Involvement of the Saccharomyces cerevisiae HDF1 Gene in DNA Double-strand Break Repair and Recombination*

(Received for publication, August 10, 1995, and in revised form, January 21, 1996)

Guenter J. Mages, Heidi M. Feldmann, and Ernst-Ludwig Winnacker†
From the Institut für Biochemie der Universität München, Würmlatalstrasse 221, 81375 München, Federal Republic of Germany

The HDF1 protein of Saccharomyces cerevisiae shares biochemical properties and structural homology with the 70-kDa subunit of the human autoantigen Ku. The Ku protein, a heterodimer composed of a 70-kDa subunit and an 80-kDa subunit, has been identified as the regulatory subunit of the DNA-dependent protein kinase. This enzyme has recently been shown to be involved in DNA repair and recombination processes in mammalian cells. Here we show that hdf1-disrupted S. cerevisiae strains are strongly sensitive toward the radiomimetic antibiotic bleomycin. In addition, mating-type switching and rates of spontaneous mitotic recombination are strongly reduced. This phenotype is similar to that of mammalian cells lacking components of the DNA-dependent protein kinase holoenzyme, suggesting that HDF1 participates in and exerts equivalent functions in S. cerevisiae.

DNA double-strand breaks (DSBs) are intermediates of recombination events in both pro- and eukaryotic cells. DSBs can also be induced by treatments with ionizing radiation and with the radiomimetic antibiotic bleomycin (1–3). In Saccharomyces cerevisiae, DSB repair is mediated by activities of the RAD52 epistasis group (4), which includes gene products RAD50-RAD57. A prototype of RAD50-RAD57 activities is the product of the RAD52 gene, which has been suggested to be required for almost all DSB events (5). Specifically, mating-type switching, which is initiated by a double-strand cleavage by the HO endonuclease at the MAT locus, is highly reduced in rad52 cells (6, 7). rad52 strains fail to undergo proper meiotic chromosome segregation and therefore are defective in meiotic recombination (8–10). Additionally, rad52 strains are defective in most mitotic recombination events (7, 11).

Homologues of S. cerevisiae RAD51, RAD52, and RAD54 proteins have been identified in various higher eukaryotes (12–15), suggesting that they play a similar functional role. Another activity involved in recombination events of higher eukaryotes has recently been attributed to the so-called Ku autoantigen. Ku is a heterodimeric protein composed of 70- and 80-kDa subunits that has recently been identified as the regulatory subunit of the DNA-dependent protein kinase (DNA-PK) (16, 17). Ku protein also binds to double-strand DNA ends, nicks, and hairpins (18–25), suggesting that Ku may be involved in DNA repair, recombination, or replication events (21, 22). This view is supported by the observation that the 80-kDa subunit of Ku is not detectable in x-ray-sensitive xrs hamster cell lines and that these cells fail to support normal V(D)J recombination (26, 27). Both mutant phenotypes are complemented by the human XRCC5 gene, which has been shown to be identical with the gene for the Ku p80 subunit (28, 29). Furthermore, the catalytic subunit of DNA-PK, p350, is a strong candidate for the afflicted gene in cells derived from severe combined immunodeficient (SCID) mice (30, 31). Like xrs cells, SCID cells are sensitive to ionizing radiation and defective in V(D)J recombination (32). The SCID defect appears to affect the rejoining of certain types of DNA strand breaks (33–36), revealing a significant overlap between the mechanisms of V(D)J recombination and the joining of DSBs during DNA repair (37).

V(D)J recombination is a specific form of DNA rearrangement that does not require extensive sequence homology. Therefore, the rejoining activity for DSBs that is deficient in the SCID and in xrs mutant cells appears distinct from the recombination repair activities in S. cerevisiae and hence might represent a specialized function of higher eukaryotes (37). On the other hand, DNA-PK activities have been detected recently in cultured cells from mouse, hamsters, Xenopus, and Drosophila. Although these activities are approximately 10–50 times less abundant than in human cells (38), their presence suggests that DNA-PK is ubiquitous in eukaryotic cells and that it may have an evolutionarily conserved basal function in DSB repair and recombination.

The most compelling argument for the ubiquitous existence of DNA-PK is the recent description of the Ku-like HDF1 protein in S. cerevisiae that binds to DNA ends and shares homology with the 70-kDa subunit of Ku (39). Here we show that disruption of the HDF1 gene in S. cerevisiae affects DSB repair, mating-type switching, and spontaneous mitotic recombination. The phenotype of hdf1 mutants is very similar to that of rodent xrs and SCID cells. This observation, together with the analogies in biochemical properties and the structural homology between HDF1 and Ku p70, suggests a functional conservation of these activities in eukaryotic organisms.

MATERIALS AND METHODS
S. cerevisiae Strains and Media—Strains used in this study are shown and characterized in Table I. Only relevant genotypes are listed. Isogenic W303rad52-4D was generated by crossing W303a and X5560-1C-1D1. α segregants were tested for ura3-1 allele and then twice back-crossed with W303-1A, resulting in W303rad15-4D. For the characterization of hdf1 rad52 double mutants, W303rad52-4D was crossed with W303Lu and spore clones of two complete tetratype tetrads (W303Lu-5 to W303Lu-8 and W303Lu-17 to W303Lu-20) were used for the assays.

Complementation analysis of hdf1 mutants have been performed with the single copy vector pRS316 carrying the genomic Xho-EcoRI fragment of the HDF1 gene. Mitotic recombination experiments were performed with strains or their derivatives generously provided by...
The shown auxotrophic mutations were used for one step gene disruption, selection for diploids and/or determination of recombination frequencies. Most strains contain various other auxotrophic mutations as well.

| Strain       | Relevant genotype          | Source            |
|--------------|----------------------------|-------------------|
| W303-1A      | a, ade2-1, his3-11,15, leu2-3,112, ura3-1 | Ref. 61          |
| W303aU       | a, hdf1::URA3, his3-11,15, leu2-3,112, ura3-1 | Ref. 39          |
| XS560-1C-1D1 | a, rad52-7::LEU2, his3-Δ, leu2-3,112, ura3-5 | Yeast Genetic Stock Center |
| W303rad52-4D | a, rad52-7::LEU2, ade2-1, his3, leu2-3,112, ura3-1 | This study       |
| W303U20      | a, hdf1::URA3, rad52-7::LEU2, ade2-1, his3, leu2-3,112, ura3-1 | This study       |
| LP2730-1A    | a, rad 6-1, his3-Δ, leu2-3,112, ura3-5 | Yeast Genetic Stock Center |
| LP2730-1A-u   | a, rad 6-1, hdf1::URA3, his3-Δ, leu2-3,112, ura3-5 | This study       |
| LP2649-1A    | a, rad3-2, his3-Δ, leu2-3,112, ura3-5 | Yeast Genetic Stock Center |
| LP2649-1A-u   | a, rad3-2, hdf1::URA3, his3-Δ, leu2-3,112, ura3-5 | This study       |
| K65-3D       | H0, leu1-c, trp5-c, tyr1-1, ura3-1 | Ref. 7          |
| K45-3A-7A    | H0, leu1-d, trp5-d, tyr1-2, ura3-313 | Ref. 7          |
| GM21         | a, leu1-c, trp5-c, tyr1-1, ura3-1 | This study       |
| GM28         | a, leu1-d, trp5-d, tyr1-2, ura3-313 | This study       |
| GM2128       | GM21 × GM28                  | This study       |
| GM21αU       | a, leu1-c, trp5-c, tyr1-1, ura3-1, hdf1::URA3 | This study       |
| GM28αU       | a, leu1-d, trp5-d, tyr1-2, ura3-313, hdf1::URA3 | This study       |
| GM2128αUαU   | GM21αU × GM28αU | This study       |
| RM37-5D      | a, rad52-1, leu1-c, trp5-c, tyr1-1, ura3-1 | Ref. 7          |
| RM37-7D      | a, rad52-1, leu1-d, trp5-d, tyr1-2, ura3-313 | Ref. 7          |
| GM3738       | RM37-5D × RM37-7D            | Ref. 40          |
| IL993-5C     | a, ilv2                     | This study       |
| KL14-4A      | a, his1, trp2                | Ref. 40          |

 RESULTS

HDF1 Gene Involvement in DNA Break Repair and Recombination

Robert Malone (7). Strains GM21 and GM28 were obtained by a sequence of crosses: K65-3D and K49-3A-7A were first crossed with IL993-5C (40) to remove the H0 allele. The non-homogeneous segregants were then back-crossed three times with RM37-5D and RM38-7D, respectively, to develop isogenic strains. Strains GM21 and GM28 were obtained by a selective transforma- tion on solid medium with and without MMS (Sigma). Survival assays were carried out on solid synthetic medium containing galactose or dextrose with 1000 cells/ml. Aliquots of the cultures were taken every hour; cells were spread on YED (1 × 10⁶) and incubated at 30 °C for 3 days. Because divergence in the portion of mating types at different time points can also be due to decelerated generation times and hence just reflect a decelerated pass through the G1 phase of the cell cycle, the number of living cells were determined for each time point. The data were taken only from cultures with similar generation times. Colonies were replica-plated on synthetic medium without amino acids with Kl14-4A and IL993-5C as mating testers. After 5 days of incubation at 30 °C, prototrophic colonies were counted. In cases of non- or double-mating colonies, single colony cross-outs were performed and mating-type tests repeated. YCp50GAL-HO is a single copy shuttle plasmid containing a transcriptional fusion between the GAL10 promoter and HO gene kindly supplied by K. Luper and I. Herskowitz.

Determination of Mitotic Recombination Frequencies—Single colonies from a freshly constructed diploid were picked into 1 ml of YPD medium and cell concentration was determined by a hemocytometer count. Approximately 500 cells/ml were inoculated into 5 ml of YPD medium, and the culture was grown at 23 °C to a culture concentration of about 2 × 10⁷ cells. Each culture was started from an independent colony. Cells were washed twice in an equal volume of sterile water and plated at various dilutions on complete medium and medium lacking the supplement for the auxotrophic marker tested. Colonies were counted after 3 days of incubation at 30 °C.

| Strain       | Relevant genotype          | Source            |
|--------------|----------------------------|-------------------|
| W303-1A      | a, ade2-1, his3-11,15, leu2-3,112, ura3-1 | Ref. 61          |
| W303aU       | a, hdf1::URA3, his3-11,15, leu2-3,112, ura3-1 | Ref. 39          |
| XS560-1C-1D1 | a, rad52-7::LEU2, his3-Δ, leu2-3,112, ura3-5 | Yeast Genetic Stock Center |
| W303rad52-4D | a, rad52-7::LEU2, ade2-1, his3, leu2-3,112, ura3-1 | This study       |
| W303U20      | a, hdf1::URA3, rad52-7::LEU2, ade2-1, his3, leu2-3,112, ura3-1 | This study       |
| LP2730-1A    | a, rad 6-1, his3-Δ, leu2-3,112, ura3-5 | Yeast Genetic Stock Center |
| LP2730-1A-u   | a, rad 6-1, hdf1::URA3, his3-Δ, leu2-3,112, ura3-5 | This study       |
| LP2649-1A    | a, rad3-2, his3-Δ, leu2-3,112, ura3-5 | Yeast Genetic Stock Center |
| LP2649-1A-u   | a, rad3-2, hdf1::URA3, his3-Δ, leu2-3,112, ura3-5 | This study       |
| K65-3D       | H0, leu1-c, trp5-c, tyr1-1, ura3-1 | Ref. 7          |
| K45-3A-7A    | H0, leu1-d, trp5-d, tyr1-2, ura3-313 | Ref. 7          |
| GM21         | a, leu1-c, trp5-c, tyr1-1, ura3-1 | This study       |
| GM28         | a, leu1-d, trp5-d, tyr1-2, ura3-313 | This study       |
| GM2128       | GM21 × GM28                  | This study       |
| GM21αU       | a, leu1-c, trp5-c, tyr1-1, ura3-1, hdf1::URA3 | This study       |
| GM28αU       | a, leu1-d, trp5-d, tyr1-2, ura3-313, hdf1::URA3 | This study       |
| GM2128αUαU   | GM21αU × GM28αU | This study       |
| RM37-5D      | a, rad52-1, leu1-c, trp5-c, tyr1-1, ura3-1 | Ref. 7          |
| RM37-7D      | a, rad52-1, leu1-d, trp5-d, tyr1-2, ura3-313 | Ref. 7          |
| GM3738       | RM37-5D × RM37-7D            | Ref. 40          |
| IL993-5C     | a, ilv2                     | This study       |
| KL14-4A      | a, his1, trp2                | Ref. 40          |
wild-type diploids displayed a resistant phenotype (Fig. 1 whereas heterozygous hdf1 or chloramphenicol at concentrations of up to 75 by either streptomycin, tetracycline, erythromycin, ampicillin, chloramphenicol sensitivities were observed for diploid be caused by loss of hdf1 HDF1 protein was related to other -deficient strains, we investigated whether the function of HDF1 activity is an important function for mating-type switching but may not be as important as the RAD52 gene product, because it has been shown by others that the rad52 mutation prevents homothallic switching altogether (7).

Mitotic Recombination Is Reduced by hdf1 Deficiency—Because the hdf1 mutation affects the rate of mating-type switching, which is a site-specific recombination event, we also studied the effect of the hdf1 mutation on spontaneous mitotic recombination. Although the proportion of spontaneous mitotic recombination events initiated by DSBs is not known, substantial evidence indicates a coupling between DSBs and mitotic recombination. Treatment of cells with x-rays, which can generate DNA DSBs, stimulates mitotic recombination more than 1000-fold (11). Furthermore, stimulation of mitotic (and meiotic) recombination by DSB was shown with systems based on the insertion of HO recognition sites into heterologous DNA and galactose-inducible expression of the HO endonuclease (45, 46). The rad52 mutation known to be involved in DSB repair also causes a substantial reduction of spontaneous mitotic recombination (7). To study the influence of hdf1 mutation on

suggesting that the RAD52 activity is a somewhat stronger factor for repair of bleomycin-induced DNA damage than the HDF1 function. Bleomycin sensitivity of hdf1-deficient strains can be restored to wild-type levels by the introduction of a functional HDF1 gene expressed from a single copy vector. The observed bleomycin sensitivity of hdf1-deficient cells thus must be caused by loss of HDF1 gene function. Comparable bleomycin sensitivities were observed for diploid hdf1/hdf1 strains, whereas heterozygous hdf1/HDF1 diploids and HDF1/HDF1 wild-type diploids displayed a resistant phenotype (Fig. 1B).

Because the hdf1 deficiency causes temperature-sensitive growth at 37 °C, bleomycin sensitivity assays were each performed at 23 and 30 °C. The results of these experiments demonstrate that bleomycin sensitivity was not temperature-dependent. No effects discriminating between hdf1-deficient and wild-type strains were observed when bleomycin was replaced by either streptomycin, tetracycline, erythromycin, ampicillin, or chloramphenicol at concentrations of up to 75 μg/ml. This indicates the specificity of the bleomycin effect on hdf1-deficient strains (data not shown).

hdf1 rad52 Double Mutants Show Increased Sensitivity for Bleomycin Treatment—In view of the bleomycin sensitivity of hdf1-deficient strains, we investigated whether the function of HDF1 protein was related to other S. cerevisiae activities causing bleomycin sensitivity or acting on repair of DNA DSBs. Several genes of S. cerevisiae, collectively known as the RAD52 epistasis group, have been shown to affect DNA DSB repair and recombination (4). Mutations in these genes cause x-ray sensitivity and cross-sensitivity to bleomycin (44). Therefore, double mutants of hdf1 and the major representative of this group, rad52, were generated and their bleomycin sensitivity was compared with strains carrying the single mutations.

Surprisingly hdf1 rad52 double mutants are significantly more sensitive to bleomycin than the isogenic rad52 single mutated clones (Fig. 2). This effect of hdf1 and rad52 deficiency on bleomycin sensitivity indicates that both gene products are involved in repair of DNA DSBs in a cumulative manner.

This hypersensitive phenotype of hdf1 rad52 double mutants could also be observed with the radiomimetic agent MMS, which is known to induce strand breaks in DNA (4) (data not shown). In contrast, double mutants of hdf1 with either the rad3 or the rad6 mutations do not show hypersensitivity for bleomycin or UV irradiation (data not shown). This indicates that the observed bleomycin hypersensitivity of the hdf1 rad52 double mutant is specific for the rad52 mutation.

hdf1 Strains Show a Significant Reduction in Mating-type Switch Events—Apart from the sensitivity for ionizing radiation, another feature of higher eukaryotic cells lacking DNA-PK activity is a defect in V(D)J recombination. This recombination event is initiated by a DSB. Similarly, the mating-type switch in S. cerevisiae is initiated by a double-strand-specific cleavage introduced by the HO endonuclease at the MAT locus, resulting in gene conversion of the MAT allele (6). We therefore investigated the ability of hdf1-deficient strain W303aL MATa to undergo mating-type switching. We used a galactose-inducible HO endonuclease gene on a single copy vector and ascertained the percentage of mating-types at different time points.

A significant reduction of mating-type switching events to 30% or less in comparison with wild-type was observed in hdf1-deficient strains after 5 h of induction of the HO endonuclease gene (Fig. 3). This indicates that the HDF1 activity represents an important function for mating-type switching but may not be as important as the RAD52 gene product, because it has been shown by others that the rad52 mutation prevents homothallic switching altogether (7).

Mitotic Recombination Is Reduced by hdf1 Deficiency—Because the hdf1 mutation affects the rate of mating-type switching, which is a site-specific recombination event, we also studied the effect of the hdf1 mutation on spontaneous mitotic recombination. Although the proportion of spontaneous mitotic recombination events initiated by DSBs is not known, substantial evidence indicates a coupling between DSBs and mitotic recombination. Treatment of cells with x-rays, which can generate DNA DSBs, stimulates mitotic recombination more than 1000-fold (11). Furthermore, stimulation of mitotic (and meiotic) recombination by DSB was shown with systems based on the insertion of HO recognition sites into heterologous DNA and galactose-inducible expression of the HO endonuclease (45, 46). The rad52 mutation known to be involved in DSB repair also causes a substantial reduction of spontaneous mitotic recombination (7). To study the influence of hdf1 mutation on

[Fig. 1. hdf1 deficiency results in an increased sensitivity toward bleomycin. A, haploid wild-type W303-1A ( ), isogenic hdf1-deficient W303aL ( ), and W303aL complemented with the wild-type HDF1 gene ( ) were plated on solid YED media containing bleomycin in concentrations of 1.88-15 mg/ml. Surviving cells were grown up as colonies and counted after incubation at 23 °C for 8 days. The data of rad52-deficient strain W303rad52-4D ( ) are shown for comparison. B, increased bleomycin sensitivity is detectable also in homozygous hdf1/hdf1. Diploid wild-type W303aL ( ), heterozygous hdf1/HDF1 W303aLwt ( ), and homozygous hdf1/hdf1 W303aLu ( ) were assayed for bleomycin sensitivity as described above.]

[Fig. 2. Haploid hdf1 rad52 double mutants exhibit hypersensitivity for bleomycin. Spore clones of complete tetraploids out of the cross of hdf1-deficient W303aU with rad52-deficient W303rad52-4D were assayed for bleomycin sensitivity as described above. The data are shown for one tetrade only. Equivalent results were obtained for the others. , W303aU-17: HDF1 RAD52; , W303aU-18: hdf1 RAD52; , W303aU-19: HDF1 rad52; , W303aU-20: hdf1 rad52.]

Downloaded from http://www.jbc.org/ by guest on April 26, 2019
Fig. 3. hdf1-deficient strains show a significant reduction in induced mating-type switching events. Haploid wild-type W303-1A (□) and isogenic hdf1-deficient W303AaL (■) were transformed with plasmid YCp50 carrying the HO endonuclease gene under the control of the galactose-inducible GAL10 promoter. Exponential cultures grown on raffinose were washed and shifted into SD medium containing galactose as carbon source. Aliquots were spread every hour on YPD media and incubated at 30°C for 5 days. Mating types were determined for at least 50 colonies of each time point.

mitotic recombination, we used the same S. cerevisiae strains introduced by Malone and Esposito (7) to quantify the influence of the rad52 mutation on spontaneous mitotic recombination. The detection of mitotic recombination events is based on the use of diploids heteroallelic for several auxotrophic loci with the number of newly generated prototrophs allowing to monitor and measure recombination events. rad52/rad52 diploids were used as reference.

Our data show a 10–40-fold reduction of spontaneous mitotic recombination events in hdf1/hdf1 diploids with respect to wild-type strains (Table II), whereas rad52/rad52 diploids showed reduction rates of 50–200-fold. Comparable values were obtained for four independent crosses of wild-type strains and four independent crosses of hdf1 mutants.

DISCUSSION

This paper describes experiments that try to understand and explain if it was assumed that the HDF1 activity is involved in the repair of bleomycin-specific DNA damages while the RAD52 activity is responsible for DSB repair. The observed effect, however, could also be due to one singular DSB repair pathway if both activities affected different steps of this pathway. Although the rad52 mutation causes drastic effects on DNA repair, homothallic mating-type switching and mitotic recombination, there still remains some residual activity (7). In vivo, the RAD52 protein interacts physically with the RAD51 protein (53, 54). The recA-like activity of RAD51 catalyzes homologous DNA pairing and strand exchange, which is ATP-dependent (55). On the other hand, the functional defect in V(D)J recombination in Ku/DNA-Pk-deficient cells seems to impair the rejoining of the free DNA ends of the coding strands (33–36). If the HDF1 protein had a comparable function in S. cerevisiae, it could act downstream of the strand exchange activity of the RAD51/RAD52 complex. Successful DSB repair and recombination in rad52 mutants would then still require the downstream acting HDF1 activity.

To investigate further phenotypic similarities between hdf1 and DNA-Pk deficient cells, we studied the effects of hdf1 deficiency on mating-type switching and spontaneous mitotic recombination. V(D)J recombination is initiated by a precise double-strand cleavage between DNA segments and their flanking heptamers (56). Similarly, the mating-type switch is initiated by a DSB introduced by the HO endonuclease at the MAT locus (6). Both recombination events only require a limited number of base pairs for site specificity. V(D)J recombination results in the deletion of spacing sequences, whereas the mating-type switch results in a gene conversion event maintaining the donor sequence. We used an expression system with the HO endonuclease gene under the inducible GAL10 promoter to study the effects of hdf1 deficiency on mating-type switching rates. The significant reduction of mating-type switches in hdf1 mutants indicates that the HDF1 activity elicits a phenotype similar to the rad52 mutants. Taken in conjunction with data reported for bleomycin hypersensitivity, this further suggests that HDF1 activity may be involved in processing of DSB events.

DNA-Pk has been suggested to affect rejoining of broken DNA strands (33–36). It seems reasonable to assume that during mating-type switching the HDF1 product might affect rejoining of donor and acceptor DNA after HO endonuclease cleavage and/or rejoining of DNA strands after resolution of the crossover fork, which occurs after strand exchange within the MAT locus. hdf1 deficiency should then also affect spontaneous mitotic recombination. The data presented in Table II demon-
strate that this is indeed the case. Therefore, we suggest that the HDF1 activity displays a very basic function in genetic recombination events.

The phenotype of hdf1-deficient strains strongly resembles that of rad52-deficient strains investigated so far, although the effect of hdf1 deficiency is not as pronounced with respect to bleomycin sensitivity, mating-type switching, and spontaneous mitotic recombination. It remains to be established whether this is a genuine feature of HDF1 or whether there is a second gene whose activities overlap with HDF function in S. cerevisiae. The fact that hdf1 and rad52 deficiencies differ phenotypically in respect to growth and viability parameters does not clarify the issue. hdf1 deficiency results in the inability to grow at 37 °C, whereas members of RAD50-7 group, e.g. rad51 and rad54, show only a modest reduction in growth rate and no obvious reduction in vegetative viability at the higher temperature (39, 37). Furthermore, hdf1/hdf1 diploids have no apparent defects in sporulation, whereas rad52/rad52 fail to undergo sporulation (8–10). Further studies are therefore required to elucidate the relationship between HDF1 and RAD50-57 activities.

The discussion above has been focused on the supposition that HDF1 has a “classical” repair function like other RAD activities of S. cerevisiae and thus led to our attempts to assign its function to known repair pathways. However, an emphasis on the homology of HDF1 protein to the Ku p70 product and hence on its function as a regulatory subunit of a putative DNA-Pk activity involved in functions similar to those in S. cerevisiae ing HDF1 activity as one essential element of this damage response. It remains to be established whether the HDF1 activity displays a very basic function in genetic recombination. The sensitivity of S. cerevisiae to bleomycin, mating-type switching, and spontaneous mitotic recombination processes. The sensitivity of hdf1-deficient strains for DNA-DNA repair and recombination may actually affect other genetic recombination processes such as gene conversion and mitotic recombination.

Acknowledgments—We thank Robert E. Malone for generously providing the strains K65-3D, K49-3A-7A, RM37-5D, and RM38-7D for critical reading the manuscript and Melanie Fischer for expert technical help.

REFERENCES

1. Ward, J. F. (1990) Int. J. Radiat. Biol. 57, 1141–1150
2. Stubble, J., and Kazarjian, J. W. (1987) Chem. Rev. 87, 1107–1136
3. Steigerh, R. J., and Povirk, L. F. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 8350–8354
4. Haynes, R. H., and Kunz, B. A. (1983) In The Molecular Biology of the YeastSaccharomyces, Life Cycle and Inheritance (Strathern, J., Jones E. W., and Broach, J. R., eds) pp. 371–414, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
5. Ozerenberger, B. A., and Roeder, G. S. (1991) Mol. Cell. Biol. 11, 1222–1231
6. Strathern, J. N., Klar, A. J. S., Hicks, J. B., Abraham, J. A. IV, J. M., Nasmyth, K. A., and McGill, C. (1982) Cell 31, 183–192
7. Malone, R. E. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 503–507
8. Game, J. C., Zamb, T., Braun, R., Resnick, M. A., and Roth, R. M. (1980) Genetics 94, 51–68
9. Malone, R. E. (1983) Mol. & Gen. Genet. 189, 405–412
10. Prakash, S., Prakash, L., Burke, W., and Montelone, B. (1980) Genetics 94, 31–50
11. Resnick, M. A. (1975) In Molecular Mechanisms for Repair of DNA (Hanawalt, P., and Setlow, R., eds) part B, pp. 549–556, Plenum Publishing Corp., New York
12. Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N., Ikeo, K., and Ogawa T. (1993) Nat. Genet. 4, 239–243
13. Bezuobova, O., Shinohara, A., Mueller, R. G., Ogawa, H., and Buerstedde, J. M. (1993) Nucleic Acids Res. 21, 1577–1580
14. Bezuobova O. Y., Schmitt, H., Ostermann, K., Heyer, W.-D., and Buerstedde J.-M. (1993) Nucleic Acids Res. 21, 5945–5949
15. Bezuobova, O. Y., and Buerstedde, J. M. (1994) Experientia 50, 270–276
16. Divir, A., Peterson, S. R., Kruth, M. W., Lu, H., and Dyan, W. S. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 11920–11924
17. Gottlieb, T. M., and Jackson, S. P. (1993) Cell 72, 131–142
18. Momoi, N., Ghoose, Y., Iso, M., Homma, M., Griffith, A., and Hardin, J. A. (1999) Proc. Natl. Acad. Sci. U.S.A. 87, 1777–1781
19. Reeves, W., and Stuehler, Z. M. (1989) J. Biol. Chem. 264, 5047–5052
20. Yoneda, M., Wen, J., Ayala, A., and Cook, R. (1989) J. Biol. Chem. 264, 13407–13411
21. Momoi, T., Hardin, J. A., and Stelzl, J. A. (1986) J. Biol. Chem. 261, 2274–2278
22. Momoi, T., and Hardin, J. A. (1986) J. Biol. Chem. 261, 10375–10379
23. Blier, P. R., Griffith, A. J., Craft, J., and Hardin, J. A. (1993) J. Biol. Chem. 268, 7594–7601
24. Paillard, S., and Strauss, F. (1991) Nucleic Acids Res. 19, 5619–5624
25. Falzon, M., Fewell, J. W., and Kuff, E. L. (1993) J. Biol. Chem. 268, 10546–10552
26. Romsdell, W. K., and Chu, G. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 7623–7627
27. Getts, R. C., and Stamatou, T. D. (1994) J. Biol. Chem. 269, 15981–15984
28. Takahashi, G. E., Gottlieb, T. M., Priéret, T., Teichmann, J., Missia, R., Lehrmann, A. R., Alt, F. W., Jackson, S. P., and Jegga, P. A. (1994) Science 265, 1442–1445
29. Schierle, V., Rathmell, W. K., Lieber, M. R., and Chu, G. (1994) Science 266, 288–291
30. Kirchgesner, 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
31. Blunt, T., Finnie, N. J., Taccioni, G. E., Smith, G. C. M., Demengot, J., Gottlieb, T. M., Mizuta, R., Varghese, A. J., Alt, F. W., Jeggo, P. A., and Jackson, S. P. (1995) Cell 80, 923–932
32. Taccioni, G. E., Rathbun, G., Oltz, E., Stamatou, T. J., Jegga, P. A., and Alt, F. W. (1993) Science 260, 207–210
33. Miyano, B. A., Blackwell, T. K., Fulop, G. M., Rathbun, G. A., Furry, A. J. W., Ferrere, P., Heinke, L. B., Phillips, R. A., Yancopoulos, G. D., and Alt, F. W. (1988) Cell 54, 453–460
34. Lieber, M. R., Hessle, J. E., Lewis, S., Bosma, G. C., Rosenberg, N., Mizuuchi, K., Reisman, G. M., and Gellert, M. (1988) Cell 55, 7–16
35. Hendrickson, E. A., Schatz, D. G., and Weaver, D. T. (1988) Genes Dev. 2, 817–829
36. Blackwell, T. K., Malynn, B. A., Pollock, R. R., Ferrere, P., Covey, L. R., Fulop, G. M., Phillips, R. A., Yancopoulos, G. D., and Alt, F. W. (1989) EMBO J. 8, 735–742
37. Carr, A. M., and Hoekstra M. F. (1995) Trends Cell. Biol. 5, 32–40
38. Finnie, N. J., Gottlieb, T. M., Blunt, T. J., Jeggo, P. A., and Jackson, S. P. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 320–324
39. Fink, H., and Winnacker, E.-L. (1987) Methods in Yeast Genetics: A Laboratory Manual, pp. 163–168
40. Shimansky, E. A., Fink, G. R., and Hids, J. B. (1983) Methods in Yeast Genetics: A Laboratory Manual, pp. 163–168, Cold Spring Harbor Laboratory, Cold
43. Kreszenman D. J., Salvo V. A., and Nunes, E. (1992) J. Bacteriol. 174, 3125–3132
44. Moore, C. W. (1978) Mutat. Res. 51, 165–180
45. Kolodkin, A. L., Klar, A. J. S., and Stahl, F. (1986) Cell 46, 733–740
46. Nickoloff, J. A., Chen, E. Y., and Heffron, F. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 7831–7835
47. Moore, C. W. (1982) Cancer Res. 42, 929–933
48. D’Andrea, A. D., and Haseltine, W. A. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3608–3612
49. Giloni, L., Takeshita, M., Johnson, F., Iden, C., and Grollman, A. P. (1981) J. Biol. Chem. 256, 8608–8615
50. Murugesan, N., Xu, C., Ehrenfeld, G. M., Sugiyama, H., Kilkuskie, R. E., Rodriguez, L. O., Chang, L.-H., and Hecht, S. M. (1985) Biochemistry 24, 5735–5744
51. Sugiyama, H., Xu, C., Murugesan, N., and Hecht, S. M. (1985) J. Am. Chem. Soc. 107, 4104–4105
52. Moore, C. W. (1991) J. Bacteriol. 173, 3605–3608
53. Shinohara, A., Ogawa, H., and Ogawa, T. (1992) Cell 69, 457–470
54. Milne, G. T., and Weaver, D. T. (1993) Genes & Dev. 7, 1755–1765
55. Sung, P. (1994) Science 265, 1241–1243
56. Alt, F. W., Blackwell, T. K., and Yancopoulos, G. D. (1987) Science 238, 1079–1087
57. Anderson, C. W. (1993) Trends Biochem. Sci. 18, 433–437
58. Ullrich, S. J., Anderson, C. W., Mercer, W. E., and Appella, E. (1992) J. Biol. Chem. 267, 15259–15262
59. Kuerbitz, S. J., Plunkett, B. S., Walsh, W. V., and Kastan, M. B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 7491–7495
60. Fiscella, M., Ullrich, S. J., Zambrano, N., Shields, M. T., Lin, D., Lees-Miller, S. P., Anderson, C. W., Mercer, W. E., and Appella, E. (1993) Oncogene 8, 1519–1528
61. Thomas, B. J., and Rothstein, R. (1989) Cell 56, 619–630
Involvement of the *Saccharomyces cerevisiae* HDF1 Gene in DNA Double-strand Break Repair and Recombination

Guenter J. Mages, Heidi M. Feldmann and Ernst-Ludwig Winnacker

*J. Biol. Chem.* 1996, 271:7910-7915.
doi: 10.1074/jbc.271.14.7910

Access the most updated version of this article at [http://www.jbc.org/content/271/14/7910](http://www.jbc.org/content/271/14/7910)

Alerts:
  - When this article is cited
  - When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 58 references, 34 of which can be accessed free at [http://www.jbc.org/content/271/14/7910.full.html#ref-list-1](http://www.jbc.org/content/271/14/7910.full.html#ref-list-1)