Journal of Cellular Biochemistry 96:47–55 (2005)

Dynamics of DNA Repair Suggested by the Subcellular Localization of Brca1 and Brca2 Proteins

L. Julie Huber and Lewis A. Chodosh*

Department of Cancer Biology, Department of Cell and Developmental Biology, Department of Medicine, Abramson Family Cancer Research Institute, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6160

Abstract The localization of proteins to specific subcellular compartments often reveals clues regarding their biological functions. Although significant progress has been made towards understanding how damaged DNA is repaired, experiments to date have primarily focused on signal transduction pathways that activate DNA repair protein complexes and on how these complexes are assembled. Current evidence suggests that certain DNA repair processes are spatially organized such that aberrant DNA structures can be brought into proximity with DNA repair proteins at fixed sites. Since biochemical evidence suggests that the tumor suppressor proteins, Brca1 and Brca2, may mediate the assembly of protein complexes involved in the repair of damaged DNA, we have performed subcellular fractionation experiments to determine the subnuclear localization of these proteins. The majority of Brca1 and Brca2 proteins were found to interact tightly with the nuclear matrix. Furthermore, within the limits of detection, localization of Brca1 and Brca2 to the nuclear matrix was not altered following treatment of cells with DNA damaging agents that activate homology-mediated double-stranded DNA break and transcription-coupled repair pathways. Our findings suggest that Brca1 and Brca2 may perform their DNA repair-related functions from positions that are anchored to the nuclear matrix. These data are consistent with proposed models that suggest that components of specific repair complexes residing on the nuclear matrix function to recruit damaged DNA. J. Cell. Biochem. 96: 47–55, 2005.

Key words: Brca1; Brca2; nuclear matrix localization; DNA Repair

The Brca1 and Brca2 tumor suppressor proteins play a functional role in DNA damage repair pathways including the homology-directed repair of double-stranded DNA breaks and transcription-coupled repair [Venkitaraman, 2002]. Brca1 also interacts with proteins associated with transcription, chromatin remodeling, mRNA processing, and cell cycle checkpoint control [Venkitaraman, 2002]. In addition, Brca1 has been shown to bind directly to specific DNA structures [Paull et al., 2001]. These findings implicate Brca1 in the activities associated with DNA repair protein complexes as well as cellular processes that are dependent on intact DNA repair pathways.

During the S-phase of the cell cycle, both Brca1 and Brca2 colocalize to discrete nuclear foci with the strand transferase Rad51 [Scully et al., 1997]. In addition, Brca1 and Brca2 colocalize with proteins involved in replication-associated repair, including PCNA and RPA, following treatment of cells with hydroxyurea or ionizing radiation [Scully et al., 1997; Wang et al., 2000; Choudhary and Li, 2002]. Moreover, DNA damage-dependent Rad51 focus formation is impaired in cells harboring either mutant Brca1 or Brca2 [Yuan et al., 1999; Bhattacharyya et al., 2000; Huber et al., 2001]. These findings are consistent with the fact that proteins involved in homologous recombination also appear to contribute to the resolution of aberrant replication forks [Kuzminov, 1999; Marians, 2000; Michel, 2000].

Grant sponsor: NIH; Grant number: CA78410; Grant sponsor: U.S. Army Breast Cancer Research Program (to LJH); Grant numbers: DAMD17-03-1-0275, DAMD17-98-1-8230.

L. Julie Huber's present address is Elixir Pharmaceuticals, Inc., One Kendall Square Bldg.1000, Cambridge, MA 02139.

*Correspondence to: Lewis A. Chodosh, 612 BRB II/III, 421 Curie Boulevard, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6160.
E-mail: chodosh@mail.med.upenn.edu

Received 10 October 2004; Accepted 7 March 2005

DOI 10.1002/jcb.20517

During the replication process, DNA has been shown to move through discrete structures that are anchored in the nucleus [Hozak et al., 1993]. Though attached, these structures change in a dynamic manner and assemble and disassemble throughout S-phase [Leonhardt et al., 2000]. These findings prompted us to consider the possibility that the subnuclear localization of Brca1 and Brca2 may provide clues as to how specific repair processes are organized. Accordingly, we have determined the localization of Brca1 and Brca2 in cell extracts fractionated into soluble, chromatin, and nuclear matrix compartments. Our results demonstrate that the majority of Brca1 and Brca2 protein exists in stable association with the nuclear matrix. We also demonstrate that the nuclear matrix association of Brca1 and Brca2 does not change following various genotoxic insults known to stimulate the activities of these proteins.

MATERIALS AND METHODS

Cell Culture

HC11 cells were grown in RPMI medium containing 10% bovine calf serum, 5 μg/ml insulin (Sigma, St. Louis, MO), 10 ng/ml epidermal growth factor (Sigma), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. HeLa cells were grown in DMEM media containing 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco-BRL, Rockville, MD).

Treatment With DNA Damaging Agents

Gamma irradiation was administered using a CIS bio international (IBL 437c) source. Ultraviolet (UV) doses were administered using a Stratalinker (Stratagene, La Jolla, CA). Hydroxyurea (HU; Sigma) was used at a final concentration of 1 mM.

Cellular Fractionation

Fractionation methods included a high salt method, preparation by amine modification, and a low-salt method [Mirkovitch et al., 1984; Reyes et al., 1997; Wan et al., 1999]. High salt method: cells were washed in phosphate buffered saline (PBS) and extracted in cytoskeleton buffer (CSK): 10 mM Pipes, pH 6.8, 100 mM NaCl, 300 mM sucrose, 3 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 0.5% Triton X-100 containing 100 μg/ml Pefabloc (Roche, Indianapolis, IN), 20 μg/ml aprotonin, 10 μg/ml leupeptin, 0.1 mM β-glycerophosphate, 50 mM NaF, and 1 mM sodium orthovanadate. After 5 min of incubation at 4°C, the insoluble fraction was obtained by centrifugation at 5,000*g* for 5 min and the supernatant (soluble fraction) was removed. In order to solubilize the chromatin, RNAse-free DNAse I (Roche) was added at a final concentration of 1 mg/ml in CSK buffer with protease and phosphatase inhibitors and extracts were incubated at 37°C for 15 min. Ammonium sulfate was added at a final concentration of 0.25M and samples were incubated for 5 min at 4°C followed by centrifugation at 5,000*g* for 5 min. The pellet was further extracted with 2M NaCl in CSK buffer. The combined supernatants were considered the chromatin fraction. Urea buffer was added to solubilize the final pellet (nuclear matrix fraction).

Amine modification method: cells were washed in PBS and extracted in CSK buffer. Following a 5 min incubation at 4°C, the insoluble fraction was separated from the soluble fraction by centrifugation at 5,000*g* for 5 min. The supernatant was removed and labeled the soluble fraction. In order to solubilize chromatin, RNase free-DNase I (Roche) was added at a final concentration of 1 mg/ml in CSK buffer with protease and phosphatase inhibitors and samples were incubated at 37°C for 15 min. Samples were then exposed to 2 mg/ml of sulfo-*N*-hydroxysulfosuccinimide acetate (sulfo-NHS, Pierce) for 20 min at room temperature. This step was repeated and cells were then washed with 10 mM glycine in CSK buffer. Urea buffer was added to the final pellet.

Low-salt method: nuclei were obtained using NE-PER (Pierce, Rockford, IL) and stabilized in isolation buffer: 3.75 mM Tris-HCl, pH 7.4, 0.05 mM spermine, 0.125 mM spermidine, 1% (v/v) thiodyglycol, 20 mM KCl, 0.1% digitonin and incubated for 20 min at 37°C. RNase-free DNase I was added at final concentration of 1 mg/ml and samples were incubated for 15 min at 37°C. Samples were then extracted with LIS buffer containing 5 mM HEPES/NaOH, pH 7.4, 0.25 mM spermidine, 2 mM EDTA/KOH, pH 7.4, 2 mM KCl, 0.1% digitonin, 25 mM 3,5-diiodosalicylic acid for 5 min at room temperature. Pellets were resuspended in urea buffer.

To confirm the extent of chromatin removal, cells were labeled with 10 μCi/ml methyl [^{3}H]

thymidine (NEN) for 24 h prior to fractionation. Trichloroacetic acid precipitated samples from each fraction were analyzed for radioactivity. Extraction buffers contained 100 μg/ml Pefabloc, 20 μg/ml Aprotonin, 10 μg/ml leupeptin, 0.1 mM β-glycerophosphate, 50 mM sodium fluoride, and 1 mM sodium orthovanadate. Cells grown on chamber slides for in situ fractionation were treated in an identical manner as cell pellets. To confirm the removal of chromatin from cells fractionated in situ preparations were stained with 4′,6-diamidino-2-phenylindole (DAPI). Samples were processed for immunoflourescence according to published protocols [Scully et al., 1997]. Affinity purified Brca1 antibody (mAb1) was used at a 1:1,000 dilution. Rad51 antibody Ab-1 (Oncogene Science, La Jolla, CA), and Mre11 antibody (Novus Biologicals, Littleton, CO), were used at a 1:1,000 dilution. Tetramethyl rhodamine (TRITC) was used at a dilution of 1:250. Images were obtained by laser scanning confocal microscopy.

Immunoblotting

Laemmli sample buffer (LSB) was added to the soluble and chromatin fractions. Insoluble fractions were resuspended in 8M urea. Equal fraction volumes were subjected to SDS–PAGE electrophoresis. Transfer to nitrocellulose was performed in buffer containing 192 mM glycine, 25 mM Tris base, and 20% methanol. Membranes were blocked for 1 h in phosphate buffered saline containing 5% nonfat dried milk and 0.5% NP-40. Primary antibodies were used at the following dilutions: Brca1 antibody (mAb1; [Huber et al., 2001]) 1:1,000, Brca2 (Ab1; [Sarkisian et al., 2001]) 1:1000, Lamin B (Santa Cruz Biotechnology, Santa Cruz, CA) 1:1,000, Histone H3 (Upstate Biotechnology, Charlottesville, VA), Rb (BD Biosciences Pharmingen, San Diego, CA), BRG1 1:2,000. Peroxidase conjugate goat anti-rabbit IgG and goat anti-mouse IgG secondary antibodies (H + L), (Jackson Immunoresearch, West Grove, PA) were used at a 1:3,000 dilution.

RESULTS

Brca1 and Brca2 Localize to the Nuclear Matrix

Although the cellular localization of Brca1 has been controversial, it is currently accepted that both Brca1 and Brca2 are localized exclusively within the nuclear compartment. To determine the subnuclear localization of Brca1 and Brca2, exponentially growing HC11 cells were subjected to nuclear fractionation by amine modification. Fractions were analyzed by immunoblotting for proteins that localize to specific subnuclear compartments in order to verify the success of the fractionation procedure. As expected, Lamin B localized exclusively to fractions containing the nuclear matrix (Fig. 1). As a marker of the chromatin fraction we employed antibodies directed against Histone H3, since histones can be released from their tight association with chromatin by digestion with restriction enzymes or DNase I and extraction with high salt. Immunoblotting revealed that most, if not all, Histone H3

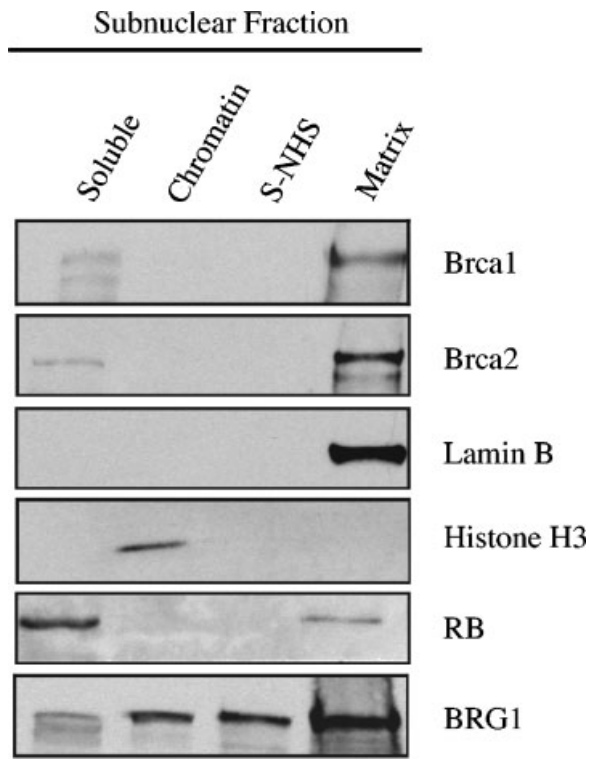

Fig. 1. Brca1 and Brca2 localize predominantly to the nuclear matrix. Nuclear extracts from exponentially growing HC11 cells were subjected to fractionation by amine modification to yield soluble, chromatin, sulfo-NHS (S-NHS), and nuclear matrix fractions. Digested chromatin, including DNA and histones, readily elutes under physiological salt conditions after sulfo-NHS treatment [Wan et al., 1999]. Equal fraction volumes were subjected to immunoblotting with the indicated antibodies. Similar results were obtained using human cells including HeLa cell as well as MCF7 cells transfected with myc-tagged human BRCA1 (data not shown).

cofractionated with chromatin (Fig. 1). Consistent with this result, experiments in which [^{3}H] thymidine-labeled cells were fractionated in an identical manner demonstrated the segregation of greater than 95% of label in the chromatin fraction (data not shown).

The localization of Histone H3 and Lamin B to the chromatin and nuclear matrix fractions, respectively, was observed using high-salt (2M NaCl) elution, low-salt (LIS) elution, and amine modification fractionation protocols (Figs. 1 and 2 and data not shown). Fractionation by amine modification, which involves treatment with sulfo-NHS, has been shown by high resolution electron microscopy to preserve the ultrastructure of the nuclear matrix, presumably due to the fact that digested chromatin readily elutes under physiological salt conditions following sulfo-NHS treatment. Fractionation by amine modification otherwise yields results similar to those obtained by other methods [Wan et al., 1999]. Notably, some differences in protein content have been reported for nuclear matrix fractions generated using amine modification as compared to high-salt elution [Wan et al., 1999]. Consequently, it has been suggested that in addition to removing residual chromatin and its associated proteins, high-salt elution may also disrupt the interactions of a subset of matrix bound proteins.

Previous experiments have determined that the retinoblastoma protein (Rb) is present in the soluble fraction in its hyperphosphorylated state, whereas the majority of hypophosphorylated Rb, as is found during the G_1 phase of the cell cycle, is associated with the nuclear lamina or nuclear matrix [Mancini et al., 1994]. Consistent with this, immunoblotting analysis of Rb in fractionated extracts derived from exponentially growing HC11 cells indicated that the majority of Rb is present in the soluble fraction (Figs. 1 and 3).

Components of the SWI/SNF chromatin-remodeling complex, such as BRG1, have been shown to be associated both with active chromatin and the nuclear matrix [Reyes et al., 1997]. In addition to these associations, we detected a small amount of soluble nuclear BRG1 (Fig. 1). This may represent the pool of BRG1 derived from mitotic cells since the SWI/SNF complex is excluded from condensed chromatin during this phase of the cell cycle [Sif et al., 1998]. We also found that a subset of BRG1 molecules were released from the nuclear matrix following elution with amine modification or high-salt suggesting that a subset of BRG1 molecules are bound to residual chromatin or are loosely associated with the nuclear matrix (Fig. 1).

Having confirmed the fractionation of the soluble, chromatin, and matrix components of the nuclear compartment, we wished to analyze the subnuclear localization of Brca1 and Brca2. Immunoblotting of fractionated extracts revealed that the majority of Brca1 is associated with the nuclear matrix (Fig. 1). The remaining fraction of Brca1 protein in the nucleus was found to be present in a soluble form. Since

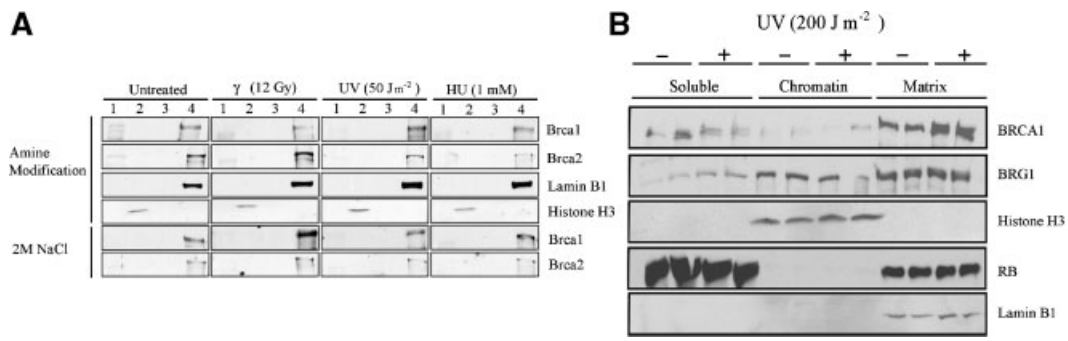

Fig. 2. Brca1 and Brca2 remain associated with the nuclear matrix following genotoxic insult. **A**: Extracts derived from exponentially growing HC11 cells subjected to fractionation by amine modification (**upper panels**) and high-salt methods (**lower panels**) were probed with antibodies listed on the right-hand side of the figure. Doses of DNA damaging agents are indicated. **Lanes 1–4** represent the soluble, chromatin, wash, and matrix fractions, respectively. Samples shown were harvested at 3 h following treatment with DNA damaging agents, however samples harvested at 1 or 6 h following treatment gave similar results (data not shown). **B**: HeLa cells subjected to 200 J m^{-2} UV were fractionated using the high-salt method as described above and were subjected to immunoblotting. The appearance of the additional band in the UV treated nuclear matrix fraction is due to the presence of phosphorylated BRCA1. Each treatment/fractionation condition is shown in duplicate.

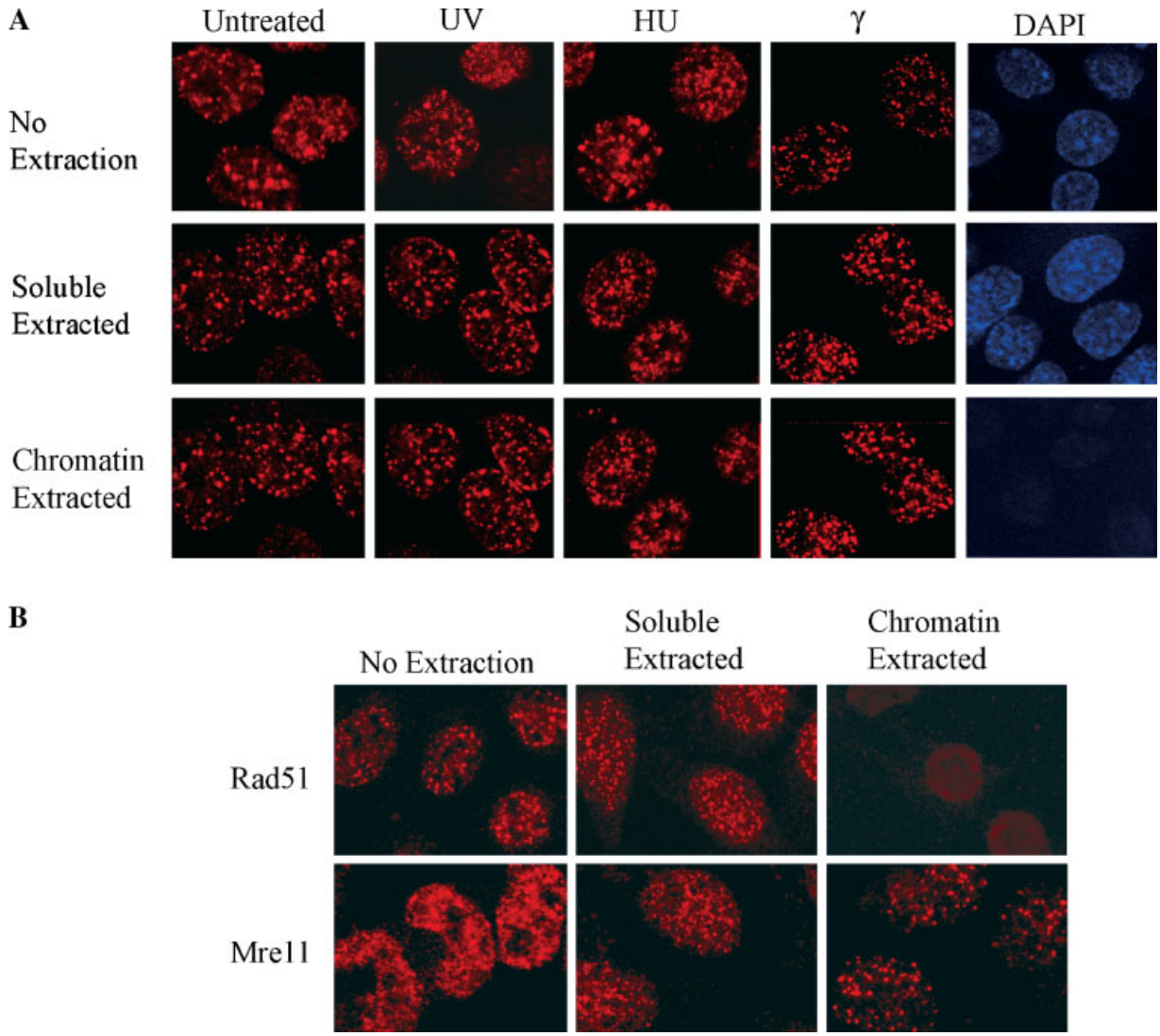

Fig. 3. Brca1 foci localize to the nuclear matrix. **A**: Exponentially growing HC11 cells grown in chamber slides were subjected to fractionation by amine modification followed by indirect immunofluorescence analysis using a murine Brca1 antibody as described in ''Materials and Methods.'' Extraction procedures were initiated 1 h following treatment with 50 J m^{-2} UV, 1 mM HU, or 10 Gy γ-irradiation. Staining with DAPI was used to confirm successful removal of chromatin. **B**: Exponentially growing HC11 cells grown in chamber slides were subjected to fractionation followed by immunofluorescence analysis using Rad51 and Mre11 antibodies as described in ''Materials and Methods.'' Extraction procedures were initiated 3 h following treatment with 12 Gy γ-irradiation.

BRG1 associates with human BRCA1, it is possible that Brca1, like BRG1, is excluded from condensed chromatin during mitosis. Alternatively, although Brca1 localizes predominantly to nuclear foci, the subset of Brca1 molecules that is detected in the soluble fraction may represent protein that is localized in a diffuse pattern throughout the nucleus. Further experiments in which cells are fractionated during the phases of the cell cycle may distinguish between these possibilities. Lastly, immunoblotting of Brca2 revealed a similar fractionation pattern to Brca1: the majority of Brca2 was found to be associated with the nuclear matrix fraction whereas a smaller amount was present in a soluble form (Fig. 1).

Brca1 and Brca2 Remain Associated With the Nuclear Matrix Following DNA Damage

Specific DNA repair proteins have been shown to bind more tightly to damaged compared to intact DNA. Brca1 is phosphorylated in

response to DNA damage, relocalizes to nuclear foci along with Brca2, and is the target of several checkpoint kinases [Gately et al., 1998]. As such, we considered the possibility that the subnuclear compartmentalization of Brca1 might change following treatment with DNA damaging agents that induce its phosphorylation. To address this possibility, we performed a comparison of fractionated nuclear extracts derived from untreated HC11 cells and cells treated with 12 Gy-irradiation, 1 mM hydroxyurea, or 50 J m^{-2} ultraviolet light. Analysis of subnuclear fractions prepared both by amine modification and high salt methods revealed that the majority of Brca1 remains tightly associated with the nuclear matrix following DNA damage (Fig. 2A). Similar to Brca1, the subnuclear distribution of Brca2 was not altered following γ-irradiation, exposure to UV or treatment with HU when examined by either fractionation procedure (Fig. 2A).

To confirm and extend these findings, we examined the subnuclear localization of human BRCA1 in HeLa cells treated with 200 J m^{-2} UV radiation using the high-salt elution method. This analysis demonstrated that, similar to murine Brca1, the bulk of human BRCA1 protein is associated with the nuclear matrix, although a fraction of BRCA1 protein was found associated with chromatin (Fig. 2B). As demonstrated for murine Brca1, the subnuclear localization of human BRCA1 was not significantly altered following treatment with DNA-damaging agents (Fig. 2B and data not shown).

Brca1 Foci Are Associated With the Nuclear Matrix

Brca1 has previously been demonstrated by indirect immunofluorescence to reside in nuclear foci in S-phase cells and to relocalize to distinct nuclear foci following DNA damage [Scully et al., 1997]. Therefore, we wished to determine the relationship between these foci and the biochemical fractionation methods employed in this study. Cells were grown in chamber slides and were subjected to biochemical fractionation procedures as described in "Materials and Methods" to sequentially extract soluble and chromatin fractions in situ. Following each fractionation step, in situ preparations were fixed for 10 min in 4% paraformaldehyde prior to being processed for immunofluorescence analysis. DAPI staining confirmed the successful removal of chromatin from cells. HC11 samples from which soluble and chromatin fractions had been extracted were probed with antibodies to murine Brca1. This analysis revealed discrete Brca1 nuclear foci in untreated cells that remained essentially unchanged following extraction of soluble nuclear components as well as following extraction of chromatin (Fig. 3A). These observations strongly suggest that Brca1 nuclear foci are tightly associated with the nuclear matrix. Furthermore, these patterns of immunofluorescence and nuclear foci did not vary significantly in cells treated with a variety of genotoxic insults, including UV, HU, and IR. These findings suggest that Brca1 foci remain associated with the nuclear matrix following treatment with DNA damaging agents.

Interestingly, we observed that following irradiation Rad51 nuclear foci are associated predominantly with the chromatin fraction rather than the nuclear matrix in irradiated cells. In contrast, only a subset of Mre11 foci was associated with the nuclear matrix (Fig. 3B). These results suggest that DNA repair proteins that function in specific repair pathways may operate within distinct nuclear compartments.

DISCUSSION

We have demonstrated that the majority of Brca1 and Brca2 protein in epithelial cells is tightly associated with the nuclear matrix. In addition, treatment of cells with agents known to generate DNA lesions that are repaired by Brca1 and Brca2-dependent pathways does not result in detectable changes in the subnuclear localization of either of these proteins. Notably, although phosphorylation of Brca1 is required for DNA repair, our results indicate that phosphorylation of Brca1 is not accompanied by movement of Brca1 or Brca2 from the nuclear matrix into the soluble or chromatin nuclear fractions. However, our results do not rule out the possibility that Brca1 or Brca2 may translocate from one position to another within this compartment, as has been observed for Mre11 following DNA damage [Nelms et al., 1998]. In addition, we can not exclude the possibility that a minor fraction of the total Brca1 and Brca2 protein redistributes following genotoxic insult.

Importantly, Brca1-interacting proteins do not necessarily undergo changes in compartmentalization in response to DNA damage. For example, the distribution of the ATM kinase within nuclear matrix and chromatin fractions does not change following exposure to ionizing radiation [Gately et al., 1998]. In addition, proteins that physically interact with Brca1 either directly or indirectly may localize to a compartment other than the nuclear matrix. This is suggested by our fractionation experiments in which the Rad51 protein, which physically interacts with Brca1 and Brca2, was found to elute with the chromatin fraction. This may be relevant to the variability in subunit composition of Brca1-associated complexes that have been reported to date. It may be particularly difficult to extract intact complexes containing Brca bound to soluble or chromatin-bound proteins; Brca1 may remain associated with the nuclear matrix whereas other components of these complexes may be more readily solubilized. Importantly, in human cells we detect a fraction of the total BRCA1 protein in the soluble and chromatin fractions and in the murine HC11 cell line a relatively small amount in the soluble fraction. We speculate that this readily extracted protein represents the fraction of BRCA1 measured using standard extraction and immunoblotting procedures.

It has previously been demonstrated that damaged DNA is recruited to the nuclear matrix [Koehler and Hanawalt, 1996]. Moreover, cells that are defective in transcription-coupled repair (TCR) exhibit a decreased ability to recruit DNA damaged by UV to the nuclear matrix [Mullenders et al., 1988]. Consistent with these results, proteins involved in excision repair have been found in the nuclear matrix including Cockayne's syndrome A (CSA), which rapidly translocates to the matrix following UV irradiation [Kamiuchi et al., 2002]. These data are consistent with the idea that components of specific repair complexes residing on the nuclear matrix function to recruit damaged DNA to sites within the matrix. Brca1 is specifically implicated in TCR and it will be informative to determine if the nuclear matrix translocation of proteins involved in this repair pathway is compromised in Brca1-deficient cells.

Another link between Brca1 and Brca2 and the concept of a protein scaffold that recruits damaged DNA to the nuclear matrix is the occurrence of BRCA1 and BRCA2 nuclear foci during the S-phase of the cell cycle. These foci have been suggested to reflect the presence of stalled replication forks, which are recognized as aberrant DNA structures by specific repair proteins. Replicating DNA has been shown to move through replication complexes that are stably anchored in the nucleus. Consistent with these data is the possibility that replication complexes that encounter stalled or collapsed replication forks contain DNA repair proteins such as BRCA1 or BRCA2. This idea is supported by the finding that the replication protein PCNA colocalizes with BRCA1 and BRCA2 following genotoxic insult during the S-phase of the cell cycle.

We propose a model in which BRCA1 and BRCA2, when present in damaged-provoked replication foci, aid in the recruitment and positioning of proteins involved in repair by bringing appropriate regions of DNA into proximity. Consistent with this model, several proteins that physically interact with Brca1 undergo changes in subnuclear compartmentalization following DNA damage. For example, the Bloom's syndrome helicase (BLM) changes its localization from an NP-40 soluble to an NP-40 insoluble fraction following treatment of cells with ionizing radiation [Dutertre et al., 2002]. BLM has been shown to associate with the nuclear matrix, interact with RAD51, and colocalize with RAD51 foci [Bischof et al., 2001]. Similarly, treatment of cells with mitomycin C results in enhanced association of Fanconi anemia proteins with nuclear matrix and chromatin fractions [Qiao et al., 2001]. BRCA1 is required for DNA damage-induced foci formation of FANCD2 and, interestingly, is impaired in Fanconi anemia primary fibroblasts, as has been observed in *Brca1*$^{-/-}$ cells, Rad51 foci formation [Garcia-Higuera et al., 2001; Digweed et al., 2002]. Thus, it is possible that nuclear matrix-associated Brca1 may serve as a scaffold to facilitate the recruitment or assembly of repair-associated protein complexes such as those proposed here as well as the BRCA1-associated genome surveillance complex (BASC) [Wang et al., 2000].

Interestingly, the expression profiles of nuclear matrix proteins are reported to be altered in human breast cancer [Spencer et al., 2001]. In addition to determining if Brca1 or Brca2 are required for the appropriate

localization of known interacting proteins, it will be informative to determine if loss of function of these tumor suppressors leads to aberrant expression profiles of nuclear matrix proteins in human breast cancers.

ACKNOWLEDGMENTS

The authors thank members of the Chodosh laboratory for helpful discussions, critical reading of the article, and reagents including anti-Brca2 antibody, which was kindly provided by Christopher Sarkisian. BRG1 antibody was kindly provided by Dr. Weidong Wang (NIH). Confocal microscopy was made possible by the help of Irina Chernysh of the Biomedical Imaging Core Facility at the University of Pennsylvania Medical Center.

REFERENCES

Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR, Bishop DK. 2000. The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. J Biol Chem 275:23899–23903.

Bischof O, Kim SH, Irving J, Beresten S, Ellis NA, Campisi J. 2001. Regulation and localization of the Bloom syndrome protein in response to DNA damage. J Cell Biol 153:367–380.

Choudhary SK, Li R. 2002. BRCA1 modulates ionizing radiation-induced nuclear focus formation by the replication protein A p34 subunit. J Cell Biochem 84:666–674.

Digweed M, Rothe S, Demuth I, Scholz R, Schindler D, Stumm M, Grompe M, Jordan A, Sperling K. 2002. Attenuation of the formation of DNA-repair foci containing RAD51 in Fanconi anaemia. Carcinogenesis 23: 1121–1126.

Dutertre S, Sekhri R, Tintignac LA, Onclercq-Delic R, Chatton B, Jaulin C, Amor-Gueret M. 2002. Dephosphorylation and subcellular compartment change of the mitotic Bloom's syndrome DNA helicase in response to ionizing radiation. J Biol Chem 277:6280–6286.

Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, Grompe M, D'Andrea AD. 2001. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. Mol Cell 7:249–262.

Gately DP, Hittle JC, Chan GK, Yen TJ. 1998. Characterization of ATM expression, localization, and associated DNA-dependent protein kinase activity. Mol Biol Cell 9: 2361–2374.

Hozak P, Hassan AB, Jackson DA, Cook PR. 1993. Visualization of replication factories attached to nucleoskeleton. Cell 73:361–373.

Huber LJ, Yang TW, Sarkisian CJ, Master SR, Deng CX, Chodosh LA. 2001. Impaired DNA damage response in cells expressing an exon 11-deleted murine Brca1 variant that localizes to nuclear foci. Mol Cell Biol 21:4005–4015.

Kamiuchi S, Saijo M, Citterio E, de Jager M, Hoeijmakers JH, Tanaka K. 2002. Translocation of Cockayne syndrome group A protein to the nuclear matrix: Possible relevance to transcription-coupled DNA repair. Proc Natl Acad Sci USA 99:201–206.

Koehler DR, Hanawalt PC. 1996. Recruitment of damaged DNA to the nuclear matrix in hamster cells following ultraviolet irradiation. Nucleic Acids Res 24:2877–2884.

Kuzminov A. 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage lambda. Microbiol Mol Biol Rev 63:751–813.

Leonhardt H, Rahn HP, Weinzierl P, Sporbert A, Cremer T, Zink D, Cardoso MC. 2000. Dynamics of DNA replication factories in living cells. J Cell Biol 149:271–280.

Mancini MA, Shan B, Nickerson JA, Penman S, Lee WH. 1994. The retinoblastoma gene product is a cell cycle-dependent, nuclear matrix-associated protein. Proc Natl Acad Sci USA 91:418–422.

Marians KJ. 2000. Replication and recombination intersect. Curr Opin Genet Dev 10:151–156.

Michel B. 2000. Replication fork arrest and DNA recombination. Trends Biochem Sci 25:173–178.

Mirkovitch J, Mirault ME, Laemmli UK. 1984. Organization of the higher-order chromatin loop: Specific DNA attachment sites on nuclear scaffold. Cell 39:223–232.

Mullenders LH, van Kesteren van Leeuwen AC, van Zeeland AA, Natarajan AT. 1988. Nuclear matrix associated DNA is preferentially repaired in normal human fibroblasts, exposed to a low dose of ultraviolet light but not in Cockayne's syndrome fibroblasts. Nucleic Acids Res 16:10607–10622.

Nelms BE, Maser RS, MacKay JF, Lagally MG, Petrini JH. 1998. In situ visualization of DNA double-strand break repair in human fibroblasts. Science 280:590–592.

Paull TT, Cortez D, Bowers B, Elledge SJ, Gellert M. 2001. Direct DNA binding by Brca1. Proc Natl Acad Sci USA 98:6086–6091.

Qiao F, Moss A, Kupfer GM. 2001. Fanconi anemia proteins localize to chromatin and the nuclear matrix in a DNA damage- and cell cycle-regulated manner. J Biol Chem 276:23391–23396.

Reyes JC, Muchardt C, Yaniv M. 1997. Components of the human SWI/SNF complex are enriched in active chromatin and are associated with the nuclear matrix. J Cell Biol 137:263–274.

Sarkisian CJ, Master SR, Huber LJ, HA SI, Chodosh LA. 2001 Analysis of murine Brca2 reveals conservation of protein-protein interactions but differences in nuclear localization signals. J Biol Chem 278:37640–37648.

Scully R, Chen J, Ochs RL, Keegan K, Hoekstra M, Feunteun J, Livingston DM. 1997. Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. Cell 90:425–435.

Sif S, Stukenberg PT, Kirschner MW, Kingston RE. 1998. Mitotic inactivation of a human SWI/SNF chromatin remodeling complex. Genes Dev 12:2842–2851.

Spencer VA, Samuel SK, Davie JR. 2001. Altered profiles in nuclear matrix proteins associated with DNA in situ during progression of breast cancer cells. Cancer Res 61: 1362–1366.

Venkitaraman AR. 2002. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell 108:171–182.

Wan KM, Nickerson JA, Krockmalnic G, Penman S. 1999. The nuclear matrix prepared by amine modification. Proc Natl Acad Sci USA 96:933–938.

Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J. 2000. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 14:927–939.

Yuan SS, Lee SY, Chen G, Song M, Tomlinson GE, Lee EY. 1999. BRCA2 is required for ionizing radiation-induced assembly of Rad51 complex in vivo. Cancer Res 59:3547–3551.