

Report

DNA repair by nonhomologous end joining and homologous recombination during cell cycle in human cells

Zhiyong Mao,[†] Michael Bozzella,[†] Andrei Seluanov and Vera Gorbunova*

Department of Biology; University of Rochester; Rochester, New York USA

[†]These authors contributed equally to this work.

Abbreviations: DSB, double-strand break; HR, homologous recombination; NHEJ, nonhomologous end joining; hTERT, human telomerase reverse transcriptase; GFP, green fluorescent protein; DsRed, red fluorescent protein

Key words: cell cycle, DNA repair, normal human fibroblasts, nonhomologous end joining, homologous recombination

DNA double-strand breaks (DSBs) are dangerous lesions that can lead to potentially oncogenic genomic rearrangements or cell death. The two major pathways for repair of DSBs are nonhomologous end joining (NHEJ) and homologous recombination (HR). NHEJ is an intrinsically error-prone pathway while HR results in accurate repair. To understand the origin of genomic instability in human cells it is important to know the contribution of each DSB repair pathway. Studies of rodent cells and human cancer cell lines have shown that the choice between NHEJ or HR pathways depends on cell cycle stage. Surprisingly, cell cycle regulation of DSB repair has not been examined in normal human cells with intact cell cycle checkpoints. Here we measured the efficiency of NHEJ and HR at different cell cycle stages in hTERT-immortalized diploid human fibroblasts. We utilized cells with chromosomally-integrated fluorescent reporter cassettes, in which a unique DSB is introduced by a rare-cutting endonuclease. We show that NHEJ is active throughout the cell cycle, and its activity increases as cells progress from G₁ to G₂/M (G₁ < S < G₂/M). HR is nearly absent in G₁, most active in the S phase, and declines in G₂/M. Thus, in G₂/M NHEJ is elevated, while HR is on decline. This is in contrast to a general belief that NHEJ is most active in G₁, while HR is active in S, G₂ and M. The overall efficiency of NHEJ was higher than HR at all cell cycle stages. We conclude that human somatic cells utilize error-prone NHEJ as the major DSB repair pathway at all cell cycle stages, while HR is used, primarily, in the S phase.

Introduction

DNA double-strand breaks (DSBs), in which both strands in the double helix are severed, are the most dangerous type of DNA lesion. If left unrepaired, or repaired incorrectly, DSBs may result in massive loss of genetic information, genomic rearrangements, or cell death.

DSBs are repaired by two major mechanisms: non-homologous end joining (NHEJ) and homologous recombination (HR).¹ The two pathways differ in their fidelity and template requirements. NHEJ modifies the broken DNA ends, and ligates them together with little or no homology, generating deletions or insertions.² In contrast, HR uses an undamaged DNA template on the sister chromatid or homologous chromosome to repair the break, leading to the reconstitution of the original sequence.³ Thus, the choice of DSB repair pathway determines the fidelity of repair, which in turn may influence the rates of aging and tumorigenesis.⁴⁻⁶

Both DSB repair pathways play important roles in mammalian DSB repair.^{7,8} The exact mechanism by which the choice between the two DSB repair pathways is made remains unclear. In part, the choice of repair pathway is determined by cell cycle stage. The dependence of DSB repair on the cell cycle was first shown by analyzing the sensitivity of chicken DT40 cells, deficient in NHEJ or HR factors, to ionizing radiation.⁹ NHEJ mutants were highly sensitive in G₁ and early S, while HR mutants were sensitive only in S/G₂. Studies in hamster CHO cell lines containing mutations in DSB repair genes showed that NHEJ-defective cells have reduced repair at all cell cycle stages, while HR-defective cells have a minor defect in G₁, and greater impairment in S/G₂/M.^{10,11} Thus, NHEJ is a major DSB repair pathway in G₁ stage, while both NHEJ and HR may be active in S/G₂/M. The studies of mutant cell lines do not allow for comparison of the contributions of each pathway, because various mutations inactivate NHEJ and HR to a different extent. Furthermore, the effect of cell cycle stage on DSB repair has not been analyzed in primary human cells, which maintain intact cell cycle checkpoints.

We recently developed sensitive fluorescent reporter assays to examine NHEJ and HR in hTERT-immortalized diploid human cells. These cells retain all characteristics of untransformed primary cells,¹² including intact cell cycle checkpoints. We show that NHEJ is active throughout the cell cycle, but is the highest in G₂/M. HR is nearly absent in G₁, most active in the S phase, and is low in G₂/M. Thus, in normal human fibroblasts NHEJ is the major DSB repair pathway, while HR primarily repairs DNA breaks that occur during replication.

*Correspondence to: Vera Gorbunova; Department of Biology; University of Rochester; 213 Hutchison Hall; Rochester, New York 14627 USA; Tel.: 585.275.7740; Fax: 585.275.2070; Email: vgorbuno@mail.rochester.edu

Submitted: 06/18/08; Revised: 07/29/08; Accepted: 07/30/08

Previously published online as a *Cell Cycle* E-publication:
<http://www.landesbioscience.com/journals/cc/article/6700>

Results

The efficiency of NHEJ and HR during cell cycle has not been examined in normal human cells. The use of normal cells is important because normal cells maintain an intact cell cycle control apparatus, which may be involved in regulating DNA repair. To examine the contribution of NHEJ and HR in DSB repair during the cell cycle we used hTERT-immortalized normal human fibroblasts (HCA2-hTERT) containing chromosomally integrated GFP-based NHEJ and HR reporter constructs. The construction of NHEJ and HR reporter cell lines has been described previously.^{13,14} Here we used two NHEJ (I9a and S13a) and two HR (H15c and H32c) cell lines for analysis of repair during the cell cycle.

The reporter cassette for detecting NHEJ¹⁵ contains a GFP gene with an artificially engineered 3 kb intron from the Pem1 gene (GFP-Pem1). The Pem1 intron contains an adenoviral exon flanked by 18 bp recognition sequences for I-SceI endonuclease (Fig. 1A). I-SceI is used to generate site-specific DSBs in vivo. In the NHEJ-I9a clone (Fig. 1A) I-SceI sites are in an inverted orientation, which generate incompatible ends (Fig. 1C), and in NHEJ-S13a (Fig. 1A) I-SceI sites are in a direct orientation, which generate compatible DNA ends (Fig. 1D). An un-rearranged NHEJ cassette is GFP negative since the adenoviral exon disrupts the GFP ORF. Upon induction of DSBs by the expression of I-SceI,¹⁶ the adenoviral exon is removed and NHEJ restores function of the GFP gene. This reporter can detect a wide spectrum of NHEJ events since the intron can tolerate deletions and insertions. The HR reporter (Fig. 1B) is built using the same GFP-Pem1 gene as the NHEJ reporter.¹⁴ In the HR reporter, the first exon of GFP-Pem1 contains a 22 bp deletion combined with the insertion of three restriction sites, I-SceI-HindIII-I-SceI, which are used for inducing DSBs. The deletion ensures that GFP cannot be reconstituted by an NHEJ event. The two I-SceI sites are in an inverted orientation, so that I-SceI digestion leaves incompatible ends (Fig. 1C). The first copy of GFP-Pem1 is followed by a promoter-less/ATG-less first exon and intron of GFP-Pem1. The intact construct is GFP-negative. Upon induction of a DSB by I-SceI digestion the functional GFP gene will be reconstituted by intramolecular or intermolecular gene conversion between the two mutated copies of the first GFP-Pem1 exon. Since the second copy of the GFP gene lacks a promoter, the first ATG codon, and the second exon, crossing over or single-strand annealing will not restore the GFP activity. This design allows for the exclusive detection of gene conversion, which is the predominant HR pathway in mammalian cells¹⁷. The H15c and H32c cell lines used in this study are two independent integrants of the HR reporter. Reconstitution of the functional GFP gene by both NHEJ or HR has been confirmed by sequencing reporter cassettes from GFP-positive clones.¹³

To study DSB repair during cell cycle we first determined the treatments that arrest HCA2-hTERT cells at various cell cycle stages. Normal cells are sensitive to contact inhibition, and arrest in G₁ stage at confluence (Fig. 2A). Treatment with a DNA polymerase α inhibitor, aphidicolin,¹⁸ arrested HCA2-hTERT in S stage (Fig. 2A). Colchicine, which prevents microtubule polymerization,¹⁹ arrested the cells in G₂/M stage (Fig. 2A). Cell cycle distribution in the treated cells was verified by propidium iodide staining and flow cytometry

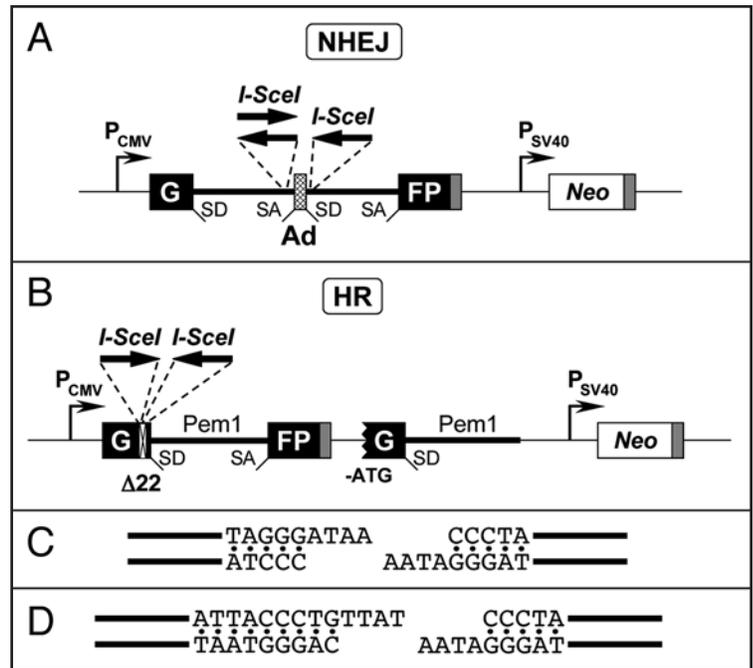


Figure 1. Reporter constructs for analysis of NHEJ and HR repair. (A) Reporter cassette for detection of NHEJ. The cassette consists of a GFP gene under a CMV promoter with an engineered intron from the rat Pem1 gene, interrupted by an adenoviral exon (Ad). The adenoviral exon is flanked by I-SceI recognition sites in inverted orientation (cell line I9a) or in direct orientation (cell line S13a) for induction of DSBs. In this construct the GFP gene is inactive; however upon induction of a DSB and successful NHEJ the construct becomes GFP⁺. SD, splice donor; SA, splice acceptor; shaded squares, polyadenylation sites. (B) Reporter cassette for detection of HR (cell lines H15c and H32c). The cassette consists of two mutated copies of GFP-Pem1. In the first copy of GFP-Pem1 the first GFP exon contains a deletion of 22 nt and an insertion of two I-SceI recognition sites in inverted orientation. The 22 nt deletion ensures that GFP cannot be reconstituted by a NHEJ event. The second copy of GFP-Pem1 lacks a promoter, the first ATG, and the second exon of GFP. Upon induction of DSBs by I-SceI, gene conversion events reconstitute an active GFP gene. (C) Incompatible DNA ends generated by digestion of two I-SceI sites in inverted orientation as in the lines NHEJ-I9a, HR-H15c, and HR-H32c. (D) Compatible DNA ends generated by digestion of two I-SceI sites in direct orientation as in the line NHEJ-S13a.

every day for 7 days following treatment. Drug-treated cells entered cell cycle arrest 2 days after treatment, and remained arrested for at least 7 days. No cell death was observed in either confluent or drug-treated cells. Confluent cells were in G₁ stage after reaching confluence, and could be maintained in G₁ indefinitely with regular media changes. Figure 2A shows cell cycle distribution on day 7 after reaching confluence and day 4 after drug treatment. This is the time point when DSB repair was taking place (discussed below).

To analyze NHEJ and HR G₁ arrested cells were co-transfected with 5 μ g of I-SceI-expressing plasmid²⁰ to induce DSBs and 0.1 μ g DsRed plasmid to normalize for the transfection efficiency. G₁-arrested cells were transfected on day 6 after cells reached confluence, and drug-treated cells were transfected on day 3 after drug treatment. In HCA2 fibroblasts I-SceI expression reaches a maximum during the first 24 hours after transfection, and then progressively declines.¹⁴ Therefore, the majority of DSBs were induced on day 7 of confluence and day 4 after drug treatment. Repair of I-SceI-induced breaks results in the appearance of GFP⁺ cells. To quantify NHEJ and HR events cells were analyzed by flow cytometry 4 days

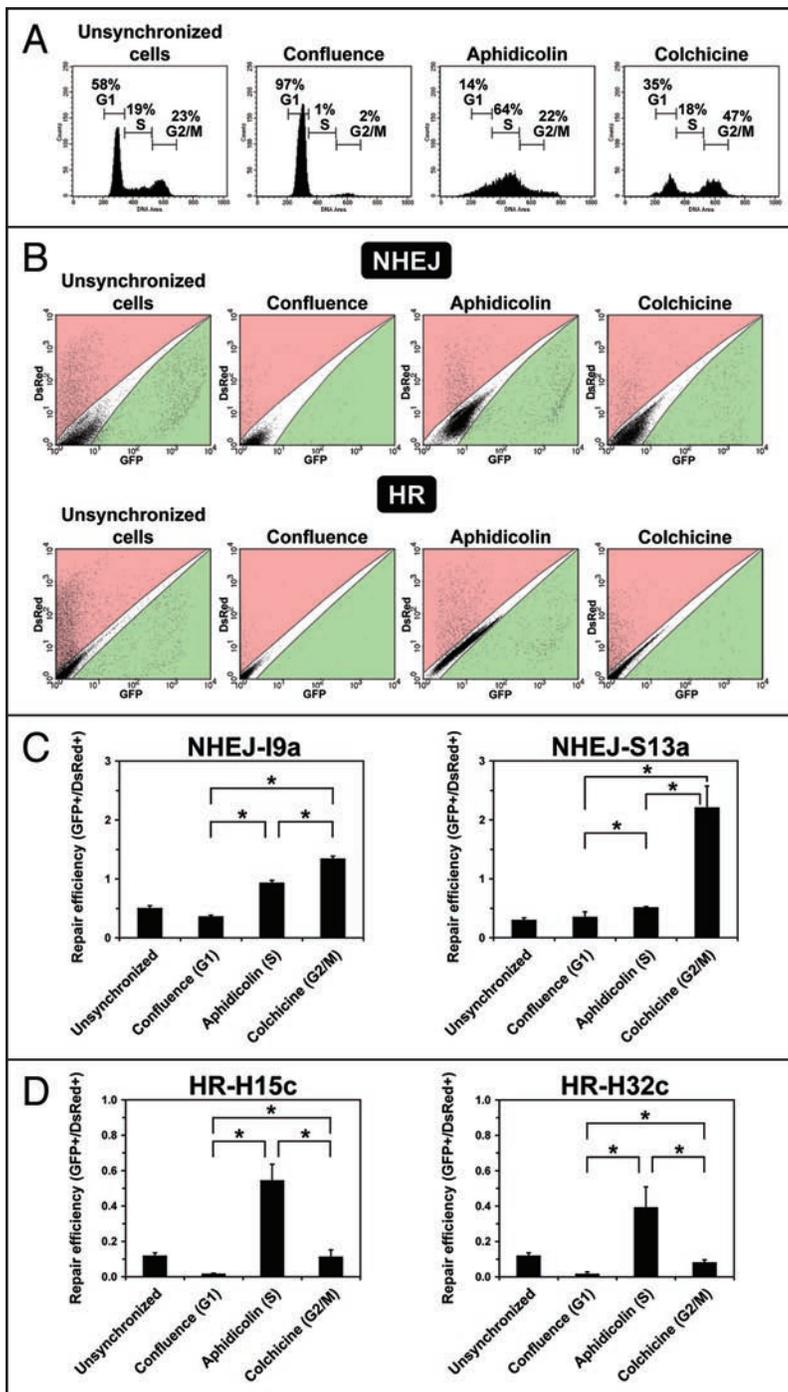


Figure 2. DSB repair pathways during cell cycle. (A) Cell cycle distribution of HCA2-hTERT cells following indicated treatments. Number of cells is plotted against DNA content determined by PI staining. Cell cycle distribution was analyzed every day for 7 consecutive days. The images show confluent cells on day 7 after they reached confluence, and drug treated cells on day 4 after treatment, which is the time point when DSB repair took place. (B) Representative FACS traces for the analysis of NHEJ and HR. Cells were co-transfected with 5 μ g of plasmid encoding I-SceI, and 0.1 μ g of a DsRed plasmid, after they entered growth arrest as shown in panel A. Cells were analyzed by flow cytometry 4 days after transfection, using green-versus-red fluorescent plot as described previously.¹⁵ Green fluorescence is plotted on the x-axis and red fluorescence is plotted on the y-axis. The narrow triangular area on the diagonal corresponds to autofluorescent cells. Typically 20,000 cells were analyzed in each sample. In the treatments where the numbers of GFP⁺ cells were low, 40,000 cells were scored. (C and D) Frequency of NHEJ and HR at various cell cycle stages analyzed in two independent NHEJ and HR reporter cell lines. Each cell line contains a single integrated copy of an NHEJ or HR reporter cassette. The ratio of GFP⁺/DsRed⁺ cells is used as a measure of repair efficiency. NHEJ and HR are shown on different scales due to the large differences in repair frequency. The experiments were repeated at least four times and error bars are s. d. Stars (*) indicate the statistically significant differences ($p < 0.05$, t-test).

In S phase the frequency of NHEJ was increased by approximately 1.5 to 3 fold relative to the G₁ stage cells (Fig. 2C). The frequency of HR in S increased 20 to 27 fold relative to G₁ phase (Fig. 2D). Despite the sharp increase in HR frequency in S phase it remained lower than S-phase NHEJ.

Interestingly, in G₂/M NHEJ frequency was further elevated 1.5 to 4 fold relative to the S phase (Fig. 2C). NHEJ of both compatible and incompatible ends was elevated in G₂/M, but the rise in compatible-end NHEJ (line S13a) was more dramatic. The frequency of HR declined in G₂/M by 5 fold relative to HR in S phase (Fig. 1D). The increase of NHEJ and decline of HR in G₂/M is an unexpected finding, since HR is believed to be efficient in G₂/M.

In summary, our results indicate that NHEJ is the major DSB repair pathway at all cell cycle stages, and is most active in G₂/M phase. HR operates predominantly in S-phase, but even in S phase HR may be less efficient than NHEJ.

Discussion

Here we report the first direct comparison of NHEJ and HR in normal human cells. We employ sensitive fluorescent reporter assays that allow for a direct comparison of the efficiencies of NHEJ and HR events upon induction of chromosomal DSBs with a rare-cutting endonuclease. Fluorescent assays score DSB repair events in thousands of cells, and are highly quantitative.

Our data shows that NHEJ is active throughout the cell cycle. Our distinct finding is that NHEJ efficiency is $G_1 < S < G_2/M$. In earlier studies the roles of NHEJ and HR during the cell cycle were analyzed by assaying the sensitivity of synchronized NHEJ or HR mutant cells to ionizing radiation.^{9-11,21-23} Most of the NHEJ-deficient cell lines are hypersensitive in G₁ phase, leading to conclusion that NHEJ acts primarily in G₁. NHEJ has been proposed to act also in S-phase,^{24,25}

post-transfection to allow for maximum GFP expression in drug-treated cells. Our pilot experiments showed no decline in GFP or DsRed signal during the first 4 days after transfection. GFP and DsRed fluorescence was analyzed using the green-versus-red plot (Fig. 2B) as described previously.¹⁵ To normalize for the efficiency of transfection, the ratio of GFP⁺ to DsRed⁺ cells was used as a measure of NHEJ and HR efficiency.

In G₁ stage NHEJ was active while HR was almost completely repressed (Fig. 2C and D). As the HR reporter contains a duplication of the GFP gene, HR can potentially occur intrachromosomally. The near absence of HR in G₁ indicates that HR does not occur when only an intrachromosomal template is available.

or in all cell cycle stages.^{10,11} It was also shown that fidelity of NHEJ is higher in G_2 than G_1 .²⁶ We show that the efficiency of NHEJ in G_2/M is 4–6 fold higher than in G_1 . Thus, NHEJ plays the major role in DSB repair in human cells and its activity is increased in S and G_2/M stages despite availability of HR. Our results also suggest that G_2/M phase of the cell cycle is characterized by the highest efficiency of DNA break repair.

We show that HR is extremely low in G_1 , is most efficient in S, and is decreased in G_2/M . The cell survival studies uniformly agree that HR is not utilized in G_1 stage.^{9–11,27} Ectopic and allelic sequences may potentially be used as templates for repair, but in organisms with large repetitive genomes this may generate chromosomal rearrangements and a loss of heterozygosity. Indeed, studies in both plants and animals show that homologous and ectopic sequences are used at very low frequencies.^{17,28,29} Theoretically, HR may occur in S, G_2 and M since sister chromatids are present. Our results indicate that HR is used more frequently in S than in G_2/M . It was shown that HR is important for repairing lesions resulting from replication block.^{10,30,31} Thus, a possible explanation for decreased HR in G_2/M is that a primary function of HR in mammalian cells is to repair DNA damage associated with DNA replication, and HR is active when DNA replication machinery is present.

Since our assay directly measures NHEJ and HR using the same type of reporter we can evaluate the contribution of NHEJ and HR at each cell cycle stage. The comparison between NHEJ and HR is summarized in a model (Fig. 3) in which NHEJ contributes to DSB repair at all cell cycle phases, but is most active in G_2/M , while HR contributes primarily in S phase, and modestly in G_2/M . Importantly, even in the S phase when HR is the most active, NHEJ is more efficient than HR. Our data is consistent with NHEJ being the major DSB repair pathway during all cell cycle stages in human somatic cells. The reason for preferential use of NHEJ by human cells is likely to be the repetitive nature of the human genome.⁵ If an incorrect template were to be used for repair, an HR event could result in a gross genomic rearrangement. Organisms with highly repetitive genomes may therefore favor NHEJ, as a small deletion associated with an NHEJ event is less deleterious than an aberrant recombination event. This strategy will protect young organisms from genomic rearrangements; however, over time mutations introduced by NHEJ will accumulate in the genome and contribute to a functional decline associated with aging.

Materials and Methods

Cell lines and culture conditions. HCA2 are human neonatal foreskin fibroblasts isolated by the laboratory of O.M. Pereira-Smith (UTHSCSA, Houston, TX). Construction of NHEJ-I9a, NHEJ-S13a, HR-H15c and HR-H32c reporter cell lines is described in.¹⁴ Cells were cultured at 37°C in a 5% CO_2 , 3% O_2 incubator, in EMEM media supplemented with 15% fetal bovine serum, 100 units/ml penicillin and 100 μ g/ml streptomycin.

Cell cycle arrest. In order to arrest the cells at various stages of the cell cycle, several growth conditions, and drug treatments were used. For G_1 arrest, cells were plated on 100 mm plates at a concentration of 5×10^5 and allowed to grow to confluence and kept there for 6 days before transfection. Following transfection, the confluent cells were replated on 60 mm plates to maintain a state of contact inhibition and therefore G_1 arrest. S-phase arrest was induced by the

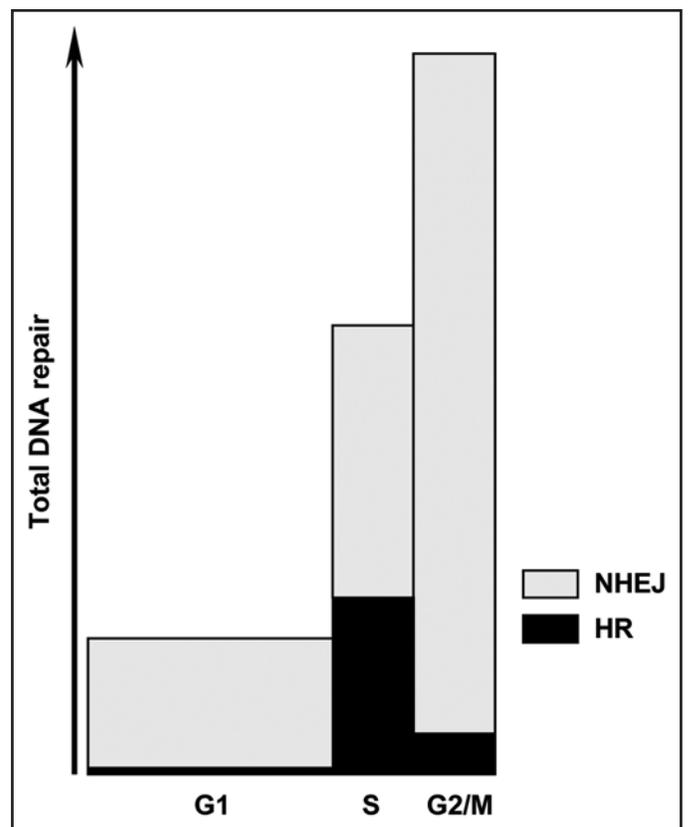


Figure 3. Model for the contribution of NHEJ and HR to repair of DSBs during cell cycle. NHEJ is active during all stages of cell cycle, but its efficiency is the highest during G_2/M . HR is active primarily in the S-phase and has lower activity in G_2/M . The length of each cell cycle stage is proportional to the number of cells at a corresponding stage in unsynchronized culture of normal fibroblasts in Figure 2A.

addition of 1 μ g/ml aphidicolin, and G_2/M arrest was induced by the addition of 0.1 mg/ml colchicine.

Transfections. The transfections were performed using an Amaxa Nucleofector; program U-20. In each transfection 2×10^6 cells were transfected with 0.1 μ g of a DsRed expressing plasmid, and 5 μ g of an I-SceI expressing plasmid.

FACS analysis. For the analysis of NHEJ and HR cells were harvested, resuspended in ~1 ml 1xPBS, put on ice, and run on a FACS Calibur instrument. GFP and DsRed fluorescence was analyzed using the red-versus-green plot as described previously.¹⁵ Cell cycle distribution was analyzed by PI staining. Data was analyzed using CellQuest software.

Acknowledgements

We thank Dara Brown, A'Shantee O'Steen and Anna Sokolov for help with construction of HR reporter cell lines. This work was supported by grants from US National Institute of Health AG027237 (V.G.), American Federation for Aging Research (V.G.), the Komen Foundation (V.G.), and Ellison Medical Foundation (V.G. and A.S.).

References

1. Haber JE. Partners and pathways repairing a double-strand break. *Trends Genet* 2000; 16:259-64.
2. Lieber MR. The mechanism of human nonhomologous DNA end joining. *J Biol Chem* 2008; 283:1-5.
3. Thompson LH, Schild D. Homologous recombinational repair of DNA ensures mammalian chromosome stability. *Mutat Res* 2001; 477:131-53.
4. Karanjawala ZE, Lieber MR. DNA damage and aging. *Mech Ageing Dev* 2004; 125:405-16.
5. Lieber MR, Karanjawala ZE. Ageing, repetitive genomes and DNA damage. *Nat Rev Mol Cell Biol* 2004; 5:69-75.
6. Thompson LH, Schild D. Recombinational DNA repair and human disease. *Mutat Res* 2002; 509:49-78.
7. Sonoda E, Hohegger H, Saberi A, Taniguchi Y, Takeda S. Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. *DNA Repair (Amst)* 2006; 5:1021-9.
8. Sargent RG, Branneman MA, Wilson JH. Repair of site-specific double-strand breaks in mammalian chromosome by homologous and illegitimate recombination. *Mol Cell Biol* 1997; 17:267-77.
9. Takata M, Sasaki MS, Sonoda E, Morrison C, Hashimoto M, Utsumi H, et al. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *Embo J* 1998; 17:5497-508.
10. Rothkamm K, Kruger I, Thompson LH, Lobrich M. Pathways of DNA double-strand break repair during the mammalian cell cycle. *Mol Cell Biol* 2003; 23:5706-15.
11. Hinz JM, Yamada NA, Salazar EP, Tebbs RS, Thompson LH. Influence of double-strand-break repair pathways on radiosensitivity throughout the cell cycle in CHO cells. *DNA Repair (Amst)* 2005; 4:782-92.
12. Morales CP, Holt I, Ouellette M, Kaur KJ, Yan Y, Wilson KS, et al. Absence of cancer-associated changes in human fibroblasts immortalized with telomerase. *Nat Genet* 1999; 21:115-8.
13. Mao Z, Seluanov A, Jiang Y, Gorbunova V. TRF2 is required for repair of nontelomeric DNA double-strand breaks by homologous recombination. *Proc Natl Acad Sci USA* 2007; 104:13068-73.
14. Mao Z, Bozzella M, Seluanov A, Gorbunova V. Comparison of nonhomologous end joining and homologous recombination in human cells. *DNA Repair (Amst)* 2008; DOI: 10.1016/j.dnarep.2008.06.018.
15. Seluanov A, Mittelman D, Pereira-Smith OM, Wilson JH, Gorbunova V. DNA end joining becomes less efficient and more error-prone during cellular senescence. *Proc Natl Acad Sci USA* 2004; 101:7624-9.
16. Jasin M. Genetic manipulation of genomes with rare-cutting endonucleases. *Trends Genet* 1996; 12:224-8.
17. Johnson RD, Jasin M. Sister chromatid gene conversion is a prominent double strand break repair in mammalian cells. *EMBO J* 2000; 19:3398-407.
18. Ikegami S, Taguchi T, Ohashi M, Oguro M, Nagano H, Mano Y. Aphidicolin prevents mitotic cell division by interfering with the activity of DNA polymerase-alpha. *Nature* 1978; 275:458-60.
19. Luduena RF, Banerjee A, Khan IA. Tubulin structure and biochemistry. *Curr Opin Cell Biol* 1992; 4:53-7.
20. Rouet P, Smih F, Jasin M. Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Mol Cell Biol* 1994; 14:8096-106.
21. Ferreira MG, Cooper JP. Two modes of DNA double-strand break repair are reciprocally regulated through the fission yeast cell cycle. *Genes Dev* 2004; 18:2249-54.
22. Stamato TD, Dipatri A, Giaccia A. Cell cycle-dependent repair of potentially lethal damage in the XR-1 gamma-ray-sensitive Chinese hamster ovary cell. *Radiat Res* 1988; 115:325-33.
23. Jeggo PA. Studies on mammalian mutants defective in rejoining double-strand breaks in DNA. *Mutat Res* 1990; 239:1-16.
24. Mills KD, Ferguson DO, Essers J, Eckersdorff M, Kanaar R, Alt FW. Rad54 and DNA Ligase IV cooperate to maintain mammalian chromatid stability. *Genes Dev* 2004; 18:1283-92.
25. Couedel C, Mills KD, Barchi M, Shen L, Olshen A, Johnson RD, et al. Collaboration of homologous recombination and nonhomologous end-joining factors for the survival and integrity of mice and cells. *Genes Dev* 2004; 18:1293-304.
26. Kruger I, Rothkamm K, Lobrich M. Enhanced fidelity for rejoining radiation-induced DNA double-strand breaks in the G₂ phase of Chinese hamster ovary cells. *Nucleic Acids Res* 2004; 32:2677-84.
27. Cheong N, Wang X, Wang Y, Iliakis G. Loss of S-phase-dependent radioresistance in *irs-1* cells exposed to X-rays. *Mutat Res* 1994; 314:77-85.
28. Puchta H. The repair of double-strand breaks in plants: mechanisms and consequences for genome evolution. *J Exp Bot* 2005; 56:1-14.
29. Shalev G, Levy AA. The maize transposable element *Ac* induces recombination between the donor site and an homologous ectopic sequence. *Genetics* 1997; 146:1143-51.
30. Saintigny Y, Delacote F, Vares G, Petitot F, Lambert S, Averbek D, et al. Characterization of homologous recombination induced by replication inhibition in mammalian cells. *Embo J* 2001; 20:3861-70.
31. Arnaudeau C, Lundin C, Helleday T. DNA double-strand breaks associated with replication forks are predominantly repaired by homologous recombination involving an exchange mechanism in mammalian cells. *J Mol Biol* 2001; 307:1235-45.