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Chromosomal double-strand break repair in Ku80-deficient cells

(*xrs-6* cells/radiation sensitive mutants/I-*Sce*I endonuclease/homologous recombination/end-joining)

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ABSTRACT **The x-ray sensitive hamster cell line *xrs-6* is deficient in DNA double-strand break (DSB) repair and exhibits impaired V(D)J recombination. The molecular defect in this line is in the 80-kDa subunit of the Ku autoantigen, a protein that binds to DNA ends and recruits the DNA-dependent protein kinase to DNA. Using an I-*Sce*I endonuclease expression system, chromosomal DSB repair was examined in *xrs-6* and parental CHO-K1 cell lines. A DSB in chromosomal DNA increased the yield of recombinants several thousand-fold above background in both the *xrs-6* and CHO-K1 cells, with recombinational repair of DSBs occurring in as many as 1 of 100 cells electroporated with the endonuclease expression vector. Thus, recombinational repair of chromosomal DSBs can occur at substantial levels in mammalian cells and it is not grossly affected in our assay by a deficiency of the Ku autoantigen. Rejoining of broken chromosome ends (end-joining) near the site of the DSB was also examined. In contrast to recombinational repair, end-joining was found to be severely impaired in the *xrs-6* cells. Thus, the Ku protein appears to play a critical role in only one of the chromosomal DSB repair pathways.**

The repair of DNA double-strand breaks (DSBs) is critical for the maintenance of genomic integrity in all organisms. Failure to repair a broken chromosome or its inappropriate repair can result in catastrophic consequences for a cell, such as the loss of genetic information and chromosome translocations. In *Saccharomyces cerevisiae*, DSBs are primarily repaired by homologous recombination, both in mitotically growing cells and in cells undergoing meiosis (1). Mammalian cells are able to repair chromosomal DSBs by both homologous recombination and nonhomologous end-joining mechanisms (2). Recombinational repair of DSBs has been demonstrated by gene targeting (3–5). Gene targeting, the homologous recombination of transfected DNA with chromosomal DNA, is enhanced two to three orders of magnitude by a break at the target locus. Two broken chromosomal ends can also be repaired by end-joining processes that involve little or no homology at the DNA ends (3, 6–8). End-joining products are frequently characterized by small deletions at the site of the break.

Detailed mechanisms of both homologous and nonhomologous repair processes are not well understood in mammalian systems. Recently, however, several radiosensitive cell mutants have been characterized that are deficient in DSB repair. These are members of ionizing radiation (IR) complementation groups 4, 5, and 7, which include the hamster XR-1 (9), *xrs-6* (10), and V3 (11) cell lines, respectively. Murine *scid* cells also belong to IR group 7 (12). The genes defective in IR group 5 and 7 mutants, *XRCC5* and *XRCC7*, respectively, encode components of a DNA-dependent protein kinase (DNA-PK), a complex possessing DNA end-binding and protein kinase activity (13–16). The *xrs-6* cells are defective in the DNA end-binding component of this complex, the Ku protein (13, 14), whereas *scid* and V3 cells are defective in the large catalytic subunit, DNA-PK_{cs} (15, 16).

Cell mutants from these IR complementation groups are also defective in V(D)J recombination, the process in which antigen receptor molecules are rearranged (17). V(D)J recombination is a site-specific recombination event initiated by the RAG1/RAG2 proteins at heptamer/nonamer signal sequences (18). The intermediates in this process are unique—hairpins at the end of the antigen receptor coding sequences and blunt ends at the adjacent signal sequences (19). Completion of the reaction leading to signal joints and coding joints requires components of the generalized cellular DSB repair machinery, including DNA-PK, although the details are not well understood.

We have begun to examine the nature of the chromosomal DSB repair defect in cell mutants using an endonuclease expression system (20). Cleavage sites for the rare-cutting endonuclease I-*Sce*I have been stably integrated into the genome of *xrs-6* cells within the context of two different repair substrates. One substrate is used to select intrachromosomal recombinational repair events at I-*Sce*I-generated DSBs and the other is used to select end-joining events near the I-*Sce*I-generated DSBs. Whereas recombinational repair of DSBs was found to occur at high levels in *xrs-6* cells, rejoining of broken chromosome ends near the site of the DSB was found to be severely impaired in the Ku80-deficient cells.

MATERIALS AND METHODS

DNA Constructions. The two selectable drug marker genes used in this study are a hygromycin resistance gene, *pgkhyg* (21), driven by the mouse phosphoglycerate kinase 1 promoter, and a neomycin phosphotransferase gene, pMC1neo (22), driven by a hybrid polyoma virus and thymidine kinase promoter. DRneo was constructed by ligating the 2.1-kb *Bgl*II fragment containing the *pgkhyg* gene into the *Bam*HI site of S2neo (5). The 700-bp 3′ *neo Pst*I fragment of pMC1neo was cloned into the *Pst*I site of pUC19 and then cleaved out as a *Sal*I fragment. It was then cloned into the *Sal*I site of the S2neo/*pgkhyg* vector. Plasmids S1neo (3) and pCMV-I-*Sce*I (20) were as described previously.

Cell Transfections. All transfections were done with uncut plasmids. Calcium phosphate transfection (3, 23) was used to introduce the DRneo, S2neo, and S1neo substrates (9 μg) into the CHO-K1 and *xrs-6* cell lines, along with 3 μg of *pgkhyg*. Hygromycin B was added to the culture medium 24 h post-transfection for a final concentration of 0.5 mg/ml for selection of CHO-K1 clones and 0.1 mg/ml for *xrs-6* clones.

In a second round of transfections, 1.6×10^7 cells were electroporated (24) with either 30 μg of plasmid DNA (pCMV-I-*Sce*I and pCMV-lacZ) or pMC1neo (1 μg). Colonies

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Abbreviations: DSB, DNA double-strand break; IR, ionizing radiation.

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were selected in 1 mg/ml G418 beginning 24 h after electroporation. Southern blot analysis was performed using 8 μg of genomic DNA according to standard procedures. The *Xho*I/*Nar*I fragment of the *neo* gene was used as the probe.

RESULTS

Recombinational Repair of Chromosomal DSBs in CHO-K1 and *xrs-6* Cells. The DRneo substrate is used to select intrachromosomal recombinational repair of I-*Sce*I-generated DSBs. It consists of two directly repeated copies of the *neo* gene (Fig. 1). One copy, called S2neo, is mutated by deletion of 4 bp within the 3′ portion of the *neo* coding region (5). At the position of the 4-bp deletion there is an insertion of the 18-bp I-*Sce*I cleavage site. Downstream of S2neo is a 0.7-kb 3′ *neo* gene fragment. The S2neo mutation can be corrected by recombination with the 3′ *neo* gene fragment, leading to a *neo*$^+$ gene. DSB-promoted homologous recombination can occur by intrachromatid or sister-chromatid interactions.

The DRneo substrate was introduced into the genome of *xrs-6* cells and the radioresistant parental CHO-K1 cell line using selection for a second marker, an *hyg*^{R} gene. In addition, as a control, the S2neo substrate was also introduced into these cell lines. Hyg^R clones were selected and analyzed by Southern blotting. Clones containing the S2neo and the DRneo substrates were expanded in media containing hygromycin and subjected to a second round of transfections, at which point hygromycin selection was removed. In this second round, the I-*Sce*I expression vector, pCMV-I-*Sce*I, was electroporated to select for DSB-promoted recombination events. A control plasmid, pCMV-lacZ, was electroporated in separate transfections to monitor spontaneous recombination events, as well as to compare transient transfection efficiencies of the cell clones. A plasmid containing an intact *neo* gene, pMC1neo, was also electroporated to monitor stable transfection efficiencies of the various cell clones. *Neo*$^+$ clones were selected with the drug G418 beginning 24 h after electroporation.

For the S2neo clones, no G418 resistant (G418^R) colonies were obtained with electroporation of pCMV-lacZ or pCMV-I-*Sce*I, as expected, since there is no homology to correct the deletion within the *neo* gene (Table 1). In the DRneo clones of both cell lines, spontaneous recombinants are detected at a low level after electroporation of pCMV-lacZ, at approximately 10^{-6}-10^{-7} of the plated cell populations.

Electroporation of the DRneo clones from both cell lines with the I-*Sce*I expression vector results in a large increase in the number of recombinants, from approximately 10^4 to almost 10^5 colonies (Table 1). For the two CHO-K1 clones, 8.3×10^3 (DRB8) and 7.7×10^4 (DRA10) G418^R colonies arise from transfection of the clones with pCMV-I-*Sce*I, whereas 0 and 10 colonies, respectively, are obtained from transfection of pCMV-lacZ. For clone DRA10, the G418^R clones are approximately 1% of the electroporated cell population. This is likely an underestimate of the number of recombinants that are possible, because cleavage of the chromosomal I-*Sce*I sites does not appear to be complete *in vivo* (F.L. and M.J., unpublished results). These results demonstrate that DSBs at a chromosomal locus can stimulate intrachromosomal recombination several thousand-fold.

Similar results are obtained with the *xrs-6* clones (Table 1). For clones DR4 and DR8, 5.1×10^4 and 2.4×10^4 G418^R colonies are obtained from transfection of the I-*Sce*I expression vector, whereas only 24 and 4 colonies, respectively, are obtained from transfection of pCMV-lacZ. This is a several thousand-fold increase in the number of recombinants. Thus, the levels of DSB-promoted recombination are not obviously reduced in the Ku80-deficient *xrs-6* cells. We are currently analyzing the structure of the locus in these colonies to determine the frequency with which recombination is associated with crossing-over between the *neo* gene repeats and whether a difference is noted between the two cell lines.

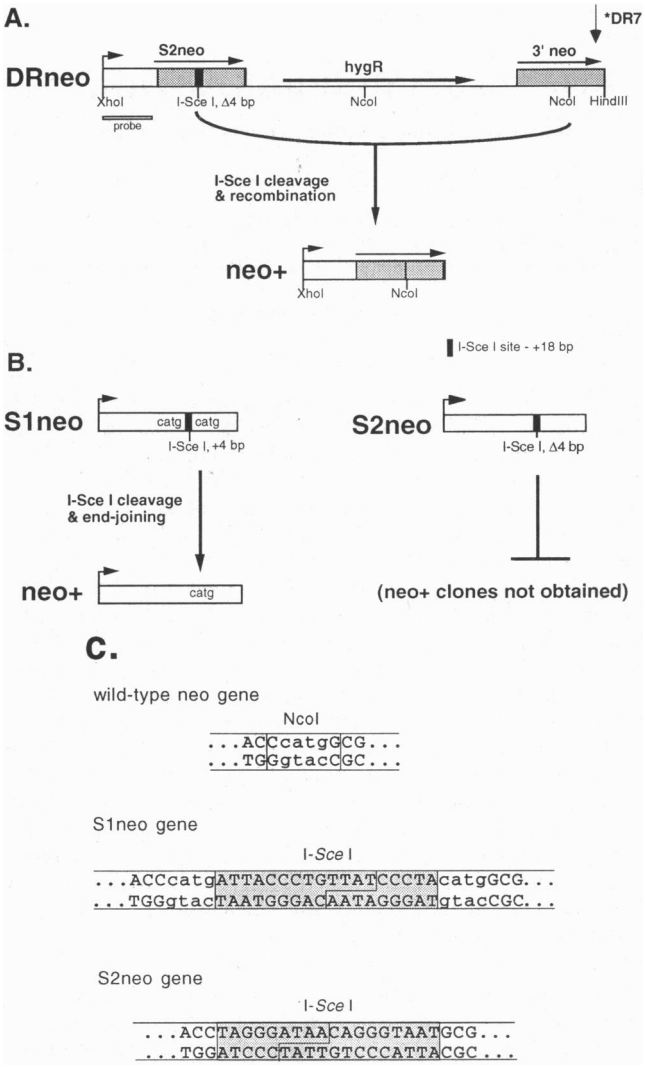

FIG. 1. DSB repair substrates. Substrates are designed to select for particular repair events after I-*Sce*I cleavage. The *neo* gene is from pMC1neo and is driven by a hybrid promoter derived from polyoma virus strain F441 and the thymidine kinase gene (22). (*A*) Recombinational repair substrate DRneo. The S2neo gene is a full-length *neo* gene except for a deletion of 4 bp at the insertion of the 18-bp I-*Sce*I site (black bar). Downstream from S2neo and in the same orientation is a 0.7-kb 3′ *neo* gene fragment that can correct the S2neo mutation. The homology between the S2neo gene and the 3′ *neo* gene fragment is indicated by the shading. Following I-*Sce*I cleavage and homologous recombination, the I-*Sce*I site is deleted and the *Nco*I site in the *neo* gene is restored from the 3′ *neo* fragment, creating a *neo*$^+$ gene. Recombination can occur by either crossing over or gene conversion (not shown). The *xrs-6* *DR7 clone contains a truncation of the 3′ *neo* fragment, as indicated. (*B*) End-joining substrate S1neo and control substrate S2neo. The S1neo gene has a duplication of 4 bp (catg) flanking the I-*Sce*I site. After I-*Sce*I cleavage and rejoining at the duplication, a *neo*$^+$ gene is recreated. (See ref. 3 for a possible mechanism.) For the S2neo gene, *neo*$^+$ clones are not obtained, since it contains a 4-bp deletion of *neo* gene sequences. (*C*) Sequence of the wild-type *neo* gene, the S1neo gene, and the S2neo gene at the position of insertion of the I-*Sce*I cleavage site. For both S1neo and S2neo, the I-*Sce*I site (shaded sequence) was inserted into the *Nco*I site of the *neo* gene, disrupting it. In S1neo, there is a duplication of the 4-bp *Nco*I overhangs (catg), which flanks the I-*Sce*I site. In S2neo, there is a deletion of the 4-bp *Nco*I overhangs. Note that the orientation of the I-*Sce*I sites are opposite in the two *neo* genes. I-*Sce*I cleaves to produce a 4 base 3′ overhang, as indicated.

A third *xrs-6* clone, *DR7, has also been examined. The 3′ *neo* fragment in the DRneo substrate in this clone is truncated (see Fig. 1*A*), such that recombination can only occur by gene

Table 1. Ku80-deficient cells are proficient at DSB-promoted recombination

Cell line	No. of G418^R colonies		
	pCMV-lacZ	pCMV-I-*Sce*I	pMC1neo
	S2neo substrate clones		
CHO-K1			
S2B2	0	0	1.77×10^3
xrs-6			
S2B8	0	0	1.68×10^3
	DRneo substrate clones		
CHO-K1			
DRA10	10	7.74×10^4	1.56×10^3
DRB8	0	8.30×10^3	1.86×10^3
xrs-6			
DR4	24	5.10×10^4	1.71×10^3
DR8	4	2.38×10^4	1.41×10^3
*DR7	0	7.08×10^3	1.59×10^3

The cell lines have integrated copies of the DRneo or S2neo substrates, as indicated. The *xrs-6* clone *DR7 is exceptional in that it has a truncated 3′ *neo* fragment at the *Hin*dIII site and can only undergo gene conversion without crossing over. Each cell line was transfected with the indicated plasmid and subsequently selected in G418. A total of 1.6×10^7 cells were electroporated in each sample, with an estimated 50% survival.

conversion without an associated crossover. Transfections of the I-*Sce*I expression vector into the *DR7 cell line also leads to a substantial increase in the number of recombinants, demonstrating that gene conversion without crossing over is not obviously impaired in the *xrs-6* cell line.

Recombination in each of the DRneo clones is expected to result in loss of the I-*Sce*I site in the S2neo gene and the recovery of the *Nco*I site from the 3′ *neo* fragment. To verify that colonies derived from pCMV-I-*Sce*I transfection are homologous recombinants, Southern blot analysis was performed. Genomic DNA derived from G418^R colonies was cleaved with *Xho*I/*Nco*I (Fig. 2). The parental clones have a 2.2-kb band with this digest. In each of the G418^R colonies, this band has been reduced to 0.9 kb, the size expected by homologous recombination between the S2neo gene and the 3′ *neo* fragment (Fig. 2). Each of these clones has also lost the I-*Sce*I site that was present at this position (data not shown). Thus, the G418^R clones derived from transfection of the I-*Sce*I expression vector have undergone homologous recombination within the DRneo substrate.

Stable transfection efficiencies were compared between the various cell clones by electroporations of uncleaved pMC1neo. No significant difference in the number of G418^R colonies was obtained between the various clones (Table 1). Thus, under these transfection conditions, Ku80 deficiency does not appear to affect the nonhomologous integration of transfected DNA. Transient transfection was also compared by assaying extracts of cells electroporated with pCMV-lacZ for β-galactosidase activity. Although as much as a 2-fold variation in transfection was noted between the various clones, no consistent difference was noted between clones derived from CHO-K1 or *xrs-6* cells.

Chromosomal End-Joining Defect in *xrs-6* Cells. To determine if the *xrs-6* cells manifest a defect in rejoining chromosomal DSBs, a second repair substrate, S1neo (3), was used. The S1neo gene, like the S2neo gene, contains an I-*Sce*I cleavage site within the 3′ portion of the *neo* coding region. Unlike S2neo, the S1neo gene contains a 4-bp repeat of *neo* coding sequence flanking the 18-bp I-*Sce*I cleavage site (Fig. 1 *B* and *C*). I-*Sce*I cleavage, followed by repair of the broken ends to retain only one copy of the 4-bp repeat, leads to a *neo*$^+$ gene, as has previously been shown in mouse 3T3 cells (3). Such a rejoining can be mediated by annealing of single strands at the 4-bp microhomology or by blunt-ended ligation, following deletion of 4 bp of the repeat. Although the S1neo substrate is designed to measure one particular rejoining product, chromosomal DSBs appear to be frequently repaired by small deletions around the break site (3, 6–8).

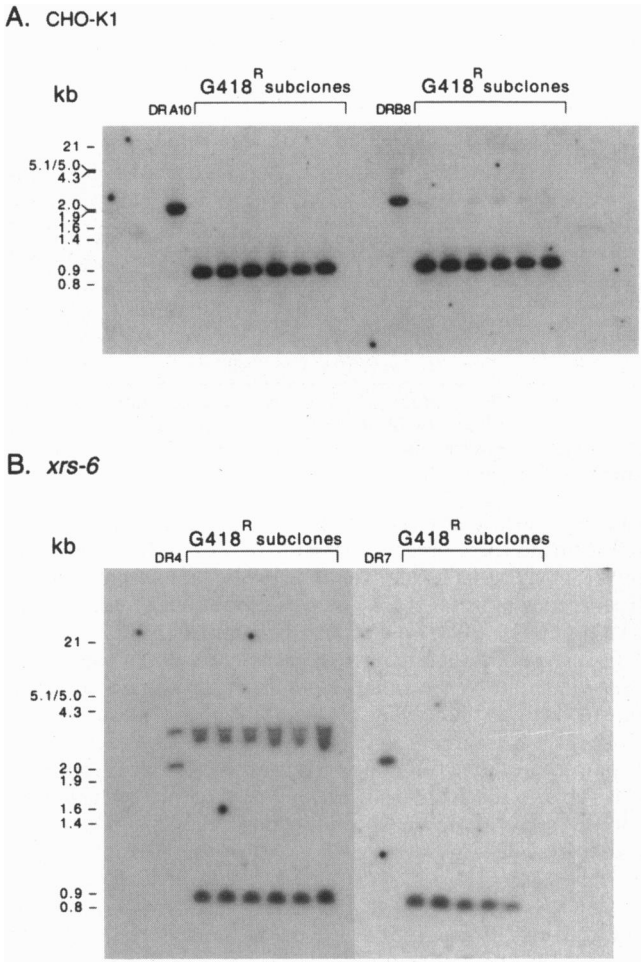

FIG. 2. Southern hybridization analysis of G418^R subclones derived from transfection of pCMV-I-*Sce*I. Genomic DNA was digested with *Xho*I/*Nco*I and probed with a 5′ *neo* gene fragment (Fig. 1*A*). Upon homologous recombination, the 2.2-kb DRneo *Xho*I/*Nco*I fragment becomes 0.9 kb. (*A*) CHO-K1 subclones derived from parental clones DRA10 and DRB8 containing an integrated DRneo substrate. (*B*) *xrs-6* subclones derived from parental clones DR4 and *DR7 containing an integrated DRneo substrate. Note that clone DR4 has a second truncated integration of the DRneo substrate, which gives rise to an additional *Xho*I/*Nco*I fragment of approximately 3.0 kb. This second band is unchanged in size in the G418^R subclones, but appears warped in this Southern blot due to electrophoresis conditions.

DNA end-joining was analyzed in CHO-K1 and *xrs-6* clones containing the integrated S1neo gene. When the I-*Sce*I expression vector was introduced into the CHO-K1 clones, hundreds of G418^R colonies were obtained from each clone (Table 2). No colonies were obtained from the pCMV-lacZ transfection, demonstrating that G418 resistance only arises from repair of I-*Sce*I-cleaved substrates. These results are similar to those previously obtained in mouse 3T3 cells (3).

Transfection of the *xrs-6* S1neo clones gave a very different result. No G418^R colonies were obtained from transfection of these clones with the I-*Sce*I expression vector (Table 2). Thus, although the Ku80-deficient *xrs-6* cells are proficient at DSB-promoted homologous recombination, they appear to exhibit a severe defect in the DNA end-joining pathway.

Gene Targeting in CHO-K1 and *xrs-6* Cells. To verify that the *xrs-6* S1neo genes were cleaved efficiently *in vivo* by I-*Sce*I, one of the S1neo clones was tested in a gene targeting assay. Although

Table 2. Ku80-deficient cells have impaired DNA end-joining

Cell line	No. of G418^R colonies		
	pCMV-lacZ	pCMV-I-*Sce*I	pMC1neo
	S1neo substrate clones		
CHO-K1			
S1B1	0	284	1.59×10^3
S1B44	0	672	1.71×10^3
S1B45	0	734	1.80×10^3
S1D3	0	552	1.80×10^3
xrs-6			
S1-2	0	0	1.62×10^3
S1-4	0	0	1.65×10^3
S1-18	0	0	1.53×10^3

The cell lines have integrated copies of the S1neo substrate. Each cell line was transfected with the indicated plasmid and subsequently selected in G418. A total of 1.6×10^7 cells were electroporated in each sample, with an estimated 50% survival.

generally inefficient in mammalian cells, gene targeting is stimulated two to three orders of magnitude by a DSB at the target locus (3–5). The *xrs-6* cells would be predicted to repair cleaved S1neo genes by gene targeting, because they are proficient at DSB-promoted intrachromosomal recombination.

To test gene targeting in the absence of a DSB, one S1neo clone and one S2neo clone from each cell line were each transfected with the plasmid p3′ neo. This plasmid contains the 0.7-kb 3′ *neo* fragment that can correct the mutation in the chromosome. Gene targeting for any of the clones is inefficient with transfection of the p3′ neo plasmid alone, such that no colonies are obtained from either the CHO-K1 or *xrs-6* cells (Table 3). The I-*Sce*I expression vector was also introduced into these clones. As seen above, electroporation of the CHO-K1 S1neo clone with pCMV-I-*Sce*I alone gives rise to hundreds of G418^R colonies, whereas the *xrs-6* S1neo clone does not give rise to colonies. Neither S2neo clone gives rise to colonies with pCMV-I-*Sce*I electroporation.

To examine DSB-promoted gene targeting, the I-*Sce*I expression vector was cotransfected with the p3′ neo plasmid (Table 3). For both the CHO-K1 and *xrs-6* clones, 10^3-10^4 G418^R colonies are obtained, indicating that there is a three to four order of magnitude stimulation of gene targeting. The absolute frequency of gene targeting for both cell lines is as high as 1 in 1000 of electroporated cells. These results confirm that the S1neo locus is expressed in the *xrs-6* S1-4 cell clone and that it is cleaved upon electroporation of the I-*Sce*I expression vector. Thus, the failure to obtain G418^R clones from transfection of pCMV-I-*Sce*I alone is a result of altered end-joining processes in the *xrs-6* cells.

Table 3. Ku80-deficient cells are proficient at gene targeting

Cell line	No. of G418^R colonies				
	pCMV-lacZ	p3′ neo	pCMV-I-*Sce*I	pCMV-I-*Sce*I + p3′ neo	pMC1neo
	S1neo substrate clones				
CHO-K1					
S1B45	0	0	638	8.88×10^3	1.86×10^3
xrs-6					
S1-4	0	0	0	1.46×10^3	1.68×10^3
	S2neo substrate clones				
CHO-K1					
S2B2	0	0	0	2.06×10^3	1.80×10^3
xrs-6					
S2B8	0	0	0	1.27×10^4	1.95×10^3

Each cell line was transfected with the indicated plasmid(s) and subsequently selected in G418. A total of 1.6×10^7 cells were electroporated in each sample, with an estimated 50% survival.

DISCUSSION

We have analyzed recombinational and end-joining repair of chromosomal DSBs in wild-type and Ku80-deficient hamster cell lines using the I-*Sce*I endonuclease expression system. Both intrachromosomal recombination and gene targeting were found to be stimulated three to four orders of magnitude by a DSB in chromosomal DNA in the CHO-K1 and the *xrs-6* cells. By contrast, rejoining of broken chromosome ends near the site of the DSB was found to be impaired in the Ku80-deficient *xrs-6* cells. These experiments implicate the Ku protein, which binds to DNA ends, in playing a critical role in nonhomologous repair pathways, but not in recombinational repair of chromosomal DSBs. These experiments also demonstrate the power of the I-*Sce*I endonuclease expression system for approaching questions of chromosomal DSB repair in mammalian cells.

Recombinational repair of DSBs in transfected DNA has previously been observed in mammalian cells (25). Because transfected DNA is introduced naked, without chromatin proteins, and is degraded soon after entry into cells (26), it does not represent an ideal model for chromosome break repair. Thus, the I-*Sce*I endonuclease system represents a significant advance for the study of how broken chromosomes are healed. In general, recombinational repair of chromosomes could occur inter- or intrachromosomally, with interchromosomal recombination expected to be rare in mitotically growing cells. Intrachromosomal recombination can occur between sister chromatids after DNA replication and between repeated sequences in the genome. Because the DRneo substrate can undergo both intrachromatid and sister chromatid recombination, it may represent a good model for repair events that naturally occur in genomes. Spontaneous recombinants are detected with the DRneo substrate at a low level in both cell lines, approximately 10^{-6}-10^{-7} of the plated cell populations. By contrast, DSB-promoted recombinants are 10^{-2}-10^{-3} of the electroporated cells. This is likely an underestimate of DSB-promoted events, because cleavage of the chromosomal I-*Sce*I site is not complete *in vivo* (F.L. and M. J., unpublished results). These results show that recombinational repair of DSBs can occur at substantial levels in mammalian genomes.

In contrast to recombinational repair, the Ku80-deficient *xrs-6* cells exhibit a clear impairment in the ability to give rise to a particular nonhomologous end-joining product after cleavage of chromosomal DNA. To generate a *neo*$^+$ gene, repair of the cleaved S1neo gene deletes 4 bp at a repeat flanking the I-*Sce*I cleavage site, as well as the I-*Sce*I site itself. Repair can be mediated either by annealing of single strands at the 4 bp repeat or by blunt-ended ligation following deletion of 4 bp of the repeat. The deficiency in *xrs-6* cells in the generation of *neo*$^+$ clones can be complemented by cotransfection of a Ku80-expression vector along with the I-*Sce*I expression vector (F.L. and M.J., unpublished results). The particular repair events leading to a *neo*$^+$ gene have previously been found to comprise a portion of the total repair events after I-*Sce*I cleavage (3). However, many of the other repair events also occur at microrepeats in the chromosome and also result in small deletions around the cleavage site (3). Thus, we expect that the specific impairment that we have observed in chromosome break rejoining reflects a more general defect in rejoining pathways.

The end-joining deficit found in *xrs-6* cells can be explained either by excessive degradation of the broken chromosome ends or by a specific block to the rejoining of DNA ends. Physical studies of the repair of chromosomal DSBs have shown that *xrs-6* cells have only a 2- to 3-fold slower repair of DSBs after ionizing radiation, suggesting that misrepair of DSBs, as well as their inability to be rejoined, contributes to the ionizing radiation sensitivity of Ku80-deficient cells (10). Studies of extrachromosomal DNA have shown that precise

rejoining of DSBs is reduced in *xrs-6* cells, with a concomitant increase in the number of end-joining products that have deletions (26). In addition, in V(D)J recombination assays, rare signal joints that are recovered in *xrs-6* cells are abnormal in that they frequently contain deletions (17). These results support the notion that degradation of the chromosome ends is increased in *xrs-6* cells, rather than there being a specific block in rejoining. Given that no deficit is observed in homologous repair of DSBs, either degradation does not occur beyond the region of homology or the Ku protein does not participate at all in this pathway.

In addition to the rejoining of broken chromosome ends, nonhomologous or illegitimate recombination events in mammalian cells can be assayed by the random integration of plasmids into chromosomal DNA. In our assays, electroporated *xrs-6* cells exhibited no deficiency in the integration of the control plasmid pMC1neo, suggesting that there may be mechanistic differences between chromosome end-joining and random plasmid integration. Previous studies in *xrs* cells using calcium phosphate transfection have reported a defect in plasmid integration that is dependent on the concentration of the transfected DNA (27, 28). At low DNA concentrations, little or no difference is observed between wild-type and mutant cell lines. At high DNA concentrations, as many as 5- to 10-fold fewer random integrants are obtained from *xrs-6* cells and other members of the IR complementation group 5, as measured by selectable drug marker expression (27, 28). These results, combined with the proficiency of these cells to undergo DSB-promoted recombination events, suggest that Ku deficient cell lines may have more desirable ratios of homologous to nonhomologous integrations in gene targeting studies, at least under some transfection conditions.

Repair of DSBs in *S. cerevisiae* has been shown to occur almost exclusively by homologous recombination (1). Although a Ku80 homolog has not as yet been identified in yeast, a putative Ku70 homolog, termed *HDF1*, has been cloned (29). Mutation of the *hdf1* gene affects radiosensitivity of yeast only in the absence of homologous recombination (30), suggesting that the Ku protein is critically involved in (minor) nonhomologous repair pathways in yeast, in concurrence with our results in mammalian cells. Recent experiments in yeast have directly determined a role for the HDF1 gene product in nonhomologous rejoining pathways (J. Haber, personal communication). A *Drosophila* homolog to the Ku70 protein has also been identified (31) and mutants in this gene, termed *mus309*, are mutagen-sensitive and have altered P element excision, which occurs through a DSB in the chromosome (32). Excision products recovered from such mutants contain large deletions, consistent with a role for the Ku protein in DNA end-protection.

In yeast, genes known to be involved in homologous repair of DSBs belong to the *RAD52* epistasis group (33). Homologs to many of these genes, including the *RAD51* (34), *RAD52* (35, 36), and *RAD54* genes (37), have been found in mammalian cells. Both *RAD51* and *RAD52* have been found to be expressed in mitotically dividing cells, as well as tissues undergoing meiosis (34–36). Considering the conservation of gene products of the *RAD52* group, as well as the Ku autoantigen, yeast and mammalian cells may have conserved homologous and nonhomologous repair pathways. A key difference between the two organisms is the relative use of the two pathways. Yeast exhibit a strong bias toward homologous repair, with an approximate 1000:1 ratio of homologous to nonhomologous events. Until now, mammalian cells have been thought to repair chromosomal DSBs almost exclusively by nonhomologous means. Although our experiments were not designed to directly determine the proportion of DSBs in mammalian cells that are healed by homologous pathways, they demonstrate that, at least in some contexts, homologous repair events constitute a significant proportion of events.

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