DNA double strand breaks (DSBs), the most serious form of DNA damage, can lead to cell death if not repaired. In mammalian cells there are two major pathways for the repair of DSBs, homologous recombination and non-homologous end joining (NHEJ). Homologous recombination repairs DNA DSBs by using an extensively homologous sequence as a DNA template. NHEJ, which is highly efficient in mammalian cells, joins two broken ends without the requirement for extended homology. NHEJ can either be error-free or mutagenic with deletions or insertions at the joint. Proteins known to be involved in NHEJ include the catalytic subunit of DNA-dependent protein kinase (DNA-PKc), Ku70/Ku80 heterodimer, XRCC4, and DNA ligase IV (for reviews, see Refs. 1 and 2). The joining of DNA termini by NHEJ is initiated by the binding of the Ku heterodimer and subsequent association with DNA-PKc (3). In addition to protecting DNA ends to which it binds, the DNA-PK complex may also facilitate their alignment and ligation by recruiting the XRCC4-ligase IV complex. However, alternative NHEJ processes that are independent of DNA-PKc/Ku and the DNA ligase IV/XRCC4 complexes have been implicated in mammalian cells (4–12). For example, the Ku80-deficient rodent cell line xrs6 (4, 6–8) was able to join DNA ends but with strongly decreased accuracy. This evidence is indicative of a Ku-independent, error-prone pathway for DSB repair. Analysis of junctions formed in Ku80-deficient cells showed extensive deletions of nucleotides at DNA ends that were likely mediated by microhomology. This microhomology-driven, error-prone end joining also occurred in mammalian cells that are deficient for the other classical NHEJ proteins XRCC4 (7) and ligase IV (10). Collectively these studies indicate that there exists a separate, microhomology-mediated end joining (MHEJ) pathway that does not require DNA-PKc/Ku and DNA ligase IV/XRCC4 complexes as does the classical NHEJ pathway. In addition to these studies using linear DNA substrates, microhomologies were observed at deletion break points at the HPRT gene in primary human fibroblasts (13) and at the Aprt gene in hamster cells (14) and in mouse cells in vivo after exposure to ionizing radiation, indicating that MHEJ contributes to radiation-induced genomic alterations at endogenous gene loci.

Deletions generated via MHEJ are also characteristic of single strand annealing (SSA), an error-prone homologous recombination repair pathway identified for yeast and mammalian cells (15–18). SSA can be preferentially initiated when a DSB lies between two direct repeat sequences such that the complementary strands can anneal to each other, (a) the generation of single strands adjacent to the break and its extension to the repeated sequences such that the complementary strands can anneal to each other, (b) annealing of single strands at homology patches, (c) removal of unpaired flap strands by endoand/or exonucleases, (d) DNA polymerase-mediated filling in of gaps, and (e) sealing of remaining nicks by a DNA ligase (17). This process results in a deletion of one of the repeats and the sequence between the repeats and thus, like MHEJ, is always mutagenic. Although some components required for SSA have been identified in yeast (18) as well as in mammalian cells (15), it remains unclear whether or not MHEJ and SSA are processed by common DNA repair components. It should be noted that whereas longer DNA repeats are used in the studies of...
SSA, repeats involved in MHEJ are typically a few base pairs in length.

Although deficiency of proteins required for classical NHEJ has been shown to shift end joining to MHEJ in mammalian cells, a systematic study of the factors that modulate the choice between the two pathways has been lacking. We postulated that both nuclear proteins and the DNA sequence context in which DSBs arise may dictate the route of their repair. In this study, we developed a cell-free DNA end-joining assay to evaluate the relative contribution of MHEJ to DNA end joining under various conditions. We found that Ku70/80 heterodimer and histone proteins are required for accurate joining of DNA ends and that flap endonuclease 1 (FEN1) protein is involved in the error-prone MHEJ pathway. The efficiency of MHEJ is also affected by the size of repeats as well as the proximity of repeats.

MATERIALS AND METHODS

Nuclear Protein Extract Preparation—Human cell line HTD114 (19) was grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. WT38 normal diploid human fibroblasts were purchased from the American Type Culture Collection (Manassas, VA) and grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Mouse bone marrow cells were obtained by flushing the medullary cavities of femurs with phosphate-buffered saline using a 25-gauge needle. The isolation and culture of mouse primary fibroblast cells has been described elsewhere (20). Nuclear extract lacking Ku70 was prepared from fibroblast cells of Ku70-deficient mice (21). The cells were harvested and washed three times in ice-cold phosphate-buffered saline. Nuclear protein extracts were prepared as described by Jessberger and Berg (22). Protein concentration was determined by the method of Bradford (23).

End-joining Substrate—The method described by Tauchi et al. (24) for the construction of linear end-joining substrates with microhomology at both ends used with modifications. Briefly the end-joining substrate pUC18PD1/4 was created by ligating oligonucleotides PD1 (5'-AGCTACATCTACGCGCTCG-3') and PD2 (5'-CTAGCAGCGGTAGATTGCTG-3') between the HindIII and XbaI sites of pUC18 (Invitrogen) followed by insertion of oligonucleotides PD3 (5'-GATC-GATATCCTACGCTG-3') and PD4 (5'-AATTCCAGTGAGATT-ATC-3') between the BamHI and EcoRI sites. After cleavage with the restriction enzymes BamHI and EcoRI, linear DNA has a 10-bp direct repeat (ATCCTACAGC) at both ends (Fig. 1A).

DNA End-joining Assays—Linearized DNA was incubated with nuclear protein extract in 50 mM Tris-HCl (pH 7.6), 10 mM MgCl₂, 1 mM dithiothreitol, 1 mM ATP, and 25% (w/v) polyethylene glycol 8000 in a total volume of 20 μl. The reaction was at 14 °C for 2 h. DNA products were purified, and sequences were analyzed by electrophoresis on 0.6% agarose gels. The intensity of DNA bands was quantified by using a Kodak Gel Logic 100 imaging system and Kodak 1D image analysis software. The efficiency of end joining was calculated as the percentage of the XcmI-digested fragments of total DNA in the reaction (monomer, dimer, and multimers).

RESULTS

The End-joining Assay—The NHEJ process requires no homology at the DNA ends, and the products formed either are perfectly rejoined or carry deletions and/or insertions at the joints. MHEJ, on the other hand, involves two direct repeats and causes deletions that span one of the two direct repeats and the intervening sequence, if any. In this study we developed a cell-free DNA end-joining assay to evaluate the relative contribution of MHEJ to DNA end joining. Plasmid pUC18PD1/4 DNA was linearized by restriction enzymes so that it had a 10-bp direct repeat at both ends (Fig. 1A). This linearized DNA was then incubated with nuclear protein extracts. The total end-joining activity was reflected by the appearance of linear dimers and multimers that were separated by electrophoresis in an agarose gel (Fig. 1B). Self-circularization was undetectable under our experimental conditions (Fig. 1B). Rejoining catalyzed by MHEJ using the 10-bp repeat generates an XcmI restriction enzyme site at the joint. No other joining reaction can create such a restriction site (Fig. 1A).

Following the end-joining reaction, a 600-bp DNA fragment containing the end-joined junction was amplified by PCR using the ligated DNA as templates. As shown in Fig. 1C, digestion of the 600-bp fragment by XcmI resulted in three bands: the 600-bp product from intact DNA and the 400- and 200-bp products from digested DNA (lane 1). Although incubation of nuclear extract and DNA substrate resulted in XcmI digestion (Fig. 1C, lane 1), the 600-bp product derived from in vitro ligation catalyzed by T4 DNA ligase, as expected, was resistant to XcmI digestion (Fig. 1C, lane 2). Thus, the relative intensity of cleaved and uncleaved fragments after XcmI digestion reflects the activity of nuclear extract in catalyzing MHEJ mediated by the 10-bp repeat.

Relative Abundance of Nuclear Protein Regulates the Pathways of End Joining—Linearized pUC18PD1/4 DNA was incubated for 2 h at 14 °C in the presence of nuclear protein extracts prepared from human HTD114 cells to detect the total end-joining activity. We observed that this DNA end-joining activity was affected by the relative abundance of DNA to total protein extract. As shown in Fig. 2A, when the amount of DNA/protein was 10:20, 10:10, or 50:10 (ng/μg), no dimers or multimers were visible (Fig. 2A, lanes 4–6). When the DNA/protein amount was 50:1 (ng/μg), ~36% of the input linear monomer DNA molecules were joined to form linear dimers and multimers (Fig. 2A, lane 7). The emergence of dimers and multimers in the presence of 1 μg, but not 10 μg, of protein with the same amount of substrate DNA (50 ng) suggests that although nuclear proteins were required for DNA end joining, an excessive amount of proteins may have inhibitory effects. Consistent with this notion, when the DNA/protein ratio was increased to 500:1 (ng/μg), more end-joined products were formed (Fig. 2, lane 8, higher intensity of dimers and multimers), although joining efficiency relative to the total substrate DNA was decreased to about 10% (Fig. 2A, lane 8).

To determine the relative contribution of MHEJ to the end-joining reactions shown above, we PCR-amplified the ligated DNA products using primers flanking the end-joined junction.
and subsequently digested the PCR products with XcmI. It should be noted that after PCR amplification we only detected products that had been joined in head-to-tail orientation (600 bp). PCR products in head-to-head or tail-to-tail orientation (400 or 800 bp) were not detected. Products joined in head-to-head and tail-to-tail orientations, if any, may be poor substrates for PCR as they may form “hairpin” structures. With the 10:20 (ng/µg/H9262 g) DNA/protein ratio, we could barely observe the XcmI-digested products (Fig. 2B, lane 1), indicating that the most end-joined products formed were not via MHEJ. With the DNA/protein ratio of 10:10 (ng/µg), end-joined products indicative of MHEJ were visible (Fig. 2B, lane 2). When the DNA/protein ratio was further increased, MHEJ became predominant (Fig. 2B, lanes 3, 4, and 5). Similar results were obtained with nuclear proteins prepared from human WI38 fibroblast cells, mouse primary fibroblast cells, splenic T cells, and bone marrow cells (data not shown).

We noted that lower DNA/protein ratios (10:20, 10:10, and 50:10 ng/µg) produced a single sharp XcmI-resistant band (Fig. 2B, lanes 1–3), whereas higher DNA/protein ratios (50:1 and 500:1 ng/µg) generated some XcmI-resistant products of smaller sizes that were suggestive of deletions (Fig. 2B, lanes 4 and 5). To determine the nature of such end-joined products, we cloned gel-purified XcmI-resistant PCR products into TOPO TA cloning vector and transformed *Escherichia coli* to produce single clones suitable for sequence analysis. Because each clone was derived from one specific PCR fragment and represented one particular end-joined product, the actual abundance of a junction from the end-joining reaction would be reflected by the frequency of specific sequences detected. As shown in Table I, all eight clones of end-joined products generated at the lower DNA/protein ratio (10:20 ng/µg) had perfect end joining. In contrast, only one of the 12 clones (8%) of end-joined products formed at high DNA/protein ratio (50:1 ng/µg) showed perfect end joining, the others (92%) showed deletions at the end-joined junction, and five of the latter (42%) apparently used microhomology of two or more nucleotides to mediate end joining. Strikingly all products with deletions had a 17-bp sequence (italic letters in Table I), which is identical to the sequence between Eco47III and EcoRV restriction sites in pUC18PD1/4 plasmids and, therefore, were probably derived from incomplete digestion by EcoRV during linearization (Fig. 1A). Still
such substrates accounted for only a very small fraction of total DNA substrates. The effect of this 17-bp "cap" on the activity of MHEJ was further studied as described below.

Taken together, our data show that when DNA substrates overwhelm nuclear proteins, DNA ends become unprotected and are exposed to exonuclease-mediated degradation. Such ends are more likely to be rejoined through an error-prone end-joining pathway either with or without the involvement of microhomology, both resulting in deletions. A relatively small amount of DNA substrate was probably protected from exonuclease attack by the presence of nuclear proteins and was rejoined by the error-free end-joining pathway.

*Ku and Histone H1 Inhibit MHEJ*—We hypothesized that the protection of DNA ends in our assay may involve the heterodimeric Ku70/80 complex, which has been reported to protect DNA ends from nucleases (25). If this notion is true, it can be expected that in the absence of Ku proteins, DNA substrates will be more likely processed by the error-prone MHEJ pathway regardless of the abundance of other nuclear proteins applied. Indeed at lower DNA/protein ratios (10:4 and 50:4 ng/µg) the XcmI-sensitive products were significantly increased in nuclear extracts prepared from Ku70-deficient mouse fibroblast cells as compared with nuclear extracts from Ku70 wild-type cells (Fig. 3A). At 10:4 ng/µg DNA/protein ratio the averages for the percentage ± S.D. of the XcmI-sensitive products were 57 ± 3 and 88 ± 1% in Ku70 wild-type and Ku70-deficient cells, respectively (Fig. 3B). At 50:4 ng/µg DNA/protein ratio the averages were 73 ± 2 and 93 ± 1% in Ku70 wild-type and Ku70-deficient cells, respectively (Fig. 3B).

We also observed that histone H1, a linker histone protein that protects DNA from degradation by nucleases (26), inhibits MHEJ at a higher DNA/protein ratio (100:1 ng/µg). As shown in Fig. 4A, the overall end-joining efficiency was significantly decreased from 32 to 4% by addition of 3 µg of histone H1. XcmI digestion of PCR products of joined DNA showed that 3 µg of histone H1 decreased the frequency of the 10-bp-mediated end joining by about 4-fold (26 versus 99% in the control) (Fig. 4B). Therefore, addition of 3 µg of histone H1 suppressed MHEJ by about 32-fold. We sequenced the XcmI-resistant PCR products (Fig. 4, lane 6) to investigate whether histone H1 had influenced the accuracy of end joining. The sequencing data, shown in Table II, revealed that 50% (12 of 24) of such products were from perfect end joining. Because only about 8% (1 of 12) of the XcmI-resistant PCR products were from perfect end joining when histone H1 was not added, even at a lower DNA/protein ratio (50:1 ng/µg) (Table I) the percentage of perfect end joining would presumably be smaller than 8% at the higher DNA/protein ratio (100:1 ng/µg) in the absence of histone H1. Taken together, these results demonstrate that the addition of histone H1 suppresses error-prone end joining.

**FEN1, but Not Mismatch Repair Proteins, Is Required for MHEJ**—Single-stranded flaps formed from broken DNA ends were proposed to be the intermediates of MHEJ (27). Such flaps were removed either by exonucleases or by endonucleases such as the structure-specific FEN1 (28). To determine whether FEN1 contributes to the MHEJ activity, we preincubated 1.2 µg of nuclear protein extract prepared from mouse bone marrow cells with monoclonal antibody raised against mouse FEN1 before initiating the end-joining assay. Although the end-joining efficiency was not significantly reduced by the addition of 1 µg of anti-FEN1 antibody (21 versus 23% in the control) (Fig. 5A), it was reduced by about 4-fold (6 versus 23% in the control) when 4 µg of antibody was added (Fig. 5A). Addition of antibody against mouse exonuclease 1, on the other hand, did not affect the end-joining efficiency (data not shown). XcmI digestion of PCR products of joined DNA showed that the addition of FEN1 antibody reduced the relative activity of MHEJ using the 10-bp repeat by about 2-fold (78 versus 37% in control) (Fig. 5B). Taking into consideration the 4-fold decrease in overall end-joining efficiency, FEN1 antibody caused an 8-fold decrease in
MHEJ mediated by the 10-bp repeat, suggesting that FEN1 is required for MHEJ.

It has been reported that mismatch repair proteins, such as MLH1, MSH2, and MSH6, are required for SSA in yeast (18). To determine whether they are required for MHEJ in mammalian cells, we performed an end-joining assay with nuclear extracts isolated from MLH1-, MSH3-, or MSH6-deficient mouse primary cells. We found that such nuclear extracts were as efficient as those from wild-type cells in catalyzing MHEJ (data not shown). Thus, mismatch repair proteins are not required for MHEJ.

Reduction of MHEJ by Intervening Sequence between Repeats—We envisaged that the process of MHEJ would include formation of single strands at each end and a search for a homologous match between the single strands. Thus, we studied how the proximity of repeat sequences and the length of such a repeat would affect the MHEJ efficiency. We used only Eco47III to obtain linearized pUC18PD1/4, thus leaving a 17-bp cap or spacer next to one of the 10-bp repeats (Fig. 1A).

As shown in Fig. 6A, at higher DNA/protein ratio (300:1 ng/μg), the 17-bp spacer reduced the end-joining efficiency by 2.8-fold (18 versus 51% when the spacer was absent). PCR amplification of end-joined junctions followed by XcmI digestion revealed that the addition of the 17-bp spacer reduced the relative activity of MHEJ using the 10-bp repeat by about 10-fold (9 versus 88% in control) (Fig. 6B). Thus, the decreased efficiency in end joining was mainly caused by a 28-fold decrease in MHEJ using the 10-bp repeat. We excised the XcmI-resistant band in lane 2 of Fig. 6B and sequenced the end-joined junctions. As shown in Table III, all junctions had 2-bp deletions as a result of MHEJ (Table III). About 92% (22 of 24) of end-joining reactions used 2-bp (GC) homologous sequences, the first two bases on either end of the DNA substrate. The other junctions (2 of 24) also resulted from a 2-bp (GC) repeat-mediated end joining, but the GC was five bases away from the end (Table III). These data suggested that DNA sequence context affects the efficiency of MHEJ. First, the proximity to repeat sequences matters. The repeats nearest to the DSB were preferentially used. Second, although repeats as small as 2-bp were effective in facilitating MHEJ, they are less efficient than 10-bp repeats. Although the GC repeats were directly exposed to each other for DNA substrates with the 17-bp spacer, end joining was only about one-third as efficient as when 10-bp repeats were directly exposed to each other (Fig. 6A).

DISCUSSION

In this study, we used a cell-free DNA end-joining assay to examine the factors that modulate the error-free and error-prone end-joining pathways for DSB repair in mammalian cells. We found that when nuclear proteins are abundant and Ku proteins are present, error-free end joining is preferred. As nuclear proteins relative to DNA decrease, MHEJ and error-prone end joining become dominant. MHEJ and error-prone end joining can be suppressed by the addition of histone H1. MHEJ can also be suppressed by inhibition of FEN1. In addition, MHEJ did not require mismatch repair proteins as are required for SSA in yeast.
were previously used to study end joining in an in vivo assay (24, 29) in which blunt-ended linear plasmids with a 6- or 10-bp repeat on both ends were transiently transfected into cells. However, as suggested by Feldmann et al. (6), the transfection procedure itself could generate some artifacts as the substrates may undergo extensive terminal degradation before they reach the nucleus. Thus, the original terminal structures may remain intact in only a fraction of the substrates, which would bias the spectrum of end-joined products toward MHEJ or error-prone end joining. Such a problem is less of a concern in our in vitro end-joining assay because DNA substrates were directly exposed to DNA repair components in nuclear extracts. Indeed, when we transfected Ku70-proficient WI38 cells with the DNA substrates having 10-bp repeats at each end, we could only detect end-joined products with deletions, which likely resulted from either MHEJ or error-prone end-joining pathway (data not shown).

Our data suggest that there are two competing pathways of DNA end joining, an error-free pathway and an error-prone pathway. The error-prone pathway probably involves exonuclease-mediated degradation of the unprotected DNA ends and the subsequent end joining with or without the involvement of microhomology, both causing deletions. We demonstrated that the Ku protein is one of the factors that affects the choice between error-free and error-prone end joining (Fig. 3). Ku heterodimers are probably involved in detecting, tethering, and protecting DNA ends as well as in recruiting other DNA repair factors (25). By protecting DNA ends from degradation by nuclease attack, as suggested in this study and previous in vivo and in vitro studies (4, 6–8, 30, 31), Ku presumably ensures the efficiency and fidelity of end joining. In the presence of Ku, DNA ends are quickly captured and joined by DNA ligase IV. This process facilitates joining of intact ends and suppresses their resection by nucleases, which might be the initial step of MHEJ. Although the end-joining substrates in our study all have blunt ends, our findings may apply to other types of DNA ends. Feldmann et al. (6) have shown that Ku80-proficient extracts were highly efficient and accurate in rejoining all types of DNA ends, including 3′ overhangs that abut a 5′ overhang or blunt end, whereas Ku80-deficient xrs6 extracts were not. Thus, in the absence of Ku or when the number of DNA ends overwhelms Ku heterodimers in the reaction, more
DNA ends are exposed and processed by exonucleases, resulting in single strands. Single strands may become stable only if they are paired with a homologous sequence. Such intermediates may be further stabilized by the binding of other proteins and eventually be converted into finished products with deletions.

Our results indicated that histone H1 is another factor that determines the choice of error-free or error-prone end joining. The linker histone H1 is involved in linking two rounds of nucleosome DNA at the surface of the nucleosome core and in the formation of higher order chromatin structures. It has been reported that the binding of H1 to DNA not only abrogates nuclease activity but also stimulates in vitro DSB ligation reactions (32, 33) probably by promoting intermolecular ligation (34). Our data clearly showed that histone H1 is capable of suppressing MHEJ presumably by protecting DNA from degradation. However, it is not clear whether Ku and histone proteins, including H1, interact in steering repair toward an error-free or an error-prone end-joining pathway. Furthermore, given their dynamic interaction with DNA during replication and transcription, the involvement of histone proteins in error-free and error-prone end joining may be different during different cell cycle stages or at gene loci that are differentially expressed in different cell types, although this model awaits testing.

We present a model, shown in Fig. 7, to interpret the results presented here and to address the roles of competing DNA end-joining pathways. This model suggests that by protecting DNA ends, Ku, histone proteins, and other unknown factors enhance the efficiency of accurate end joining and suppress error-prone end joining. In the absence of end protection, DSBs

![Fig. 5. Requirement of FEN1 in MHEJ. A, nuclear extract from mouse bone marrow (1.2 μg of total protein) was incubated for 30 min on ice with the antibody against mouse FEN1 and then incubated with 440 ng of linear DNA at 14 °C. After 1-h incubation, DNA products were analyzed by agarose gel electrophoresis. B, PCR products of end-joined products shown in A were digested with XcmI. EJ, end joining.](image)

![Fig. 6. The effect of a spacer between homologous sequences on MHEJ. A, 1 μg of extract from mouse bone marrow cells was incubated with 300 ng of pUC18PDI/4 linearized with both EcoRI and EcoRV (lane 1) or 300 ng of pUC18PDI/4 linearized with EcoRI only (lane 2). B, PCR products of end-joined products shown in A were digested with XcmI. EJ, end joining.](image)

![Fig. 7. Steps of end-joining repair of DSBs. In the error-free pathway, the Ku protein, histone proteins, and other factors bind to the ends of a broken DNA duplex and protect them from nuclease. This binding may also serve to maintain the proximity of ends. Together with DNA-PKcs, DNA ligase IV-XRCC4 complex accurately joins two ends. In the error-prone pathway, DNA ends are processed by exonucleases, resulting in single strand flaps. Single-stranded DNA may search for and anneal with a homologous sequence to form a stable intermediate. Unpaired flap strands are then removed by FEN1 and/or other nucleases followed by filling in of gaps by DNA polymerases and sealing of remaining nicks with a DNA ligase. When local homology is not found, the DNA ends may be joined with deletions/insertions at the junction. EJ, end joining.](image)
are repaired by an error-prone pathway that is initiated with nucleotide resection. This pathway relies on microhomology-mediated annealing of single strands and other unknown protein factors to stabilize the intermediates.

It is reasonable to propose that exonuclease(s), endonuclease(s), DNA polymerase(s), DNA ligase(s), and proteins that stabilize the annealed intermediates would be required for MHEJ. Our data indicate that FEN1 is required for the MHEJ pathway (Fig. 5). Eukaryotic FEN1 (Rad27) protein is a highly conserved structure-specific 5’-unannealed flap DNA structure of any stability (for a review, see Ref. 35). Although FEN1 acts as an endonuclease on a 5’-unannealed flap DNA structure of any stability (Ref. 35). Although FEN1 acts as an endonuclease during the resection step of MHEJ. It most likely acts as an endonuclease to remove the flaps. Interestingly FEN1 (Rad27) was shown to be involved in the processing of aligned end joining intermediates with 5’ flaps in yeast (36, 37). It is also possible that FEN1 plays other roles in MHEJ through its functional and/or physical interaction with other proteins such as polymerases (38, 39). It should be noted that although the data from our cell-free assay suggest that FEN1 may be required for MHEJ in mammalian cells, Chen and Kolodner (40) have reported that mutations in the Saccharomyces cerevisiae RAD27 gene greatly increased microhomology-mediated gross chromosomal rearrangements, such as translocations, deletions, or inversions, suggesting a Rad27-dependent suppression of gross chromosomal rearrangements in yeast. Whether or not FEN1 (Rad27) plays different roles in maintenance of genomic stability in mammalian and yeast cells is currently not known.

Our data indicate that DNA sequence strongly influences the efficiency of MHEJ. Importantly the level of MHEJ between two repeats can be greatly reduced by insertion of an intervening sequence. Furthermore when multiple repeats are available, the repeat nearest to the DSB is used preferentially (Fig. 6B and Table III, the GC repeats and the 10-bp repeats). These results indicate an orderly searching process in which sequences adjacent to the DSB on one side are compared with sequences on the other side until a homologous match is found. Another important observation in this study is that even a 2-bp repeat sequence was effective in facilitating MHEJ. This 2-bp repeat length may represent the minimum length needed to form an intermediate structure on which protein factors can act. Still a 2-bp repeat is much less efficient than longer repeats (Fig. 6B and Table III, the GC repeats and the 10-bp repeats). Pairing between shorter (2-bp) repeats probably provides less stability compared with pairing between longer (10-bp) repeats with or without association of DNA-binding proteins.

Our results also suggest that MHEJ and SSA may represent distinct processes, although they both result in deletion of one repeat in the joined product. Although mismatch repair proteins are required for SSA in yeast, they are not required for MHEJ in mammalian cells as extracts from mismatch-deficient cells were equally efficient in catalyzing MHEJ. However, it remains to be determined whether DNA substrates with repeats longer than those we used are processed in the same manner.

Recent studies showed that genomic alteration as a result of MHEJ underlies some tumorigenesis in human and mouse models (41–43). For example, Zhu et al. (49) showed that pro-B lymphomas in mice deficient for both p53 and DNA Ligase IV, XRCC4, or Ku undergo translocations via the MHEJ pathway, resulting in co-amplification of c-myc (chromosome 15) and IgH (chromosome 12) sequences. Further study of factors involved in this mutagenic DNA repair pathway will shed more light on the mechanisms of this carcinogenic process.

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Modulation of DNA End Joining by Nuclear Proteins
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