RINT-1, a Novel Rad50-interacting Protein, Participates in Radiation-induced G₂/M Checkpoint Control*

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Rad50, an structural maintenance of chromosomes (SMC) protein family member, participates in a variety of cellular processes, including DNA double-strand break repair, cell cycle checkpoint activation, telomere maintenance, and meiosis. Disruption of Rad50 in mice leads to lethality during early embryogenesis, indicating its essential function in normal proliferating cells. In addition to its ability to form a complex with the DNA double-strand break repair proteins Mre11 and NBS1, Rad50 may interact with other cellular proteins to execute its full range of biological activities. A novel 87-kDa protein named RINT-1 was identified using the C-terminal region of human Rad50 as the bait in a yeast two-hybrid screen. Human RINT-1 shares sequence homology with a novel protein identified in Drosophila melanogaster, including a coiled-coil domain within its N-terminal 150 amino acids, a conserved central domain of about 350 amino acids, and a C-terminal region of 90 amino acids exhibiting 35–38% identity. The conserved central and C-terminal regions of RINT-1 are required for its interaction with Rad50. While Rad50 and RINT-1 are both expressed throughout the cell cycle, RINT-1 specifically binds to Rad50 only during late S and G₂/M phases, suggesting that RINT-1 may be involved in cell cycle regulation. Consistent with this possibility, MCF-7 cells expressing an N-terminally truncated RINT-1 protein displayed a defective radiation-induced G₂/M checkpoint. These results suggest that RINT-1 may play a role in the regulation of cell cycle control after DNA damage.

The RAD50 gene was first identified in Saccharomyces cerevisiae based on its role in DNA repair (1). It is a member of the RAD52 epistasis group, members of which function in recombinational DNA repair. Yeast strains carrying rad50 mutations exhibit hypersensitivity to γ-irradiation and radiomimetic chemicals such as methyl methanesulfonate and only slight sensitivity to UV irradiation (1). In addition, rad50 mutations also lead to telomere shortening and meiotic failure (1). The 153-kDa Rad50 protein contains both an N-terminal Walker A and an C-terminal Walker B NTP binding domains, linked by two extensive coiled-coil regions that are characterized by leucine heptad repeats (2). This sequence architecture is similar to that found in other members of the structural maintenance of chromosomes (SMC) protein family (3, 4). The first 35 heptad repeats span amino acids 177–421 of the Rad50 sequence, whereas the second 37 heptad repeats span amino acids 743–995. These heptad repeat sequences exhibit length and significant sequence similarity to the S2 domain of rabbit myosin (2). Mutations within the Rad50 Walker A domain that alter conserved amino acids resides within the NTP binding site confer a null phenotype, and several mutations near the nucleotide binding sites cause defects in meiosis (5).

The RAD50 gene product is highly conserved among species. Human and yeast Rad50 share more than 50% sequence identity within their corresponding N- and C-terminal regions as well as a similar coiled-coil structure in between. A null mutation of the mouse Rad50 gene is associated with an embryonic lethal phenotype, and cells derived from early embryos are hypersiveitiv to ionizing radiation, suggesting that Rad50 is essential for normal cell proliferation and DNA double-strand break repair (6). Yeast Rad50 forms a complex with Mre11 and Xrs2 (7). Similarly, human Rad50 forms a complex with hMre11 and NBS1, the potentially functional homologue of yeast Xrs2 (8, 9). It is believed that the Mre11-Rad50-NBS1 protein complex plays a central role in the cellular response to DNA damage. The NBS1 gene is mutated in Nijmegen Breakage syndrome (9, 10), which is phenotypically characterized by radiosensitivity and increased chromosomal instability upon ionizing radiation. Mre11 possesses DNA end-holding as well as endonucleolytic and exonucleolytic activities (11–15) that may be utilized during the initial steps of DNA double-strand break repair. Mouse embryonic stem cells deficient in Mre11 are not viable (16), suggesting that Mre11, like Rad50, is essential for normal proliferating cells and DNA double-strand break repair. Thus, while it is clear that these three proteins function as a triplex in DNA double-strand break repair, it is also very likely that these proteins function in association with other partners to have different biological functions.

To explore other potential functions of Rad50, we have identified a novel Rad50-interacting protein, named RINT-1. These two proteins specifically interact during S and M phases of the cell cycle. Expression in human breast cancer MCF7 cells of an N-terminally truncated RINT-1 protein that is capable of binding to Rad50 leads to failure of G₂/M, but not G₁/S, checkpoint control. These results suggest that RINT-1 may play a role in cell cycle checkpoint control.

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1 The abbreviations used are: SMC, structure maintenance of chromosomes; GFP, green fluorescence protein; BrdUrd, bromodeoxyuridine; Tet, tetracycline; GST, glutathione S-transferase; mAb, monoclonal antibody. NTP, nucleoside triphosphate.
Rint-1, a Novel Rad50-binding Protein

EXPERIMENTAL PROCEDURES

Cell Lines, Culture Conditions, and Synchronization—Cell lines including T24 and 5637 (human bladder carcinoma cell lines); Soas-2 (a human osteogenic sarcoma cell line); HeLa (a human cervical carcinoma line); MB231, ZR75, and T47D (human breast cancer lines); SW837 (a rectal carcinoma line); and GM09607A and GM09637G (cell lines with mutated and wild-type ataxia telangiectasia gene [ATM]), respectively (Coriell Cell Repositories) were cultured in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum. Molt-4, a lymphoblastic leukemia cell line, and LEM, a human B cell lymphoma line, were cultured in RPMI 1640 medium plus 10% fetal bovine serum and 5% CO2. MCF10A, a breast epithelial cell line, was cultured in Dulbecco's modified Eagle's medium/Ham's F-12 with 20 ng/ml epidermal growth factor, 100 ng/ml cholera toxin, 0.01 mg/ml insulin, 500 ng/ml hydrocortisone, and 5% horse serum. MCF10A-derived cell line, 5637, was added to the culture medium for 8 h prior to harvest as described (17).

To identify the cellular RINT-1 protein, about 1 × 106 cells were metabolically labeled with [35S]methionine for 3 h and lysed in ice-cold Lysis250 buffer (50 mM Tris-HCl, pH 7.4, 50 mM NaCl, 5 mM EDTA, 0.1% Nonidet P-40, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 μg/ml pepstatin) for 10 min at room temperature. The lysates were then incubated with an equal amount of glutathione-coupled galactocytoxanase assay as described (20).

Plasmid Construction and Expression of Fusion Proteins in Escherichia coli—Plasmids for the bacterial expression of several glutathione-S-transferase (GST) fusion proteins were constructed following standard protocols (22). GST-PIN and GST-PIC contain translational fusions of GST with amino acids 1–256 and 501–792 of RINT-1, respectively. For Rad50-GST fusion, a Rad50 plasmid pAS-R50PB (56) was used as the bait in a yeast two-hybrid screen of a human B-cell library. The plasmids identified in the screen were sequenced by the dideoxynucleotide termination method (21). For binding assays, three Rad50 deletion constructs were fused with the GST DNA-binding domain in frame, creating pAS-R50PB (amino acids 753–1312), pAS-R50PB-6 (amino acids 753–953), and pAS-R50PB1.2 (amino acids 953–1312). Various RINT-1 deletion mutants (RINT-H, amino acids 1–227; RINT-RV, amino acids 1–466; RINT-R, amino acids 1–706; RINT-PCR3, amino acids 1–550) were fused with the GST DNA-binding domain in frame, creating pAS-R50PB (amino acids 753–1312), pAS-R50PB-6 (amino acids 753–953), and pAS-R50PB1.2 (amino acids 953–1312) were constructed. Expression of the fusion proteins was induced by the addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 0.1 mM. Cell expression was quantified by measuring growing culture bacteria at 50°C. Bacteria were collected and lysed as described. Fusion proteins were purified by affinity chromatography using glutathione-coupled agarose beads (23).

In Vitro Binding Assays—For in vitro transcription and translation of RINT-1 protein, the cDNA that begins with the first ATG start codon (Fig. 1) was subcloned into pBSK and transcribed and translated in vitro using the TNT-coupled reticulocyte lysate system (Promega, Madison, WI). Glutathione-Sepharose beads containing about 5 μg of GST or GST fusion proteins were initially precipitated with Tris-buffered saline-bovine serum albumin buffer (25 mM Tris-HCl, pH 8.0, 120 mM NaCl, 10% bovine serum albumin, 1 μg/ml protease inhibitors including leupeptin, antipain, aprotinin, and pepstatin) for 10 min at room temperature. The beads were then incubated with an equal amount of in vitro translated products in lysis buffer (100 mM NaCl, 50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 0.5% Nonidet P-40, and the protease inhibitors listed above) for 1 h at room temperature with rotation. Complexes were washed extensively with the lysis buffer, resolved by SDS-polyacrylamide gel electrophoresis, and detected by fluorography.

Cell Lysate Preparation, Immunoprecipitation, and Immunoblotting—Cytoskeletal RINT-1 was prepared by labeling with [35S]methionine for 3 h and lysed in ice-cold Lysis250 buffer (50 mM Tris-HCl, pH 7.4, 250 mM NaCl, 5 mM EDTA, 0.1% Nonidet P-40, 50 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml aprotinin) (24). Equal amounts of clarified lysates (14,000 × g for 5 min) were incubated with anti-PIC antibodies at 4 °C for 1 h, protein A-Sepharose beads were added, and the mixture was incubated for another 1 h with constant rotation. Finally, the beads were collected and washed with Lysis250 buffer five times, and the immunoprecipitates were separated by 7.5% SDS-polyacrylamide gel electrophoresis and detected by fluorography. For double immunoprecipitations, the immunoprecipitates were boiled in 200 μl of dissociation buffer (20 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1% Igepal, and 5 mM dithiothreitol) and diluted with 1 μl of Lysis250 buffer and immunoprecipitated once again with the same antibodies as previously described (24).

For immunoprecipitation, synchronized T24 cells were lysed and clarified as described above, and the supernatant was diluted with lysis buffer without salt (the final concentration of NaCl was 180 mM) before the addition of antibodies. For double immunoprecipitations, the immunoprecipitates were separated by SDS-polyacrylamide gel electrophoresis, transferred to Immobilon-P membranes (Millipore Corp., Bedford, MA), and immunoblotted with the indicated antibodies. The immunoblots were then probed with alkaline phosphatase-conjugated secondary antibody and visualized with 5-bromo-4-chloro-3-indolyl phosphate toluidinium and nitro blue tetrazolium (Promega, Madison, WI). In some experiments, p84, a nuclear matrix protein (26), served as an internal control.

Isolation of Cell Clones with Expression of the NH2-terminal Truncated RINT-1 Protein—To establish cell clones that conditionally express the modified RINT-1 (named RINT-XS, from amino acid 257 to 792), a UHD10–3-base plasmid, pUHD10–3/GFP-RINT-XS, that will control dimeric proteins containing GFP with a nuclear localization signal and a Myc epitope (27) fused to the RINT-XS controlled by a Tet-responsive promoter, was constructed. This plasmid was cotransfected into MCF-7 cells with a second plasmid, pCHTV, which contains a hygromycin resistance gene and a cytomegalovirus-controlled tetra-cycline repressor–VP16 fusion transcription unit (27). For constitutive expression of the RINT-XS protein, a CHPl-GFP-base plasmid (28), with identical structure of pUHD10–3/GFP-RINT-XS, except controlled by CMV promoter and with the hygromycin resistance gene as selection marker, was used. Cell clones resistant to hygromycin (100 μg/ml) were subsequently isolated and examined for either constitutive or tetracycline-regulated expression of the GFP-RINT-XS proteins. Expression of these fusion proteins in these clones was examined by immunoprecipitation with anti-Myc mAb and blotting with anti-GFP aAb (CLONTECH, Palo Alto, CA). Two constitutively expressing clones, clones 1 and 12, and one conditionally expressing clone, clone 122, were established.

Cell Cycle Checkpoint Analysis—Cell cycle checkpoints were determined according to the procedures described (27, 29). For the G2/S checkpoint assay, cells in logarithmic growth were seeded on coverslips and after 24 h, the presence or absence of tetrazycline and mock-exposed or treated with 12 gray of γ-irradiation. After 24 h, cells were incubated with 10 μM BrdUrd for 4 h and fixed for BrdUrd staining using a cell proliferation kit (Amersham Pharmacia Biotech). More than 1000 cells were counted for each clone under different conditions, and BrdUrd-positive cells were quantified and expressed as a fraction of the total cells. For the G2/M checkpoint, cells were seeded on the six-well dish with a density of 105 cells/well in the presence or absence of tetracycline for 24 h. Cells were then irradiated with 4 Gy and fixed with 4% paraformaldehyde at indicated time points. Alternatively, cells were fixed 2 h after treatment with various doses of irradiation. During the fixation process, cells were stained with 4,6-diamidino-2-phenylindole. About 200–400 cells in a field under a fluorescence microscope were imaged, and the mitotic phase including prophase, metaphase, anaphase, and telophase was counted. More than 6000 cells under different microscopic fields were counted for each time point and expressed as a percentage of mitotic index compared with the cells without treatment.

RESULTS AND DISCUSSION

RINT-1 Encodes a Novel Human Protein That Shares a Significant Homology with a Putative Drosophila Protein—To identify proteins that potentially interact with human Rad50, a fragment of Rad50 containing the second of two leucine heptad repeats and the Walker B NTP binding domain was used as the bait in two-hybrid experiments with a human genome-wide expressed sequence tag library. The six strongest positive clones identified in this screen were sequenced and found to be identical. Subsequent data base searches revealed that the insert cDNA encodes a novel protein that we named hsRINT-1, which stands for Rad50 interacting protein 1. The complete sequence of the longest cDNA encompasses 2,855 nucleotides and contains a putative translation

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initiation codon, the first methionine, at position 111 with an open reading frame of 792 amino acids (Fig. 1A). With the exception of several leucine heptad repeats in the N terminus of the predicted open reading frame, no other known functional domains have been identified in the predicted 87-kDa protein. However, significant sequence homology was found between RINT-1 and the CG8605 gene product in Drosophila melanogaster (30), which we refer to as dmRINT-1 (Fig. 1B). The predicted sequences of both of these proteins share similar leucine heptad repeats within their corresponding N-terminal region (box A). Two additional regions designated box B, which spans amino acids 220–565 of hsRINT-1, and box D, which spans amino acids 659–751 of hsRINT-1, exhibit 35–39% homology to the corresponding regions of dmRINT-1. hsRINT-1 has a unique sequence, box C, that lies between the two conserved regions. The biological function of the dmRINT-1 is unknown at the present time.

A search of the NCBI human genome data base revealed that hsRINT-1 maps to chromosome segment 7q22.1, with an open reading frame of 792 amino acids (Fig. 1A). With the exception of several leucine heptad repeats in the N terminus of the predicted open reading frame, no other known functional domains have been identified in the predicted 87-kDa protein. However, significant sequence homology was found between RINT-1 and the CG8605 gene product in Drosophila melanogaster (30), which we refer to as dmRINT-1 (Fig. 1B). The predicted sequences of both of these proteins share similar leucine heptad repeats within their corresponding N-terminal region (box A). Two additional regions designated box B, which spans amino acids 220–565 of hsRINT-1, and box D, which spans amino acids 659–751 of hsRINT-1, exhibit 35–39% homology to the corresponding regions of dmRINT-1. hsRINT-1 has a unique sequence, box C, that lies between the two conserved regions. The biological function of the dmRINT-1 is unknown at the present time.

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RINT-1 a Novel Rad50-binding Protein

FIG. 2. Interaction between RINT-1 and Rad50. A, human Rad50 binds RINT-1 in a yeast two-hybrid assay. The indicated regions of human Rad50 were fused to the DNA-binding domain of GAL4 in pAS1. RINT-1 was fused to the activation domain of GAL4 in pSE1107. These plasmids were cotransformed into yeast strain Y153, and the Rad50 were fused to the DNA-binding domain of GAL4 in pAS1. pAS-R50PB (amino acids 753–1312) and pAS-R50PB0.6 (amino acids 753–953) derivatives bind to RINT-1. B, RINT-1 binds human Rad50. The indicated regions of RINT-1 were fused to the activation domain of GAL4 in pAS1107, and these plasmids were cotransformed with pAS-R50PB containing amino acids 753–1312 of human Rad50 as in A. Note that deletions of amino acids in the conserved region of RINT-1 (boxes B and D) abrogated its binding to human Rad50 in this assay. C, human Rad50 binds RINT-1 by GST pull-down assay. The expressed and purified human Rad50-GST fusions are shown in the upper panel. Stars indicate each corresponding full-length GST fusion protein. In vitro translated RINT-1 was used to bind to GST fusions, and the results are shown in the lower panel. The R50PB (amino acids 753–1312) and R50PB0.6 (amino acids 753–953) derivatives bind to RINT-1 (lanes 2 and 3) but not the GST or R50PB1.2 (lanes 1 and 4) proteins. Lane 5 shows 10% of the total input of the in vitro translated RINT-1.

Identification of the RINT-1 Gene Product as an 87-kDa Cellular Protein—To identify cellular proteins encoded by the novel RINT-1 gene, polyclonal antibodies were raised against two RINT-1 protein fragments expressed as GST fusions, GST-PIN (amino acids 1–256) and GST-PIC (amino acids 501–792). Metabolically labeled cellular proteins derived from human acute lymphoblastic leukemia Molt-4 cells were immunoprecipitated with anti-PIN or anti-PIC immune sera or preimmune sera. A protein doublet exhibiting an electrophoretic mobility consistent with the predicted 792-amino acid RINT-1 protein was specifically immunoprecipitated with both anti-PIN and anti-PIC antibodies (Fig. 3A, lanes 3 and 8), but not with preimmune antiserum. However, preincubation of these two specific antibodies with GST-PIN (lane 5) or GST-PIC (lane 10) antigen, but not GST (lanes 4 and 9), completely abolished the immunoprecipitation of the protein doublet. In addition to the 87-kDa doublet, other cellular proteins were also observed in the immunoprecipitates. Using the rigorous protocol of reimmunoprecipitation, each of the anti-RINT-1 antibodies detected only the 87-kDa protein doublet (lanes 6 and 11). The RINT-1 protein synthesized by coupled in vitro transcription and translation using a cDNA template that specifies translation initiation from the first methionine in the RINT-1 sequence exhibits a mobility upon SDS-polyacrylamide gel electrophoresis similar to that of the faster migrating cellular RINT-1 protein (Fig. 3A, compare lane 1 with lane 3). To determine whether the slower migrating form of RINT-1 is the product of post-translational modification, immunoprecipitated RINT-1 protein was treated with alkaline phosphatase. No mobility change was observed following phosphatase treatment, suggesting that the slower migrating form may arise from other cellular processes (data not shown). Alternatively, differential initiation of translation using a non-AUG codon could explain the appearance of the slower migrating form of RINT-1 protein. A longer RINT-1 cDNA including sequence upstream of the first ATG codon was in vitro transcribed and translated (Fig. 1A, the amino acid sequence). While the majority of the product thus synthesized in vitro exhibited an electrophoretic mobility corresponding to the faster migrating form, a distinct product exhibiting a mobility corresponding to the slower migrating form of the cellular RINT-1 protein was also detected (Fig. 3B, compare lane 2 with lane 3). These results suggest that the slower migrating form of the RINT-1 doublet corresponds to a polypeptide whose translation begins at an upstream non-AUG codon. Analysis of the 5′ RINT-1 cDNA sequence for non-AUG start codons (32) revealed the presence of two potential CUG start sites (see the two circled leucines in Fig. 1). Both of these potential codons are flanked by purines at the −3- and +4-positions, indicating that either or both may be used for initiation of translation.

We next examined the expression profile of the RINT-1 protein doublet in a panel of human cell lines. These include a breast epithelial immortalized line MCF10A; three human breast cancer lines, T47D, MB231, and ZR75; two human bladder carcinoma lines, T24 and 5637; a rectal carcinoma line, SW837; and two immortalized fibroblast lines, GM09607A and GM09637G, carrying mutated and wild-type ATM gene, respectively. RINT-1 doublet was detected in all cell lines (Fig. 3C), indicating that RINT-1 is expressed in many different kinds of human cells.

RINT-1 is Expressed throughout the Cell Cycle but Interacts with Rad50 Only at Late S and G2/M Phase—To further explore the interaction of RINT-1 and Rad50 in cells, human bladder carcinoma T24 cells synchronized by density arrest and subsequent release were harvested at different time points. Cell lysates were subjected to immunoprecipitation with anti-RINT-1 or anti-Rad50 antibody, followed by immunoblot analysis using antibodies specific for RINT-1 and Rad50. The specific cell cycle stage(s) from which the lysates were prepared at each time point was determined by probing immunoblots with anti-RB mAb as previously described (17). The amount of total lysate used for each time point was normalized by immunoblotting with an antibody specific for a nuclear matrix protein, p84 (26) (Fig. 4C, bottom panel). While the 87-kDa doublet was detected in immunoprecipitates using anti-RINT-1 antibody using all stages of the cell cycle, an interaction with Rad50 was observed only during late S, G2/M, and M phases (Fig. 4A, lanes 4–6 in the top panel). Reciprocally, an interaction was also detected only after late S phase by immunoprecipitation with anti-Rad50 mAb. Interestingly, both bands of the RINT-1 doublet were coimmunoprecipitated with Rad50, although the slower migrating form of RINT-1 may preferentially bind to Rad50. Nevertheless, the specific interaction between RINT-1 and Rad50 at late S and G2/M suggests
that RINT-1 may play a role at these time windows during cell cycle progression.

Expression of an N-terminally Truncated RINT-1 Protein Leads to G2/M Checkpoint Failure—To test the possibility that RINT-1 functions in DNA damage-induced cell cycle checkpoint control, we established several stable breast cancer MCF7 clonal cell lines that express GFP fusions containing only the Rad50 binding region of RINT-1 (named GFP-RINT-XS). Among these, clone 122 constitutively expressed the GFP-RINT-XS fusion protein, while expression of the fusion protein in clone 122 was conditionally regulated by the presence of tetracycline (Fig. 5). The GFP-RINT-XS protein could be coimmunoprecipitated along with Rad50 (Fig. 5). It is possible that this N-terminally truncated protein may disturb the biological function(s) of the wild-type RINT-1 by binding to Rad50. Hence, we reasoned that this dominant negative mutant of
RINT-1 might reveal the potential function of RINT-1 in cell cycle progression. To test this possibility, the clonal cell lines expressing the GFP-RINT-XS fusion, as well as a control clonal cell line (5UGN) expressing GFP alone, were examined for the integrity of their respective radiation-induced G1/S and G2/M checkpoints. Clone 122, the control clone 5UGN, and the parental MCF7 cell lines all exhibited reduced BrdUrd-positive cells when treated with 12 grays of γ-irradiation, independent of the GFP fusion protein expression (Fig. 6A). These results indicate that expression of the truncated RINT-1 protein did not interfere with the radiation-induced G1/S checkpoint. To examine whether these cells exhibit differences in G2/M checkpoint control, the mitotic index of each cell culture at various time points after γ-irradiation was scored (Fig. 6B). In cells expressing GFP alone (5UGN, −Tet), a sharp reduction in mitotic index was observed, which was similar to that observed in cells without induced expression of GFP alone or GFP-RINT-XS (5UGN or clone 122, +Tet). In contrast, cells expressing the GFP-RINT-XS fusion protein (clone 122, −Tet) showed a comparatively delayed and significantly increased mitotic index (Fig. 6B). In a parallel experiment, mitotic cells were scored 2 h following treatment with various doses of γ-irradiation (Fig. 6C). Clone 122 cells induced to express GFP-RINT-XS exhibited a higher percentage of mitotic cells than cells without induced expression of the fusion protein or GFP alone. Similar results were also obtained using cells constitutively expressing GFP-RINT-XS (data not shown). Collectively, these results suggest that expression of an N-terminally truncated RINT-1 protein retaining its Rad50 binding region leads to an immediate delay of G2/M checkpoint following DNA damage. It is thus very likely that the RINT-1 and Rad50 complex may play an important role in control of the G2/M checkpoint in response to DNA damage.

In addition to their function in DNA double-strand break repair, recent data have suggested that Rad50, together with Mre11 and NBS1, may also play a role in cell cycle checkpoint control (reviewed in Ref. 33). In yeast, an unrepairable double-strand break in Ku70 mutant cells causes a permanent G2/M cell cycle arrest, while mutations in MRE11 suppress the inability of the cells to overcome double strand-break-induced cell cycle arrest (34). In NBS cells, a defect in the S phase checkpoint leading to radioreistant DNA synthesis and failure to activate checkpoints at the G1/S and G2/M boundaries in response to ionizing radiation has been observed (reviewed in Ref. 35). Furthermore, NBS cells exposed to ionizing radiation exhibit a prolonged accumulation of cells in G2 phase (36). Interestingly, BRCA1, a tumor suppressor linked to familial breast and ovarian cancer (37), was found to associate with Rad50 (25, 38). In addition to its participation in transcriptional regulation, cell growth and differentiation, and DNA repair processes, BRCA1 has also been implicated in cell cycle checkpoint control, since deletions of exon 11 of BRCA1 display a defective G2/M checkpoint after ionizing radiation and methyl methanesulfonate treatments (39). However, direct evidence to show Rad50 in G2/M checkpoint control remains lacking. It is possible that RINT-1 functions independently or, alternatively, in association with a Rad50-BRCA1 complex to ensure appropriate G2/M checkpoint control. Regardless, the identification of this novel Rad50-interacting protein represents an important step toward a more complete description of
the activities through which proper cell cycle checkpoint control is achieved.

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