *hdf1* mutation on illegitimate recombination

To investigate the effect of mutation of the *HDF1* gene on illegitimate recombination, the *hdf1* disruptant YT444 was constructed from DH6.61D, a *can1 cyh2* mutant, and YCpL2, which is a YCp plasmid carrying the *CAN1*, *CYH2* and *URA3* genes (see Materials and Methods), was introduced into the YT444 cells. The Ura$^+$ transformants grown in liquid SD minus uracil medium were plated on SD plates containing canavanine and cycloheximide. It was found that cells resistant to both canavanine and cycloheximide appeared at the rate of 4.4×10^{-9}/cell/division cycle in the *hdf1* disruptant (Fig. 1). The rate in the *hdf1* disruptant was ~5% of the rate in the isogenic *HDF1* strain (8.5×10^{-8}/cell/division cycle). The result indicates that Hdf1 is involved in illegitimate recombination.

To analyse deletion mutations formed in the *hdf1* disruptant, the recombinant plasmids rescued from five Can^R Cyh^R colonies of the disruptant were analysed by PCR as described previously (2). Four out of the five plasmids were found to have various sizes of deletions, but one of them, 1HX2, was indistinguishable from the parental plasmid YCpL2 by 0.7% agarose gel electrophoresis (Fig. 2A). When 1HX2 was again introduced into the *can1 cyh2* strain YT444, the transformant was resistant to both canavanine and cycloheximide and, therefore, 1HX2 may have point mutations or small rearrangements in both the *CAN1* and *CYH2* genes. The nucleotide sequences of the recombination junctions of the four deletion plasmids were determined and those of the

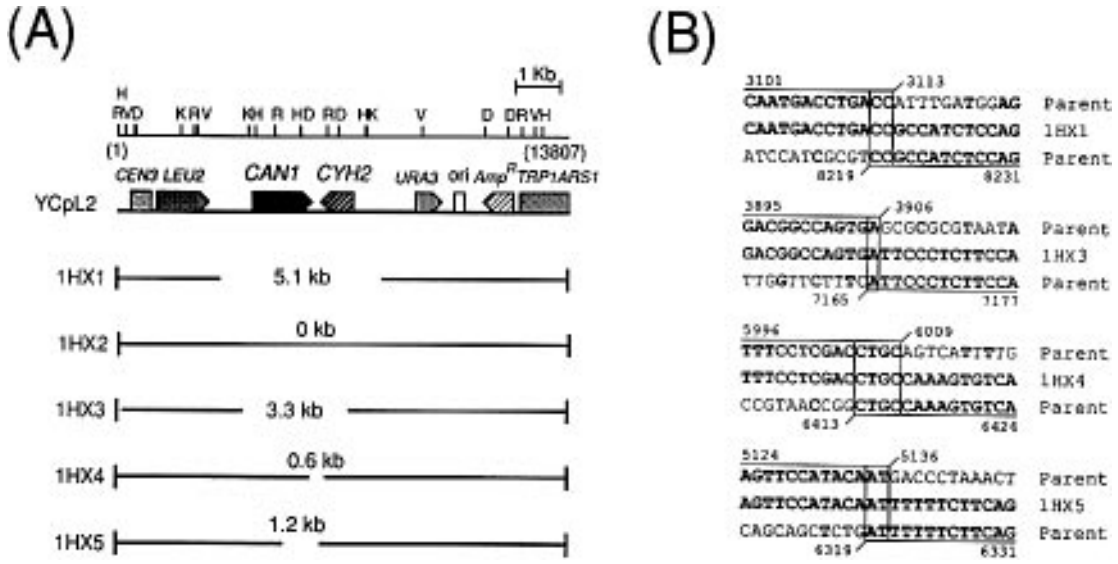

Figure 2. Structure and junction sequences of the recombinant plasmids rescued from Can^R Cyh^R cells of the *hdf1* mutant. (**A**) Structure of recombinant plasmids. Sequences present in the plasmids are represented by a line. The numbers indicate the length of the deletion. The *Eco*RI site between *ARS1* and *CEN3* on YCpL2, whose length is 13807 bp, is defined as position 1. Restriction sites shown are *Eco*RI (R), *Hin*dIII (H), *Kpn*I (K), *Eco*RV (V) and *Dra*I (D). (**B**) Junction sequences of recombinant plasmids. The top sequence represents the parental sequence corresponding to the left side of the junction, the middle sequence represents the sequence of the recombinant and the bottom sequence represents the parental sequence corresponding to the right side of the junction. The site of the junction is represented by a box. Homologous sequences around a junction are represented by bold letters. The orientation of DNA is 5′→3′ from left to right. Numbers represent the map coordinates of the YCpL2 sequence.

parental recombination sites were estimated. There were short regions of homology (1–4 bp) between the parental recombination sites of the deletion mutation formed in the *hdf1* disruptant (Fig. 2B). This result was comparable with those obtained from the wild-type strain (see also 2). From sequence analysis of the recombination junctions in the present and previous works, the sequences of the parental recombination sites in the *hdf1* disruptant were indistinguishable from those in the wild-type strain.

Table 2. Frequency of mitotic and meiotic homologous recombination between *his1-1* and *his1-7*

Strain	Culture	Frequency of His$^+$ cells		Fold
		0 h	24 h	induction
YT511 (*HDF1*/*HDF1*)	1	2.5×10^{-5}	2.4×10^{-3}	96
	2	2.7×10^{-5}	3.7×10^{-3}	140
	3	1.4×10^{-5}	3.2×10^{-3}	230
	4	3.5×10^{-5}	3.1×10^{-3}	89
	5	1.6×10^{-5}	2.7×10^{-3}	170
YT521 (*hdf1*/*hdf1*)	1	3.2×10^{-5}	3.7×10^{-3}	120
	2	0.6×10^{-5}	2.1×10^{-3}	350
	3	1.3×10^{-5}	2.5×10^{-3}	190
	4	1.8×10^{-5}	1.4×10^{-3}	78
	5	3.9×10^{-5}	4.0×10^{-3}	100

Five independent cultures of each strain were synchronously sporulated as described in Materials and Methods. Before and after 24 h in sporulation medium, cells were plated to determine the frequency of His$^+$ cells. The average fold inductions of His$^+$ recombinants for each set of five cultures were 150 for YT511 and 170 for YT521.

Effect of the *hdf1* mutation on homologous recombination

To observe the effect of the *hdf1* mutation on homologous recombination during both mitosis and meiosis, we measured recombination frequency between two heteroalleles at the *his1* locus. The frequency of mitotic recombination in the *hdf1* diploid was comparable with that in the wild-type strain (Table 2, 0 h). A similar result was obtained for recombination during meiosis (Table 2, 24 h). Sporulation efficiency of the wild-type and the *hdf1* diploids was nearly 90% at 24 h after the shift to sporulation medium. We also measured UV-induced homologous recombination between two *his1* heteroalleles. When the cells plated on SD minus histidine were irradiated by UV (20 J/m^2), the frequency of His$^+$ recombinants was increased by ~20 times in the *hdf1* diploids, an elevated frequency that was comparable with that in the wild-type strain (data not shown).

It is known that the factors involved in homologous recombination between direct repeats are different from those involved in recombination between two heteroalleles (28). We have constructed a plasmid system for detecting homologous recombination between direct repeats. A plasmid YCpD2, which carries the *CYH2* gene between *CAN1* gene repeats on aYCp plasmid, was introduced into haploid *cyh2* strains (Fig. 3A). The transformants were sensitive to cycloheximide. When a deletion mutation is formed by homologous recombination between the *CAN1* repeats, the cell will become resistant to cycloheximide. The rate of deletion mutation can be measured by plating YCpD2 transformants on SD plates containing cycloheximide. Structural analysis of the recombinant plasmids rescued from Cyh^R cells revealed that five out of the five plasmids rescued had a deletion of the *CYH2–CAN1* segment. It was found that Cyh^R cells of the *hdf1* disruptant appeared at the rate of 9.0×10^{-5}/cell/division

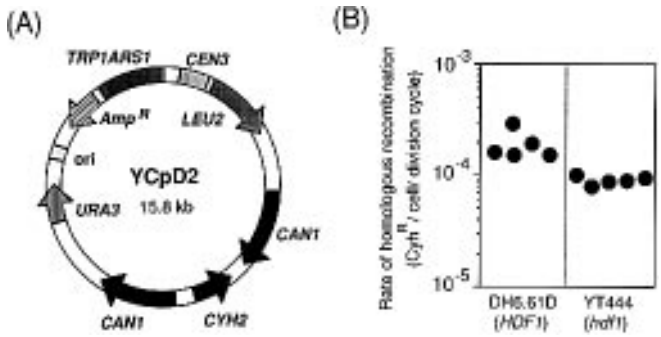

Figure 3. The rate of homologous recombination between direct repeats on plasmid YCpD2. (**A**) Plasmid YCpD2 carries the *CYH2* gene between direct repeats of the *CAN1* gene. (**B**) The rate of plasmid deletion between direct repeats on plasmid YCpD2. The rate of homologous recombination between direct repeats was determined by fluctuation analysis as a determination of the rate of illegitimate recombination (26,27). The average rates of plasmid deletion ± SD are as follows: *HDF1* strain, $1.9 \times 10^{-4} \pm 0.5 \times 10^{-4}$/cell/division cycle; *hdf1* mutant, $9.0 \times 10^{-5} \pm 0.7 \times 10^{-5}$/cell/division cycle.

cycle, which was comparable with that in the wild-type strain (1.91×10^{-4}/cell/division cycle) (Fig. 3B), while the rate of deletion was reduced by 10-fold in a *rad52* mutant (2.1×10^{-5}/cell/division cycle). These results indicate that Hdf1 is not involved in mitotic and meiotic homologous recombination between two heteroalleles or homologous recombination between direct repeats.

The present result that Hdf1 participates in illegitimate recombination, but not in homologous recombination, may suggest that it has a role in double-strand break repair via end-joining activity. We examined the sensitivity of the *hdf1* disruptant to methyl methanesulfonate (MMS) and UV irradiation and found that it had essentially the same sensitivity as the wild-type strain (data not shown). Siede *et al.* (21) also indicated that an *hdf1* mutant does not exhibit sensitivity to MMS and ionizing radiation. Therefore Hdf1 may play a minor role, if any, in double-strand break repair mediated by end-joining activity.

DISCUSSION

The present study shows that Hdf1 is involved in the formation of deletions caused by illegitimate recombination. However, Hdf1 is not involved in mitotic and meiotic homologous recombination between two heteroalleles or homologous recombination between direct repeats and a mutant is not sensitive to UV irradiation or MMS treatment. In a previous paper, we showed that Rad52, Rad50, Mre11 and Xrs2 participate in illegitimate recombination (2). Rad52 is known to be involved in both mitotic and meiotic homologous recombination and in DNA double-strand break repair (30–34). Rad50, Mre11 and Xrs2 are involved in meiotic homologous recombination, though they do not participate in mitotic homologous recombination (32,35–38). All these proteins but Hdf1 are involved in both illegitimate recombination and homologous recombination, with the Hdf1 protein having a unique function specifically involved in illegitimate recombination in yeast.

Hdf1 is the 70 kDa subunit of the yeast Ku-protein homologue, Hdf, which consists of 70 and 85 kDa subunits and has DNA end-binding activity (20). In mammalian cells, the Ku-protein is known to play roles in double-strand break repair and both RS and coding joint formation during V(D)J recombination (12–14,17,18). Ku-protein might mediate a common reaction during double-strand break repair and V(D)J recombination because it is known that Ku-protein has strong binding activity to the ends of double-stranded DNA produced by double-strand breaks and the hairpin structure produced by Rag1 and Rag2 during V(D)J recombination (4,39–44). It is thought that Ku-protein might be involved in DNA end-protection in both processes. In *xrs6* mutants, which lack the Ku80 subunit, the frequencies of both RS and coding joints were reduced during V(D)J recombination. RS junctions contain various sizes of deletion in *xrs6* mutants, while most RS were joined precisely without loss of nucleotides in the wild-type strain (12). Another model is that Ku-protein might be involved in regulating a cell cycle checkpoint mediated by DNA-PK in double-strand break repair and V(D)J recombination (14). Based on the present results and these considerations, we constructed models in which Hdf1 plays a role in the illegitimate recombination process in yeast (Fig. 4).

The first model is that the yeast Ku-protein homologue binds to the ends of DNA double-strand break to protect them from degradation by DNA exonuclease until DNA end-joining (Fig. 4A). The fact that Ku-protein is known to bind to the ends of DNA might support this first model. The second model is that two Ku-protein homologues, which bind to the ends of DNA double-strand breaks, associate with each other to form a tetramer composed of two Ku70 and two Ku80 (Fig. 4B). Formation of the tetramer has been reported previously (45,46). As a consequence of tetramer formation, the ends of the DNA double-strand break would be assembled and it may help in efficient rejoining of the DNA ends, as shown in Figure 4B. The third model is that the Ku-protein homologue unwinds DNA from the ends to expose single-stranded DNA tails (Fig. 4C). DNA end-joining may be promoted by interaction between these single-stranded tails. The fourth model is that the Ku-protein homologue, which binds to the ends of DNA double-strand breaks, may associate with the catalytic subunit of DNA-PK and DNA-PK may activate a factor which is involved in DNA end-joining or regulate expression of them (Fig. 4D). The fact that Ku70 and Ku80 are components of DNA-PK might be consistent with this fourth model. In conclusion, the third model is most likely because there are short regions of homology at most recombination junctions (2,47,48). The fact that mammalian Ku-protein has DNA helicase activity might also support this third model, although it has not been shown whether yeast Hdf1 also has DNA helicase activity.

It should be noted that the sensitivity of the yeast *hdf1* mutant to UV radiation, MMS and ionizing radiation was essentially the same as that of the wild-type (this study, 21), while mammalian *XRCC5* and *XRCC6* mutants, which are defective in Ku80 and Ku70, are severely sensitive to ionizing radiation. This may suggest that, in yeast, mitotic homologous recombination may play a major role in double-strand break repair, while Hdf-dependent illegitimate recombination may play a minor role in it. In fact, the *hdf1* mutation exhibits additional sensitivity to MMS and ionizing radiation in a *rad52* background, which confers a defect in double-strand break repair (21). In *S.cerevisiae*, it is thought that mitotic homologous recombination takes part in most double-strand break repair. Haploid cells in the G_1 phase are particularly sensitive to ionizing radiation, indicating that a homologous DNA molecule is required in the repair of double-strand breaks (49). Haber and co-workers also showed that HO

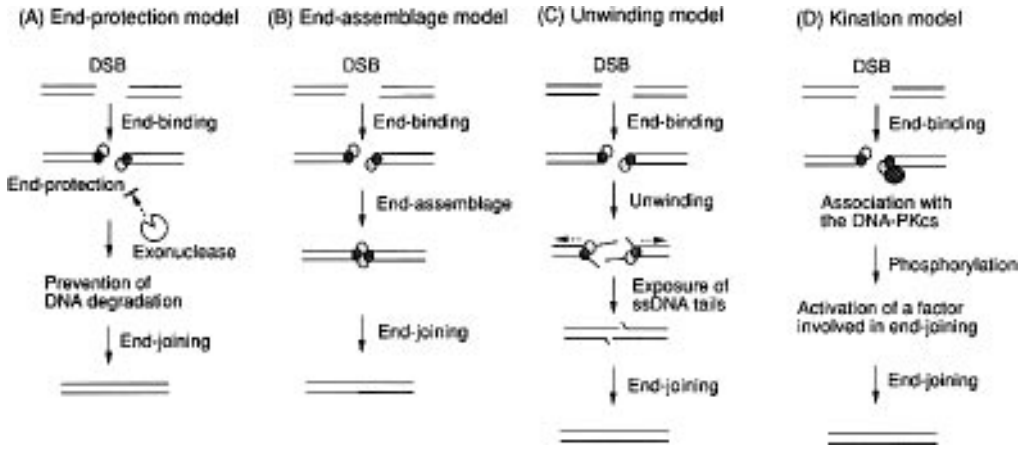

Figure 4. Models for the role of Hdf1 in illegitimate recombination. (**A**) In the end-protection model, the Ku-protein protects DNA ends from DNA exonuclease attack. (**B**) In the end-assemblage model, the two Ku-proteins associate with each other and promote rejoining of DNA ends. (**C**) In the unwinding model, the Ku-protein unwinds DNA ends to expose single-stranded DNA tails and promotes rejoining of DNA ends in a short homology-dependent manner. (**D**) In the kination model, the Ku-protein bound to broken DNA ends associates with a catalytic subunit of DNA-PK (DNA-PKcs) and the DNA-PK activates the factor which is involved in DNA end-joining. DSB represents a double-strand break.

endonuclease-induced double-strand breaks are effectively repaired by homologous recombination using a physical assay of recombination intermediates (50). On the other hand, in mammalian cells, illegitimate recombination plays the most important role in double-strand break repair. A linear DNA fragment transfected in mammmalian cells is effectively rejoined by illegitimate recombination (51,52). Crude extract from mammalian cells also contains strong DNA end-joining activity that does not require a long homology during the joining reaction (53). In gene targeting, most DNA molecules are integrated by illegitimate recombination in mammalian cells (54). These results suggest that, in *S.cerevisiae*, homologous recombination plays a major role in double-strand break repair, while, in mammalian cells, illegitimate recombination, in which Ku-protein may play a critical role, takes the major role in repair. Hence, the different effects on radiation sensitivity between the *S.cerevisiae hdf1* mutant and mammalian *XRCC5* and *XRCC6* mutants may reflect a difference in the major repair pathways between yeast and mammals.

Further genetic and biochemical studies on the role of Hdf1 in illegitimate recombination will help to clarify the mechanisms of double-strand break repair and V(D)J recombination.

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REFERENCES

1 Schiestl,R.H., Zhu,J. and Petes,T.D. (1994) *Mol. Cell. Biol.*, **14**, 4493–4500.
2 Tsukamoto,Y., Kato,J. and Ikeda,H. (1996) *Genetics*, **142**, 383–391.
3 Reeves,W.H. (1985) *J. Exp. Med.*, **161**, 18–39.
4 Mimori,T. and Hardin,J.A. (1986) *J. Biol. Chem.*, **261**, 10375–10379.
5 Tuteja,N. *et al.* (1994) *EMBO J.*, **13**, 4991–5001.
6 Lees-Miller,S.P., Chen,Y.-R. and Anderson,C.W. (1990) *Mol. Cell. Biol.*, **10**, 6472–6481.
7 Iijima,S., Teraoka,H., Date,T. and Tsukada,K. (1992) *Eur. J. Biochem.*, **206**, 595–603.
8 Peterson,S., Dvir,A., Anderson,C. and Dynan,W. (1992) *Genes Dev.*, **6**, 426–438.
9 Gottlieb,T.M. and Jackson,S.P. (1993) *Cell*, **72**, 131–142.
10 Dvir,A., Peterson,S.R., Knuth,M.W., Lu,H. and Dynan,W.S. (1992) *Pcoc. Natl. Acad. Sci. USA*, **89**, 11920–11924.
11 Lieber,M.R., Hesse,J.E., Lewis,S., Bosma,G.C., Rosenberg,N., Mizuuchi,K., Bosma,M.J. and Gellert,M. (1988) *Cell*, **55**, 7–16.
12 Taccioli,G.E., Rathbun,G., Oltz,E., Stamato,T., Jeggo,P.A. and Alt,F.W. (1993) *Science*, **260**, 207–210.
13 Taccioli,G.E., Gottlieb,T.M., Blunt,T., Priestley,A., Demengeot,J., Mizuta,R., Lehmann,A.R., Alt,F.W., Jackson,S.P. and Jeggo,P.A. (1994) *Science*, **265**, 1442–1445.
14 Smider,V., Rathmell,W. K., Lieber,M.R. and Chu,G. (1994) *Science*, **266**, 288–291.
15 Blunt,T. *et al.* (1995) *Cell*, **80**, 813–823.
16 Weaver,D.T. (1995) *Trends Genet.*, **11**, 388–392.
17 Rathmell,W.K. and Chu,G. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 7623–7627.
18 Boubnov,N.V. *et al.* (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 890–894.
19 Kirchgessner,C.U., Patil,C.K., Evans,J.W., Cuomo,C.A., Fried,L.M., Carter,T., Oettinger,M.A. and Brown,J.M. (1995) *Science*, **267**, 1178–1183.
20 Feldmann,H. and Winnacker,E.L. (1993) *J. Biol. Chem.*, **268**, 12895–12900.
21 Siede,W., Friedl,A.A., Dianova,I., Eckardt-Schupp,F. and Friedberg,E.C. (1996) *Genetics*, **142**, 91–102.
22 Grant,S.G.N., Jessee,J., Bloom,F.R. and Hanahan,D. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 4645–4649.
23 Rothstein,R.J. (1983) *Methods Enzymol.*, **101**, 202–211.
24 Kane,S.M. and Roth,R. (1974) *J. Bacteriol.*, **118**, 8–14.
25 Clark,A.B., Dykstra,C.C. and Sugino,A. (1991) *Mol. Cell. Biol.*, **11**, 2576–2582.
26 Luria,S.E. and Delbruck,M. (1943) *Genetics*, **28**, 491–511.
27 Lea,D.E. and Coulson,C.A. (1948) *J. Genet.*, **49**, 264–285.
28 McDonald,J.P. and Rothstein,R. (1994) *Genetics*, **137**, 393–405.
29 Firmenich,A.A., Elias-Arnanz,M. and Berg,P. (1995) *Mol. Cell. Biol.*, **15**, 1620–1631.
30 Ho,K.S.Y. (1975) *Mutat. Res.*, **30**, 327–334.
31 Resnick,M.A. and Martin,P. (1976) *Mol. Gen. Genet.*, **143**, 119–129.
32 Game,J.C., Zamb,T.J., Braun,R.J., Resnick,M. and Roth,R.M. (1980) *Genetics*, **94**, 51–68.
33 Prakash,S., Prakash,W., Burke,W. and Montelone,B.A. (1980) *Genetics*, **94**, 31–50.
34 Adzuma,K., Ogawa,T. and Ogawa,H. (1984) *Mol. Cell. Biol.*, **4**, 2735–2744.

35 Alani,E., Padmore,R. and Kleckner,N. (1990) *Cell*, **61**, 419–436.
36 Malone,R.E., Ward,T., Lin,S. and Waring,J. (1990) *Curr. Genet.*, **18**, 111–116.
37 Ajimura,M., Leem,S.-H. and Ogawa,H. (1992) *Genetics*, **133**, 51–66.
38 Ivanov,E.L., Korolev,V.G. and Fabre,F. (1992) *Genetics*, **132**, 651–664.
39 McBlane,J.F., van Gent,D.C., Ramsden,D.A., Romeo,C., Cuomo,C.A., Gellert,M. and Oettinger,M.A. (1995) *Cell*, **83**, 387–395.
40 Falzon,M., Fewell,J.W. and Kuff,E.L. (1993) *J. Biol. Chem.*, **268**, 10546–10552.
41 Blier,P.R., Griffith,A.J., Craft,J. and Hardin,J.A. (1993) *J. Biol. Chem.*, **268**, 7594–7601.
42 Morozov,V.E., Falzon,M., Anderson,C.W. and Kuff,E.L. (1994) *J. Biol. Chem.*, **269,** 16684–16688.
43 Paillard,S. and Strauss,F. (1991) *Nucleic Acids Res.*, **19**, 5619–5624.
44 de Vries,E., van Driel,W., Bergsma,W.G., Arnberg,A.C. and van der Vliet,P.C. (1989) *J. Mol. Biol.*, **208**, 65–78.
45 Genersch,E., Eckerskorn,C., Lottspeich,F., Herzog,C., Kuhn,K. and Poschl,E. (1995) *EMBO J.*, **14**, 791–800.
46 Griffith,A.J., Blier,P.R., Mimori,T. and Hardin,J.A. (1992) *J. Biol. Chem.*, **267**, 331–338.
47 Kramer,K.M., Brock,J.A., Bloom,K., Moore,J.K. and Haber,J.E. (1994) *Mol. Cell. Biol.*, **14**, 1293–1301.
48 Schiestl,R.H. and Petes,T.D. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 7585–7589.
49 Chlebowicz,E. and Jachymczyk,W.J. (1979) *Mol. Gen. Genet.*, **167**, 279–286.
50 Fishman-Lobell,J., Rudin,N. and Haber,J.E. (1992) *Mol. Cell. Biol.*, **12**, 1292–1303.
51 Roth,D.B., Porter,T.N. and Wilson,J.H. (1985) *Mol. Cell. Biol.*, **5**, 2599–2607.
52 Roth,D.B. and Wilson,J.H. (1986) *Mol. Cell. Biol.*, **6**, 4295–4304.
53 North,P.N., Ganesh,A. and Thacker,J. (1990) *Nucleic Acids Res.*, **18**, 6205–6210.
54 Roth,D. and Wilson,J. (1988) In Kucherlapati,R. and Smith,G.R. (eds), *Genetic Recombination.* American Society for Microbiology, Washington, D.C., pp. 621–653.