BRCA1 Facilitates Microhomology-mediated End Joining of DNA Double Strand Breaks*

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BRCA1 is critical for the maintenance of genomic stability, in part through its interaction with the Rad50-Mre11-Nbs1 complex, which occupies a central role in DNA double strand break repair mediated by nonhomologous end joining (NHEJ) and homologous recombination. BRCA1 has been shown to be required for homology-directed recombination repair. However, the role of BRCA1 in NHEJ, a critical pathway for the repair of double strand breaks and genome stability in mammalian cells, remains elusive. Here, we established a pair of mouse embryonic fibroblasts (MEFs) derived from 9.5-day-old embryos with genotypes Brca1+/−: p53−/− or Brca1−/−:p53−/−. The Brca1−/−:p53−/− MEFs appear to be extremely sensitive to ionizing radiation. The contribution of BRCA1 in NHEJ was evaluated in these cells using three different assay systems. First, transfection of a linearized plasmid in which expression of the reporter gene required precise end joining indicated that Brca1−/− MEFs display a moderate deficiency when compared with Brca1+/− cells. Second, using a retrovirus infection assay dependent on NHEJ, a 5–10-fold reduction in retroviral integration efficiency was observed in Brca1−/− MEFs when compared with the Brca1+/− MEFs. Third, Brca1−/− MEFs exhibited a 50–100-fold deficiency in microhomology-mediated end-joining activity of a defined chromosomal DNA double strand break introduced by a rare cutting endonuclease I-SceI. These results provide evidence that Brca1 has an essential role in microhomology-mediated end joining and suggest a novel molecular basis for its caretaker role in the maintenance of genome integrity.

Inactivation of the breast cancer susceptibility gene, BRCA1, accounts for a significant portion of familial breast cancer (1, 2). Chromosome aneuploidy has been reported in human breast cancer cells, HCC1937, which contain a C-terminal truncated BRCA1 (3). Similarly, murine fibroblasts containing a deletion within exon 11 of BRCA1 display extensive chromosomal abnormality (4). Both Brca1-deficient murine and human cells have been shown to be sensitive to DNA-damaging agents, including ionizing radiation (5–7), suggesting that BRCA1 may play an essential role in DNA double strand break (DSB) repair. DSBs can be repaired through homologous recombination (HR) or nonhomologous end joining (NHEJ) to ensure the maintenance of genome integrity in eukaryotic organisms (8). More significantly, deficiencies in the mammalian NHEJ pathway can lead to an increase in the frequency of chromosomal translocations and the rate of neoplastic transformation (9–13), thus emphasizing the importance of this DSB repair pathway in the maintenance of genome integrity.

BRCA1 binds to the Rad50-Mre11-Nbs1 complex and can form radiation-induced foci with this complex (14). The yeast counterpart of this complex, Rad50-Mre11-Xrs2, is required for both NHEJ and HR (15). BRCA1 has been implicated in homology-based recombination repair (16). In the study described by Moynahan et al. (16), a reporter substrate containing two differentially mutated neomycin phosphotransferase gene (neo) placed in tandem, with one harboring an I-SceI cleavage site, was integrated into the genome of both wild-type or Brca1 exon 11 deleted embryonic stem (ES) cells. Upon expression of the I-SceI endonuclease, ES cells containing the Brca1 exon 11 deleted mutant, but not wild-type Brca1, showed a deficiency in intra- and interchromosomal recombination (16). Interestingly, these Brca1 mutant cells showed a slight increase in nonhomologous repair processes characterized by nucleotide deletion or addition (16). However, neither precise end joining, which may account for nearly 60% of the end-joining events in the I-SceI-inducible DNA DSB repair (17), nor a microhomology-mediated end-joining activity that may recover a functional neo gene independent of gene conversion was taken into consideration in this assay system (16). Moreover, it is likely that repair by HR contributes much more than NHEJ in ES cells because of their relatively short cell cycle duration (18). Thus, the potential role of BRCA1 in NHEJ may not be revealed by this reported assay system (16). In BRCA1-deficient HCC1937 cells, a much slower rate and less extensive amount of DSB repair, as measured by pulse field gel electrophoresis (PFGE) at 6 h after ionizing irradiation, has been observed as compared with the same cells expressing wild-type BRCA1 protein (19). The altered kinetics of the PFGE assay primarily reflects the inefficiency of chromosomal break rejoining, which is commonly observed in mutant cells deficient in NHEJ mutant but not in HR (20). This finding, albeit circumstantial, suggests that BRCA1 may participate in NHEJ.

To address the potential role of BRCA1 in NHEJ, we utilized three independent in vivo approaches demonstrating that BRCA1 promotes NHEJ mediated by microhomology. Trans-
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Most importantly, Brca1-deficient MEFs exhibit severely impaired DNA repair, which depends on an intact NHEJ pathway. Western blotting using anti-mouse Brca1 antibody. In MEFs, the expression of Brca1 in wild-type, Brca1<sup>+/+</sup>, Brca1<sup>−/−</sup>, and Brca1<sup>+/−</sup> MEFs. Cell lysates were prepared from these MEFs for protein analysis by immunoprecipitation followed by Western blotting using anti-mouse Brca1 antibody. In Brca1<sup>−/−</sup> MEFs, the full-length Brca1 protein was not detected. C, Brca1<sup>−/−</sup> MEFs are hypersensitive to γ-irradiation. Brca1<sup>+/−</sup>, Brca1<sup>+/+</sup>, and Brca1<sup>−/−</sup> MEFs were either exposed to different doses of γ-irradiation as indicated or mock-exposed. Surviving colonies were counted 10 days later. The results shown represent the mean ± standard deviation of three independent experiments.

Fection of a linearized plasmid in which expression of the reporter gene required precise end joining indicated that Brca1<sup>−/−</sup> MEFs display a moderate deficiency when compared with Brca1<sup>+/+</sup> MEFs. Further, Brca1 promotes efficient retroviral infection, which depends on an intact NHEJ pathway. Most importantly, Brca1-deficient MEFs exhibit severely impaired end-joining activity mediated by microhomology in response to I-SceI restriction endonuclease induced chromosomal double-stranded DNA break. These results support a role for Brca1 in NHEJ and provide a biochemical basis for the caretaker function of BRCA1 in the maintenance of genome integrity.

MATERIALS AND METHODS
Mouse Embryonic Fibroblast
To prepare Brca1<sup>−/−</sup>:p53<sup>−/−</sup> and p53<sup>−/−</sup> mouse embryonic fibroblasts (MEFs), Brca1<sup>+/+</sup> mice derived from a Brca1-ko<sup>o</sup>3 targeted ES mice were used. The Brca1<sup>−/−</sup>:p53<sup>−/−</sup> and p53<sup>−/−</sup> MEFs were generated from a Brca1-ko<sup>o</sup>3 targeted ES cell line as described previously (21). The Brca1<sup>−/−</sup>:p53<sup>−/−</sup> and p53<sup>−/−</sup> MEFs were seeded in identical plates at 4000 cells/well-90-mm dish in medium for 24 h. Cells were then irradiated with 0, 1, 2, 4, and 8 gray by using a Mark 1, model 68A, irradiator. After 14 days, the colonies were fixed and stained with 2% methylene blue in 50% ethanol and then counted.

Adenovirus I-SceI Construct
pPKG3XNLS-I-SceI was kindly provided by P. Berg and G. Donoho (Stanford University). The entire I-SceI DNA fragment was digested with SaI and PstI and cloned into the pBlueScript vector, referred to as pBSK-I-SceI. This plasmid was then digested with NolI and XhoI to release I-SceI and inserted into the AdTrack-CMV plasmid (a gift from B. Vogelstein) to form the AdTrack-CMV-I-SceI plasmid. This adenovirus was then produced following the protocol as described previously (24).

Mouse Brca1 Antibodies, Immunoprecipitation, and Western Blotting
Mouse Brca1 cDNA-encoded amino acids 788–1135 was translationally fused to glutathione S-transferase in-frame, and the bacterially generated fusion protein derived from this construct was used as an antigen for producing mouse polyclonal antibodies. Immunoprecipitation and Western blotting were performed as described previously (14).

Transfection and Luciferase Activity Assay
pGL2 plasmid (Promega) was completely linearized by restriction endonuclease HindIII or EcoRI and confirmed by agarose gel electrophoresis. The linearized DNA was subjected to phenol/chloroform extraction, ethanol-precipitated, and dissolved in sterilized water. DNA was then transfected into cells with FuGene 6 following the procedures described by the supplier (Roche Molecular Biochemicals). The transfected cells were harvested and assayed for luciferase activity as described (22).

Retroviral Infection Assay
MEFs were plated at 5 × 10<sup>5</sup>/10-cm dish and infected with a 477H recombinant retrovirus carrying a hygromycin resistance gene for 24 h. The infected cells were then selected by hygromycin (200 μg/ml) for 10–14 days. The resulting hygromycin-resistant colonies were counted on three plates for each infection titer, and the experiments were repeated three times.

Chromosomal DNA End-joining Assay
Substrate Construction—pBBSK-SHygro was constructed by inserting an endonuclease I-SceI recognition sequence into the unique NolI site of pBSK-PGKhygro as described below. The NolI site was filled in using Klenow fragment and ligated with an 18-bp I-SceI recognition sequence of 5'CATGATACGCGTATTATGCTTAAC-C'. A DNA cassette containing puromycin resistance gene driven by the SV40 T antigen promoter was then inserted into pBSK-SHygro. The entire SHygro with puromycin gene was propagated as a plasmid, and the plasmid DNA was then transfected into MEFs followed by a selection with 2 μg/ml puromycin. Cell clones that contained one to two copies of SHygro identified by genomic DNA blotting analysis were selected for further experiments.

SHygro Retrovirus—The entire SHygro with puromycin gene was released and inserted into an engineered murine leukemia virus (MuLV)-based retroviral vector. The SHygro virus, produced by transfecting the constructed viral vector DNA into Phoenix Amphi packag-
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**RESULTS**

**Brc1 Null MEFs Are Hypersensitive to Ionizing Irradiation**—Previously, mice carrying a mutated Brc1 allele with a small 5’ portion of exon 11 replaced by a neomycin gene were generated and shown to be viable (21). However, mice with both alleles mutated were embryonic lethal at postnatal days 5–7 (22). This phenotype was much more severe when compared with mice carrying both alleles of Brc1 deleted for exon 11, which displayed an extended embryonic life span (4, 27, 28).

Therefore, the MEFs derived from our Brc1 knock-out mice may be useful in exploring the full function of the Brc1 gene. Because the mutant Brc1 embryos died too early to generate MEFs, survival of Brc1+/– mutant embryos could be extended with the ablation of the p53 gene (4, 29). MEFs with Brc1+/–/p53+/– or p53+/– genotype were then established from E9.5 embryos (Fig. 1A) derived from a cross of Brc1+/–:p53+/– mice (21). Protein analysis by immunoprecipitation followed by Western blotting revealed no full-length BRCA1 protein in these Brc1+/–:p53+/– MEFs (Fig. 1B). Importantly, the Brc1-deficient MEFs exhibited hypersensitivity to γ-irradiation when compared with the p53+/– MEFs (Fig. 1C). The degree of γ-irradiation sensitivity of this Brc1+/–:p53+/– MEF is greater than mouse embryonic stem cells or MEFs carrying a Brc1 exon 11 splice variant (5, 6).

**Brc1 Affects Precise End Joining rather than Overall End Joining**—To examine the potential contribution of Brc1 in DSB repair, the established Brc1-deficient MEFs were used in a plasmid end-joining assay utilizing transient transfection of a linearized pGL2 plasmid harboring a luciferase reporter gene. If the reporter plasmid was linearized with restriction endonuclease HindIII, which cleaved at the linker region between promoter and coding sequence, any end-joining activity with small deletion or insertion would not affect the expression of the luciferase gene and could be considered an overall end-joining activity. However, if the reporter plasmid was digested with EcoRI at the luciferase coding region, only precise end joining would restore the original sequence (Fig. 2). Repair efficiency was calculated from the luciferase activities of linearized reporter constructs compared with that of the intact plasmid. We observed no difference in the overall end-joining activity between the Brc1+/–:p53+/– and p53+/– MEFs, but Brc1-deficient MEFs exhibited about a 50% reduction in precise end-joining activity (Fig. 2).

**BRC1 Is Required for Efficient Retroviral Infection Mediated by NHEJ**—It has been reported that cells deficient in proteins involved in NHEJ show a reduced efficiency of retroviral infection (30, 31), suggesting that NHEJ is critical for the resolution of DNA DSBs that arise during retroviral infection. The first report suggested that NHEJ is required for retroviral DNA integration that proceeds through the repair of gapped intermediates arising from the linkage of viral and host cell DNA in an early reaction catalyzed by the viral integrase protein. The repair of gapped intermediates, which consist of nonjoined viral DNA 5’ ends, results in a 4–6-base pair repeat of host cell DNA flanking each proviral end (30). The second report demonstrated that cells with mutated NHEJ proteins suffer a high level of cell death upon retroviral infection (31) and proposed that, instead of being involved in an early step of retroviral integration, the NHEJ proteins are required to clear the linear viral DNA to conceal the exposed DSBs to prevent subsequent apoptosis (31). In either case, NHEJ appears to be essential for retroviral infection.

Based on these findings, we decided to examine the efficiency of retroviral infection in the Brc1 deficient cells. Following infection with a retrovirus, 477H, carrying a hygromycin resistance gene (Hygro’), the frequency of hygromycin-resistant colonies was found to be 5–10-fold lower in the Brc1+/–:p53+/– MEFs as compared with the matched Brc1+/+:p53+/– MEFs (Fig. 3, A and B). Similarly, a 3–5-fold reduction in the number of hygromycin-resistant colonies was also observed in infected Ku80+/–:p53+/– MEFs (13) as compared with Ku80+/+::p53+/– MEFs (Fig. 3B). These results are consistent with the notion that DNA end-joining activity is significantly reduced in Brc1-deficient cells.

**Brc1 Promotes Nonhomologous End joining of Chromosomal DSBs**—In an attempt to further substantiate the contribution of Brc1 in NHEJ, we modified a DSB repair assay system (32) that permitted us to measure the frequency of NHEJ at a defined chromosomal DSB. For this purpose, we established p53+/– and Brc1+/–:p53+/– MEF cell lines that carried a single integrated copy of a defined DSB repair substrate. This substrate, S hygro, contained one copy of the hygromycin resistance gene (Hygro’) carrying an 18-base pair recognition sequence for the rare cutting restriction endonuclease I-SceI inserted into a naturally occurring NcoI site (Fig. 4), therefore leaving 4 bp of CATG microhomologies (residual NcoI site) flanking the I-SceI site (Fig. 4A). This modification resulted in the inactivation of the hygromycin resistance gene. However, upon I-SceI endonuclease cleavage, hygromycin-resistant activity could be restored, if this defined DSB was resected to a point at which the CATG microhomologies could be detected, and rejoined to restore the NcoI site, thereby restoring activity. The relative end-joining efficiency was calculated by comparing luciferase activity expressed in MEFs transfected with HindIII- or EcoRI-digested DNA with that of the intact plasmid. The results shown were from three independent transfection experiments.

**Microhomology-mediated End-joining Assay**—MEFs containing a single copy of S hygro substrate were transfected with pPGK3X表达-I-SceI or infected with I-SceI adenovirus to express I-SceI endonuclease that cleaves the I-SceI site at the proviral hygromycin DNA. The cells were subsequently selected in medium with hygromycin (200 μg/ml) for 14 days, and the hygromycin-resistant colonies were counted. To examine whether these resistant colonies arose from the microhomology-mediated end-joining repair, genomic DNA was extracted for PCR analysis using the primers hygro-4 (5’-CTCTGGTGATATAGTTGCCCAGATG-3’) and hygro-5 (5’-CATACAAGCCACCAGGCTCCAG3’) within the hygromycin resistance gene to generate a 595-bp DNA fragment that included the original inactivated NcoI and I-SceI sites. Recovery of the NcoI site by microhomology-mediated end joining was indicated by the cleavage of the 595-bp fragment into a 379- and a 216-bp fragment following NcoI restriction enzyme digestion.
generating an intact hygromycin resistance gene (Fig. 4A). The original sequence was unlikely to be restored by homologous recombination due to the absence of homologous sequence. Importantly, this type of NHEJ repair promoted by an I-SceI-induced DSB at the S1hygro gene could be assayed by selecting for hygromycin-resistant cell clones and physically analyzing the repair products by genomic DNA blotting or by direct PCR followed by digestion with NcoI endonuclease. Using a similar system, the Ku80 protein has been demonstrated to be crucial for NHEJ of a defined chromosomal DSB in vivo (32).

The S1hygro DSB repair substrate containing a puromycin selection cassette was stably integrated into the genome of wild-type, p53−/−, and Brca1−/−:p53−/− MEFs by either plasmid transfection or retroviral infection. Puromycin-resistant clones were selected and analyzed by Southern blotting and PCR. MEF clones containing a single copy of S1hygro were chosen for the chromosomal DSB-promoted NHEJ repair assay as described above. To generate DSBs, I-SceI endonuclease was introduced by transfection of an I-SceI expression vector (33) into the MEFs. In the mock transfection experiment, few or no hygromycin-resistant colonies were obtained following a selection against hygromycin. However, expression of the I-SceI endonuclease in either the wild-type or the p53−/−:p53−/− MEFs promoted NHEJ-mediated repair of the S1hygro substrate. Two identical plates of MEFs with a single integrated copy of S1hygro substrate were transfected with an I-SceI expression vector (+) or control vector (−). The dishes were fixed and stained with methylene blue. Representative dishes are shown. C, physical analysis of NHEJ products. PCR reaction using a pair of primers within the hygromycin gene generates a DNA fragment of ~573–595 bp (a). If this fragment contained a recovered NcoI site, digestion with restriction enzyme NcoI would produce two fragments of 368 (b) and 205 bp (c), respectively. D, physical analysis of genomic DNA extracted from both hygromycin-resistant clones (hygro r) and parental cells containing S1hygro. PCR reactions were performed using primers flanking the I-SceI or NcoI site as shown in panel C. Upon I-SceI endonuclease digestion, the PCR product (a) from the parental clone cleaved into two fragments, b* (379 bp) and c* (216 bp), indicating the intact I-SceI site. However, I-SceI failed to cleave the PCR fragments (a*) from the 10 hygromycin-resistant clones (lanes 1–10). Conversely, upon NcoI restriction digestion, 9 of 10 clones (except lane 7) gave rise to the b and c fragments, which were not seen in the parental clone. Clone 7 apparently went through a different repair mechanism because it lost both the I-SceI and NcoI sites but kept hygromycin resistance.
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**TABLE I**

| Genotype | No. of Hygro® colonies**a** | Mock-transfected | I-Sce1 |
|----------|-----------------------------|------------------|--------|
| **WT-1** | Wild type | 1 ± 1 | 50 ± 3 |
| **p53**- | p53-/-/Breca1--/+ | <1 | 86 ± 19 |
| **p53**- | p53-/-/Breca1--/+ | <1 | 90 ± 16 |
| **1H-5** | p53-/-/Breca1--/+ | <1 | <1 |

**Number of Hygro® colonies**

| Genotype | Mock-transfected | I-Sce1 |
|----------|------------------|--------|
| **RP-3** | p53-/-/Breca1--/+ | <1 | 45 ± 17 |
| **RP-4** | p53-/-/Breca1--/+ | <1 | 82 ± 41 |
| **RP-6** | p53-/-/Breca1--/+ | <1 | 45 ± 15 |
| **RH-1** | p53-/-/Breca1--/+ | <1 | <1 |
| **RH-3** | p53-/-/Breca1--/+ | <1 | 1 ± 1 |
| **RH-6** | p53-/-/Breca1--/+ | <1 | <1 |

* 1–2 copies of S1hygro were introduced into the cells by plasmid transfection.

* Single copies of S1hygro were introduced into the cells by retroviral DNA integration.

Adenovirus infection and expression. We observed that the expression of I-Sce1 in either wild-type or p53-/- MEFs yielded 45–82 hygromycin-resistant colonies/10^6 cells (4.5 × 10^−8.2 × 10^−3). By contrast, only background levels of hygromycin-resistant colonies were obtained after transfection of I-Sce1 in Breca1--/-/p53-/- MEFs (Table I). The transfection efficiency of the parental Breca1--/-/p53-/- MEF was 2-fold lower than Breca1 wild-type cells. However, each individual clone harboring S1hygro had a varied transfection efficiency of between 2-fold lower (RH3) to 3-fold higher (RH1) when compared with the clones derived from Breca1--/-/p53-/- MEF (RP3 and RP4), as measured by a transient transfection assay with a SV40 promoter-driven luciferase reporter (data not shown). Only background levels of hygromycin-resistant colonies were formed in a Breca1--/-/p53-/- MEF clone (RH1) despite its highest transfection efficiency. Therefore, mutation of Breca1 decreased the formation of hygromycin-resistant colonies by 1–2 orders of magnitude.

The repair of DSBs by NHEJ in the hygromycin-resistant clones would be expected to restore the naturally occurring NcoI restriction site, which was destroyed by the insertion of the 18-bp I-Sce1 recognition site during construction of the S1hygro substrate. To verify that the hygromycin-resistant colonies derived from I-Sce1-induced DSB repair did in fact arise from NHEJ, a 595-bp DNA fragment of the hygromycin resistance gene encompassing the inactivated NcoI and I-Sce1 restriction sites was amplified by PCR and subjected to NcoI restriction enzyme digestion (Fig. 4C). Nine of 10 PCR products generated from the hygromycin-resistant colonies were completely cleaved by NcoI but were resistant to I-Sce1 digestion. In contrast, PCR products derived from parental clones harboring S1hygro were readily cleaved by I-Sce1 but not by NcoI (Fig. 4D). These results indicated that cleavage followed by end processing must have occurred in these cells to restore the NcoI site.

To ensure a high level of I-Sce1 expression, an adenovirus, AdTrack-CMV-I-Sce1, encoding I-Sce1 and GFP under two distinct promoters was generated (Fig. 5A). We then tested this adenovirus over a series of m.o.i. by tracing the fluorescence derived from GFP to monitor the infection efficiency. As shown in Fig. 5B, the expression of GFP in Breca1 mutant or wild-type MEFs was comparable following the infection of AdTrack-CMV-I-Sce1 in the range of m.o.i. used, suggesting that there was no significant difference between these two MEFs in terms of adenovirus infection and expression. We observed that the hygromycin-resistant colonies increased proportionally to the m.o.i. level in RP3 and RP4 but was undetectable in RH3 and RH1 MEFs. Infection with Adeno-I-GFP that expressed only GFP alone did not increase any hygromycin-resistant colonies in either type of MEF.

![Graph showing efficiency of I-Sce1 delivery by adenovirus](image-url)
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As probe. The gel shown in Fig. 6A, lane 1-7 and RH1 (Brca1-p53-/-, lanes 8-14) MEFs infected with different m.o.i. of AdTrack-CMV-I-SceI as indicated were used as templates to generate 573–595-bp PCR fragments as described in Fig. 4. The PCR products were digested with I-SceI endonuclease, separated by agarose gel electrophoresis, and stained with ethidium bromide. Lane M shows the DNA size marker. The appearance of two DNA fragments (a and c) from NcoI digestion (lane 2) indicates the presence of the recovered NcoI site through microhomology-mediated end joining. D, DNA blotting analysis using hygromycin resistance gene cDNA as probe. The gel shown in C was analyzed by Southern blotting with p32-labeled cDNA of the hygromycin resistance gene as described (21).

Fig. 6. Analysis of earlier time points for chromosomal end joining induced by I-SceI DSBs by transient transfection. A, illustration of the potential processes for repairing I-SceI-induced DSB and the properties of the end products derived from each repair process. The first pathway is mediated by a precise end joining that will regenerate the I-SceI site; its end product is indistinguishable from the original substrate. This pathway cannot be analyzed by this method. The second pathway functions through MHEJ, which restores the NcoI site. The third pathway is mediated by imprecise end joining, and the end product loses the I-SceI site and does not restore the NcoI site. B, physical analysis of the nonhomologous end-joining products. Genomic DNA from RP3 (Brca1-p53-lanes 1 and 2) or RH1 (Brca1-p53-/-, lanes 3 and 4) MEFs infected with 20 m.o.i. of AdTrack-CMV-I-SceI (lanes 2 and 4) or Adeno-I-GFP (lanes 1 and 3) were used as templates to generate the PCR fragments as described above. The PCR products (lanes 2 and 4) were digested with NcoI endonuclease, analyzed by agarose gel electrophoresis, and stained with ethidium bromide. Lane M shows the DNA size marker. The appearance of two DNA fragments (a and c) from NcoI digestion (lane 2) indicates the presence of the recovered NcoI site through microhomology-mediated end joining. D, DNA blotting analysis using hygromycin resistance gene cDNA as probe. The gel shown in C was analyzed by Southern blotting with p32-labeled cDNA of the hygromycin resistance gene as described (21).

Fig. 7. Proposed multiple functions of BRCA1 in DNA double strand break repair. A, BRCA1 is important in homology-based repair and microhomology-mediated end joining, possibly through its direct interaction with the Rad50-Mre11-Nbs1 complex (R/M/N), and/or indirect association with Rad51, and/or other unknown factors (X). BRCA1 may also function in the suppression of nonhomologous end joining through Rad50-Mre11-Nbs1 or other proteins. B, BRCA1 may bind to a microhomology-paired double-stranded DNA to stabilize it transiently before recruiting other repair factors involved in further processing.

DISCUSSION

BRCA1 plays an essential role in DNA DSB repair either through HR or NHEJ. The results described above suggest that Brca1 may have an important role in NHEJ. Using a plasmid end-joining assay, it was shown that Brca1 plays a moderate role in precise end joining rather than in overall NHEJ. Brca1 was also found to promote efficient retroviral infection, likely reflecting a role in retroviral DNA end-joining activity. Furthermore, repair of a defined chromosomal DSB mediated by microhomology annealing is severely impaired in Brca1-deficient MEFs. Taken together, these observations suggest that BRCA1 has a critical role in microhomology-mediated end joining rather than in overall nonhomologous end-joining activity. These results are consistent with the recent observation that cell extracts derived from Brca1-deficient MEFs significantly reduced end-joining activity with 4-bp homology in vitro (34).

Mechanistically speaking, NHEJ can proceed through distinct subpathways determined by the nucleotide sequence immediately surrounding the break site. Often, NHEJ generates junctions with sequence homologies consisting of only a few nucleotides. DNA ends can be joined either precisely, without nucleotide loss through sequence homology at the DNA termini, or imprecisely, through nucleotide deletion or addition, to generate microhomologies flanking the break site. Alternatively, broken DNA ends can be joined without microhomology (15). In an assay for gross chromosomal rearrangements (GCRs), the rate of non-homology-mediated GCRs was increased ~600-fold in yeast rad50, mrx11, and xrs2 mutants (35), suggesting that the Rad50-Mre11-Xrs2 complex appears to be important in suppressing non-homology-mediated end-joining processes. Previous work has demonstrated that BRCA1
physically interacts with the Rad50-Mre11-Nbs1 complex in vivo and in vitro (14). Furthermore, BRCA1 can be isolated from cells in a high molecular complex with Rad50-Mre11-Nbs1 (36). These observations suggest that the function of BRCA1 in DSB repair is mediated, at least in part, through its association with the Rad50-Mre11-Nbs1 complex (Fig. 7A), although the possibility exists that BRCA1 functions through other associated repair component such as FANC2D (37, 38).

The function of the mammalian Rad50-Mre11-Nbs1 complex in DSB repair is not entirely known at present. However, substantial evidence has suggested a functional conservation between the mammalian Rad50-Mre11-Nbs1 and yeast Rad50-Mre11-Nbs1 complex (Fig. 7B). The Rad50-Mre11-Nbs1 complex contains two conserved DNA repair enzymes, ligase IV and Xrcc4, which are critical for DNA repair in mammalian cells (14). Furthermore, BRCA1 can be isolated from cells in a high molecular complex with Rad50-Mre11-Nbs1 (36). These observations suggest that the function of BRCA1 in DSB repair is mediated, at least in part, through its association with the Rad50-Mre11-Nbs1 complex (Fig. 7A), although the possibility exists that BRCA1 functions through other associated repair component such as FANC2D (37, 38).

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