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Nuclear foci of mammalian Rad51 recombination protein in somatic cells after DNA damage and its localization in synaptonemal complexes

(DNA repair/irradiation/meiotic prophase/RecA protein homolog/protein immunofluorescence)

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ABSTRACT **Rad51 protein of *Saccharomyces cerevisiae* is a structural homolog of the *Escherichia coli* recombination enzyme RecA. In yeast, the Rad51 protein is required for mitotic and meiotic recombination and for repair of double-strand breaks in DNA. We have used antibodies raised against the homologous human protein, HsRad51, expressed in *E. coli*, to visualize the spatial distribution of the protein in mammalian somatic and meiotic cells. In cultured human cells, the HsRad51 protein is concentrated in multiple discrete foci in the nucleoplasm; it is largely absent from cytoplasm and nucleoli. After treatment of cells with methyl methanesulfonate, ultraviolet irradiation, or ^{137}Cs irradiation, the percentage of cells with HsRad51 protein immunofluorescence increases; the same cells show unscheduled DNA synthesis. Induction of Rad51 foci is blocked by inhibitors of transcription. In mouse pachytene spermatocytes, the mouse homolog of Rad51 protein is highly enriched in synaptonemal complexes that are formed between the paired homologous chromosomes during meiotic prophase. We conclude that the mammalian proteins homologous to yeast Rad51 are involved in repair of DNA damage and recombinational repair during meiosis.**

Meiosis is a special form of cell division in sexually reproducing eukaryotes which reduces the chromosome number of diploid cells to a haploid one. Prophase of meiosis I involves pairing of homologous chromosome segments, formation of the synaptonemal complex (SC), and high levels of genetic recombination (1–3). The SC is a meiosis-specific proteinaceous structure that forms along the length of the paired homologous chromosomes. At the ultrastructural level, it consists of two parallel lateral elements and a central element. SC formation is generally considered necessary to bring homologous DNA segments into close enough contact for crossing-over and recombination to occur. Monoclonal antibodies elicited to isolated rat SCs and human autoantibodies have enabled the identification of several protein antigens located in the lateral elements and synapsed regions of meiotic prophase chromosomes (4–6).

The molecular basis of homologous pairing and meiotic recombination is poorly understood. It has been hypothesized that proteins with structural similarity to the recombination enzyme RecA from *Escherichia coli* may provide the enzymatic functions for recognition of homology and DNA strand exchange in recombination and recombinational repair (3). In *E. coli*, RecA protein polymerizes on DNA, searches for homologous regions between two double-stranded DNA molecules, catalyzes their pairing, and promotes strand exchange (7–10). Moreover, it is involved in recombinational repair of double-strand breaks. In yeast, the structural RecA protein homolog Rad51 protein plays a role in DNA double-strand break repair and genetic recombination during mitosis and meiosis (11). DMC1, a meiosis-specific Rad51 homolog of *Saccharomyces cerevisiae*, is required for SC formation and recombination during meiosis (12).

Genes homologous to *E. coli recA* and yeast *rad51* have now been isolated from all groups of eukaryotes, including mammals (13–15). The human HsRad51 and mouse MmRad51 proteins are highly similar (83% identical) with the yeast Rad51 protein but less homologous with DMC1 protein. All these Rad51 proteins share significant homology with residues 33–240 of the *E. coli* RecA protein, which have been identified as a "homologous core" region (13–15). The rather high expression of the mouse *MmRad51* gene in meiotic tissues points to a specific role of MmRad51 protein in meiotic recombination (13, 14). To date, however, it is unclear where the *MmRad51* gene is expressed at highest levels and in which subcellular compartment the resulting protein is localized during meiotic prophase. Thus, all data suggesting a relationship between MmRad51 protein and meiotic recombination have been circumstantial. To examine the role of mammalian Rad51 protein in repair and recombination, we have analyzed the intranuclear distribution and amount of HsRad51 protein in cultured human cells treated with different DNA-damaging agents and the localization of MmRad51 protein in mouse pachytene spermatocytes.

MATERIALS AND METHODS

Cloning of the *HsRad51* Gene. The whole coding sequence of *HsRad51* (*RAD51* in standard human gene nomenclature), the human gene homologous to yeast recombination gene *rad51*, was amplified by PCR from a human thymus cDNA library. Sequences of the upstream and downstream primers are TCCCCCGGGATCCGATGGCAATGCAGATGCAGCTTG and CTAGGTTCGAACAGCTGCAGTCAGTCTTTGGCATCTCCCACT. Underlined sequences are homologous to the published sequence of the *HsRad51* gene (16). For PCR, 0.5 μg of DNA was used in a mixture containing each primer at 200 nM, 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, each of the four dNTPs at 200 μM, and 2.5 units of AmpliTaq polymerase (Perkin–Elmer). The reaction mixture (100 μl) was heated at 95°C for 3 min and used in a PCR consisting of 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The resulting DNA fragment was inserted into expression vector pTrcHisB (Invitrogen) in frame with a 5′-end sequence coding for a series of six histidine residues that function as a metal-binding domain in the translated fusion protein and an epitope for commercially

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Abbreviations: AMD, actinomycin D; CCD, charge-coupled device; CENP, centromeric protein; DAPI, 4′,6-diamidino-2-phenylindole; DRB, 5,6-dichloro-β-D-ribofuranosylbenzimidazole; MMS, methyl methanesulfonate; SC, synaptonemal complex.

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available monoclonal antibody (Novogen). Identity of the inserted sequence to the published coding sequence of the *HsRad51* gene was confirmed by dideoxynucleotide chain-termination sequencing performed with a Sequenase kit (United States Biochemical).

Isolation of HsRad51 Protein. Plasmid pEG916, which carries the whole coding sequence of the *HsRad51* gene, was introduced into *E. coli* DH10B (BRL). Synthesis of the fusion protein is under control of the *Trc* promoter and can be induced by isopropyl β-D-thiogalactopyranoside (IPTG). For isolation of HsRad51 protein, *E. coli* DH10B/pEG916 cells were grown in SOB medium (24) at 37°C to OD_{590} = 0.6 and induced with 1 mM IPTG. Cells were harvested 4 h after induction and lysed by lysozyme treatment, followed by three cycles of freezing and thawing and sonication. After precipitation of nucleic acid–protein complexes by 0.43% polyethylenimine P and protein extraction in 20 mM sodium phosphate buffer (pH 7.0) containing 1 M NaCl, proteins were precipitated by ammonium sulfate at 0.31 g/ml. Upon dialysis against 20 mM sodium phosphate buffer (pH 7.0) most of the HsRad51 protein precipitated. The precipitate was redissolved in denaturing buffer [20 mM sodium phosphate buffer (pH 7.8)/6 M guanidine hydrochloride/0.5 M NaCl]. HsRad51 protein was purified by nickel-chelate affinity chromatography (ProBond Nickel Column; Invitrogen). The isolated HsRad51 protein was near homogeneous (Fig. 1) and used for preparation of rabbit polyclonal antibodies (24).

Expression of HsRad51 Fusion Protein in Human Cells. Human kidney cells (line 293, ATCC CRL1573) were stably transformed by plasmid pEG915. This plasmid carries the whole coding sequence of the *HsRad51* gene inserted in frame with the 5′-terminal sequence of vector pEBVHisB (Invitrogen), which is analogous to the 5′-terminal sequence of the above-mentioned plasmid pTrcHisB.

Induction of Endogenous HsRad51 Protein in Cultured Cells After DNA Damage. Human fibroblasts (strain Hs68) and 293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics. To induce double-strand breaks in DNA and recombinational repair, the x-ray-mimetic drug methyl methanesulfonate (MMS; Fluka) was added to the culture medium 3 h before cell harvest at a final concentration of 0.005% and 0.01%. In different experiments, cell cultures were exposed to a cesium (^{137}Cs) irradiator at doses of 10–900 rad (1 rad = 0.01 Gy) and to an ultraviolet (UVC) irradiator at doses of 15 J/m^2 and then allowed to recover for various times. The cells were detached from culture flasks by gentle trypsination, pelleted, and resuspended in phosphate-buffered saline [PBS; 136 mM NaCl/2 mM KCl/10.6 mM Na_2HPO_4/1.5 mM KH_2PO_4 (pH 7.3)] prewarmed at 37°C.

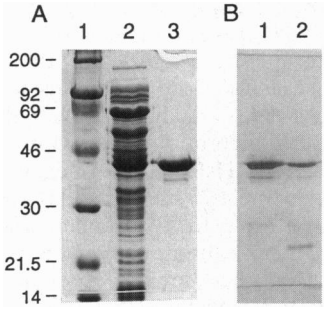

FIG. 1. Electrophoretic analysis of isolated HsRad51 protein. HsRad51 protein, expressed in *E. coli* as a fusion protein, was isolated and electrophoresed on an SDS/12% acrylamide gel. Proteins were visualized directly by Coomassie blue staining (*A*) or after Western blotting (*B*) by using mouse monoclonal antibody against an epitope of the N-terminal domain of the fusion protein. (*A*) Lane 1, protein standards (numbers at left in kDa); lane 2, crude lysate of *E. coli* DH10B/pEG916; lane 3, HsRad51 protein. (*B*) Lane 1, HsRad51 protein; lane 2, crude bacterial lysate.

To block protein synthesis, the transcriptional inhibitors actinomycin D (AMD; Serva) (17) and 5,6-dichloro-β-D-ribofuranosylbenzimidazole (DRB; Fluka) (18) were added to the culture medium of ^{137}Cs-irradiated cells at final concentrations of 25 and 50 μg/ml, respectively.

Preparation of Mouse Spermatocytes. Immediately after the animal was sacrificed, the testes were removed and placed in PBS. The tissue was cut into small pieces with a pair of fine scissors, and the seminiferous tubules were emptied by squeezing with a forceps. The resulting cell suspension was transferred to a centrifuge tube and allowed to settle down for 10–15 min. The supernatant was then carefully removed with a pipette and transferred to a new centrifuge tube. Premeiotic, meiotic, and postmeiotic cell types were present in this supernatant in adequate numbers.

Immunofluorescent Staining with Anti-HsRad51 Protein Antibodies. The density of somatic or spermatogenic cells was adjusted to ≈10^5 cells per ml in PBS. Aliquots (0.5 ml) of the cell suspension were centrifuged onto clean glass slides at 800 rpm for 4 min, in a Cytospin (Shandon, Pittsburgh). After cytocentrifugation, the slides were fixed in −20°C methanol for 30 min and then immersed in ice-cold acetone for a few seconds to permeabilize cells for antibody staining. Following three washes with PBS, the preparations were incubated at 37°C with rabbit anti-HsRad51 antiserum, diluted 1:50 with PBS containing 0.5% bovine serum albumin, in a humidified incubator for 30 min. In some experiments, human autoimmune sera specifically directed against SC and centromeric proteins (CENPs) were used for SC and centromere staining (6). The slides were washed three times for 10 min each and then incubated for 30 min with differently fluorescent labeled (rhodamine- and fluorescein-conjugated) anti-rabbit IgG and anti-human IgG, diluted 1:20 with PBS. After three washes with PBS, the preparations were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; 0.1 μg/ml for 1 min) and mounted in antifade {90% (vol/vol) glycerol/0.1 M Tris·HCl (pH 8.0)/2.3% 1,4-diazabicyclo[2.2.2]octane (DABCO)}.

Labeling Sites of Unscheduled DNA Synthesis. Hs68 fibroblasts were irradiated with ^{137}Cs with a dose of 900 rad and then cultured for additional 24 h. 5-Bromodeoxyuridine (BrdUrd) was added to the culture medium at 10 μg/ml 2 h before cell harvesting. At the end of the labeling period, slides were prepared as described above. After HsRad51 protein staining, the preparations were again fixed in a 3:1 (vol/vol) mixture of methanol and acetic acid for several hours at −20°C. Since the anti-BrdUrd antibody recognizes BrdUrd incorporated into chromosomal DNA only if the DNA is in the single-stranded form, the slides were denatured in 70% (vol/vol) formamide/2× SSC for 1 min at 80°C and then dehydrated in an ethanol series. Sites of unscheduled DNA synthesis (BrdUrd incorporation) were visualized by indirect anti-BrdUrd antibody staining. First, the preparations were incubated with mouse monoclonal anti-BrdUrd antibody (Boehringer Mannheim), diluted 1:50 with PBS, for 30 min. The slides were washed with PBS and then incubated with rhodamine-conjugated anti-mouse IgG, diluted 1:20 with PBS, for another 30 min.

Digital Imaging Microscopy. Images were taken with a Zeiss epifluorescence microscope equipped with a thermoelectronically cooled charge-coupled device (CCD) camera (model PM512; Photometrics, Tucson, AZ), which was controlled by an Apple Macintosh computer. Gray scale source images were captured separately with filter sets for rhodamine, fluorescein, and DAPI. Source images were saved as gray scale data files by using the CCD Image Capture software. Merging and pseudocoloring were accomplished by using Gene Join. Software packages are available through the Office of Cooperative Research, Yale University. It is worth emphasizing that

although a CCD imaging system was used, all antibody signals were clearly visible by eye through the microscope.

RESULTS

To study the *in situ* localization of Rad51 protein homologs in mammalian cells, a specific rabbit antiserum raised against human HsRad51 protein expressed in *E. coli* was used (Fig. 1). Western blotting experiments reveal that these antibodies do not react significantly with any proteins present in human somatic cells and mouse seminiferous tubule cells except HsRad51 and MmRad51 (Fig. 2*A*). The mammalian *Rad51* gene is transcribed at high levels in thymus, spleen, intestine, ovary, and testis and at lower levels in most other tissues (13, 14). Using reverse PCR and Western blotting, we have additionally demonstrated expression of the gene in a large variety of mammalian cell cultures. In fact, 20 cultured cell types of widely different tissues all exhibited detectable amounts of *Rad51* mRNA and/or Rad51 protein (data not shown). This possibly implies that the mammalian *Rad51* gene is expressed in rapidly dividing cells.

Induction of HsRad51 Protein Foci in Somatic Cells. The anti-HsRad51 antiserum was used to visualize the distribution of HsRad51 protein *in situ* in diploid human fibroblast and lymphoblast cells. We find detectable immunolabeling of nuclei (above background) in only a small percentage (10% or less) of cells (Table 1). In these immunopositive cells, the HsRad51 protein is focally concentrated in small and discrete sites throughout the nucleoplasm that number several dozen to several hundred. This HsRad51 protein is largely excluded from the nucleoli and cytoplasm. When fibroblasts are exposed to 0.01% MMS for 3 h and the distribution of HsRad51 protein is then visualized by specific antibodies, most nuclei give specific immunolabeling and contain a large number of fluorescing HsRad51 protein foci (Fig. 3*A*, Table 1). Use of preimmune serum, as well as omission of either the primary or secondary antibody, results in the absence of focally concentrated nuclear immunofluorescence. MMS-treated fibroblasts labeled with preimmune serum show only a faint staining of cytoplasm that is due to nonspecific antibody binding and also to the presence of autofluorescing material in cultured cells. However, discrete nuclear foci are never observed (Fig. 3*B*).

We next investigated the effects of ionizing and UV irradiation on nuclear HsRad51 protein labeling. Both human fibroblasts and 293 cells were exposed to a ^{137}Cs irradiator at a dose of 900 rad, which kills 99% of cells, or to a lethal dose (15 J/m^2) of UVC light. These cells were then cultured for various times to allow repair of induced DNA damage to occur. Compared with controls, irradiated cell cultures exhibit a dramatically increased percentage of nuclei with very brightly fluorescing foci that are highly enriched with HsRad51 protein (Figs. 3*C* and 4*A* and *B*). Foci induced by irradiation are larger but fewer on the average than after MMS treatment. Table 1 reveals that the induction of focally concentrated HsRad51 protein by ionizing and UV radiation is time and dose dependent. The highest number of nuclei showing this effect and the strongest immunofluorescence of HsRad51 protein foci is observed 3–24 h after ^{137}Cs irradiation with doses >300 rad.

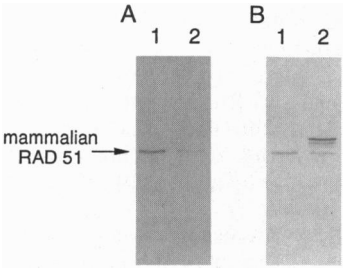

FIG. 2. Western blotting of mammalian Rad51 protein. Mammalian cells were lysed, and the contents were electrophoresed on an SDS/12% acrylamide gel and Western blotted. Mammalian Rad51 protein bands were visualized by using polyclonal rabbit anti-HsRad51 antiserum. (*A*) Lane 1, human 293 cells; lane 2, mouse seminiferous tubule cells. (*B*) Lane 1, 293 cells; lane 2, 293 cells, stably transformed by plasmid pEG915. Fusion protein encoded by pEG915 has a molecular mass of 42 kDa (upper band in lane 2), which is 3 kDa higher than endogenous HsRad51 protein. The faint intermediate band in lane 2 may be a partial degradation product of the fusion protein.

Table 1. Percentage of nuclei containing discrete foci enriched with HsRad51 protein

Cells	Treatment	% nuclei with HsRad51 immunofluorescence		
		No foci	Type I*	Type II*
Hs68	None	90	10	<0.4
	3 h after 0.005% MMS	78	15	7
	3 h after 0.01% MMS	38	55	7
	10 min after 900 rad ^{137}Cs	89	10	1
	3 h after 900 rad ^{137}Cs	58	23	19
	7 h after 900 rad ^{137}Cs	83	5	12
	24 h after 900 rad ^{137}Cs	80	8	12
	7 h after 900 rad ^{137}Cs + AMD	98	1	1
	24 h after 900 rad ^{137}Cs + AMD	98	1	1
	7 h after 900 rad ^{137}Cs + DRB	97	2	1
	24 h after 900 rad ^{137}Cs + DRB	97	2	1
	6 h after 10 rad ^{137}Cs	96	4	<0.4
	6 h after 50 rad ^{137}Cs	96	4	<0.4
	6 h after 150 rad ^{137}Cs	92	7	1
	6 h after 450 rad ^{137}Cs	88	8	4
	6 h after 900 rad ^{137}Cs	91	4	5
	3 h after 15 J/m^2 UVC	90	3	7
	24 h after 15 J/m^2 UVC	77	6	17
	48 h after 15 J/m^2 UVC	91	7	2
293	None	75	23	2
	3 h after 900 rad ^{137}Cs	38	27	35
	24 h after 900 rad ^{137}Cs	32	24	44
	48 h after 900 rad ^{137}Cs	70	16	14
	168 h after 900 rad ^{137}Cs	82	10	8

At least 250 nuclei were analyzed for each experiment.
*Type I nuclei show only a few (<10) foci and/or weak-to-medium HsRad51 immunofluorescence, whereas type II cells show many and/or strongly fluorescing foci.

We noticed that many HsRad51 protein foci in irradiated nuclei have a double-dot appearance (Fig. 4 *A* and *B*), typical of newly replicated DNA segments (19). To determine if the HsRad51 foci correspond to sites of unscheduled DNA synthesis, BrdUrd was incorporated into DNA of ^{137}Cs-irradiated cells and visualized by using BrdUrd-specific antibodies. Simultaneous HsRad51 protein immunofluorescence and anti-BrdUrd antibody staining demonstrates that nuclei with focally concentrated HsRad51 do undergo repair DNA synthesis: 22% of the 200 nuclei analyzed show both HsRad51 foci and sites of BrdUrd incorporation. In contrast, only 2% of nuclei are found to be HsRad51-foci negative and BrdUrd-staining positive, while 6% are HsRad51-foci positive and BrdUrd-staining negative. In 70% of nuclei HsRad51 foci and BrdUrd staining are both absent. In this context, it is important to note that BrdUrd incorporation does not occur at sites enriched with HsRad51 protein (Fig. 4*C*).

Experiments with transcriptional inhibitors reveal that *de novo* protein synthesis is required for the induction of focally concentrated HsRad51 protein in cells that have sensed DNA damage. Immediately before irradiation, fibroblast cells were placed in medium containing AMD at 25 μg/ml or DRB at 50 μg/ml to inhibit transcription by RNA polymerase II and reduce protein synthesis (to <5% of normal). Neither AMD- nor DRB-treated cells show an increased number of cells with

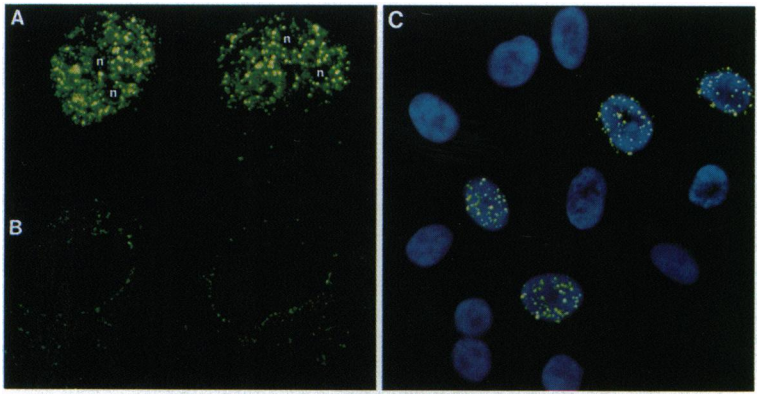

FIG. 3. *In situ* localization of HsRad51 protein in MMS-treated and ^{137}Cs-irradiated human Hs68 fibroblasts. (*A*) Two representative MMS-treated cells labeled with anti-HsRad51 antibodies (green fluorescein fluorescence). Nuclei contain a large number of discrete foci enriched with HsRad51 protein. HsRad51 immunofluorescence is excluded from nucleolar domains (n). MMS was added to the culture medium 3 h before cell harvest at a final concentration of 0.01%. (*B*) MMS-treated cells labeled with preimmune serum alone. The image is intentionally overexposed to visualize the nuclear outline. Note: Specific nuclear immunofluorescence is absent. (*C*) HsRad51 protein staining of cells 3 h after ^{137}Cs irradiation with a dose of 900 rad. Induction of focally concentrated HsRad51 protein is seen in four nuclei. Nuclei are counterstained with DAPI. (×1000.)

focally concentrated HsRad51 protein after ^{137}Cs irradiation at a dose of 900 rad (Table 1).

Human 293 cells were transfected with plasmid pEG915, which encodes HsRad51 protein fused to a T7-tag epitope. Cells constitutively expressing this protein (Fig. 2*B*) were stained with rabbit anti-HsRad51 antiserum and a mouse monoclonal antibody against the N-terminal domain in the fusion protein. The two antibody probes produce identical immunofluorescence patterns (Fig. 4*D*), confirming the specificity of the HsRad51 antiserum.

Association of MmRad51 Protein with SCs. Immunofluorescence microscopy on mouse testicular cells also demonstrates a punctate nuclear staining pattern in various meiotic and postmeiotic cell types. Immediately striking, however, is a very strong fluorescent staining of the thread-like SC structures in essentially all pachytene spermatocytes (Fig. 5). These cells are easily identified by their abundance, size, nuclear morphology, and heterochromatic chromocenters (6, 20). Simultaneous incubation with a human autoantibody labels synapsed regions of the SC. The centromeric regions of telocentric mouse SCs are additionally labeled by human CREST autoantibody (6). In each case the SC structures labeled by anti-HsRad51 antiserum and human autoantibodies colocalize. It is worth pointing out that in well-spread SCs the MmRad51 protein immunofluorescence exhibits a conspicuous beads-on-a-string nature. The longitudinal SC appears to consist of small granular subunits of MmRad51 protein. The unpaired axes of the X and Y chromosomes (Fig. 5 *A* and *A'*) are not strongly labeled by anti-HsRad51 antibodies. The most straightforward interpretation of our data is that the SC serves as a structural support of the recombination machinery.

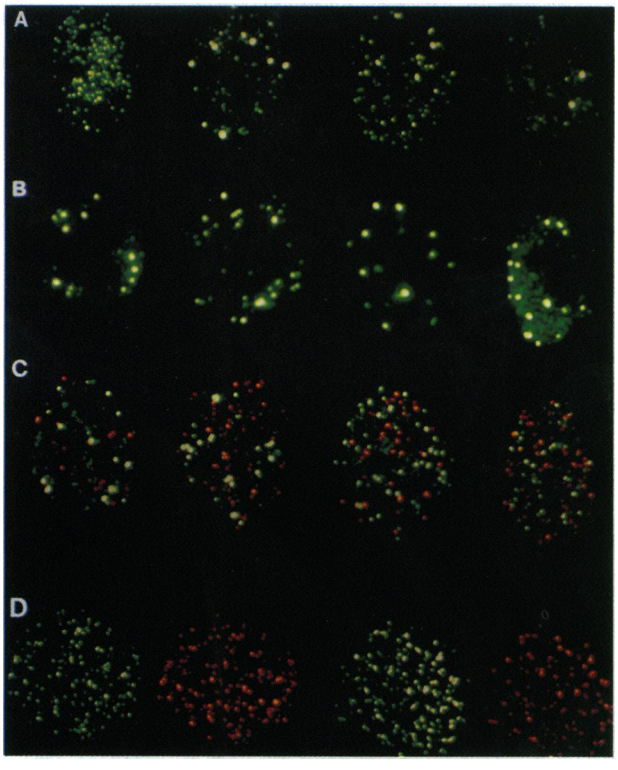

FIG. 4. (*A*) Patterns of HsRad51 protein immunofluorescence in fibroblasts UV-irradiated with a dose of 15 J/m^2 and then cultured for 24 h. (*B*) HsRad51 protein staining of fibroblast cells 24 h after ^{137}Cs irradiation with a dose of 900 rad. (*C*) Simultaneous visualization of focally concentrated HsRad51 protein (green) and sites of unscheduled BrdUrd incorporation (red) in ^{137}Cs-irradiated cells. (*D*) Two-color protein immunofluorescence of HsRad51 in cells constitutively expressing HsRad51 fusion protein. Each nucleus is seen after staining with rabbit anti-HsRad51 antiserum (green) and with mouse monoclonal antibody against the N-terminal domain of the fusion protein (red). Note: Both antibodies produce identical nuclear staining patterns. (×1000.)

Given the observation that human and mouse homologs of Rad51 protein are highly homologous proteins that differ by only four amino acids (13, 14), it is not unexpected that, in the immunofluorescent experiments reported here, antibodies to the human protein bind mouse MmRad51 protein with high affinity. Mock immunofluorescence with the preimmune serum shows low-intensity nonspecific signals throughout the nucleus and cytoplasm of pachytene spermatocytes. However, no SC staining was observed in several independent experiments (data not shown).

DISCUSSION

Structural RecA and Rad51 protein homologs have been highly conserved throughout evolution, from yeasts to mammals, and are therefore assumed to be key players for recombination in lower and higher eukaryotes (13–15). Our results show a dramatic increase of HsRad51 foci after DNA damage introduced into the genome of somatic cells by various agents. By analogy with the structural homologs of yeast (11, 12), the human HsRad51 protein may be involved, directly or indirectly, in repair of double-strand breaks in DNA. Interestingly, we find several dozen to several hundred focal sites of HsRad51 protein scattered throughout the nucleus. It is tempting to speculate that these sites represent repair domains where an underlying structure organizes damaged DNA and enzymes required for repair into functional complexes and where DNA damage is repaired. By immunolabeling sites of unscheduled BrdUrd incorporation, we showed that repair DNA synthesis occurs in cells with focally concentrated HsRad51 protein, but the HsRad51 foci and sites of BrdUrd incorporation do not colocalize. One possibility is that HsRad51 protein participates early in a multistep process before repair DNA synthesis occurs. Alternatively, foci enriched with HsRad51 protein may only serve as a stock of repair enzymes in cells that have sensed DNA damage. Al-

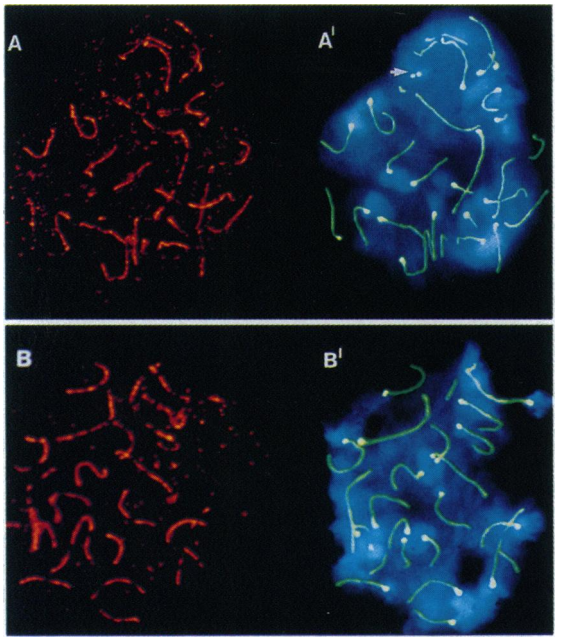

FIG. 5. *In situ* localization of MmRad51 protein in mouse pachytene spermatocytes. Rabbit antiserum against HsRad51 protein, detected with rhodamine-conjugated anti-rabbit IgG (red fluorescence), is used in conjunction with human autoimmune sera against SCs and CENPs, detected by fluorescein-conjugated anti-human IgG (green). (*A* and *B*) MmRad51 protein staining of two representative pachytene cells. MmRad51 protein is concentrated in the SC structures and additionally distributed in numerous fluorescing foci. Note: The SC immunofluorescence appears as beads on a string. (*A'* and *B'*) The same pachytene cells after simultaneous staining of chromatin with DAPI (blue fluorescence) and of SCs/CENPs with specific human autoantibodies (green). The X and Y chromosomes are not synapsed. Therefore only the X and Y centromeres are visible (arrow). MmRad51 protein colocalizes with the SC autoantigen. Note: MmRad51 protein is absent from the unsynapsed lateral axes of the X and Y chromosomes. (×500.)

though the cell nucleus has no membrane-bounded compartments, all basic nuclear processes such as transcription, transcript processing and transport through the nucleoplasm, and chromatin replication seem to be confined to particular locations within the nucleus (21). In this light, it is not surprising that the repair of damaged DNA also may be compartmentalized within a structural and functional domain within the highly ordered interphase nucleus. The isolation of such a hypothetical "repair complex" containing HsRad51 protein and ancillary repair/recombination proteins would be of considerable interest.

High levels of genetic recombination occur during meiotic prophase while homologous chromosomes are organized on specific SC structures. Double-strand breaks widely induced during meiosis in *S. cerevisiae* (22) appear to be early events in the meiotic recombination process that can occur before the SC formation (23). The Rad51 protein may be involved in a step that converts meiotic double-strand breaks to the next recombination intermediate on the way to crossovers (11). Consistent with this observation, yeast mutants lacking DMC1, a meiosis-specific homolog of Rad51, are defective in both reciprocal recombination and SC formation (12). Thus, evidence is accumulating from several directions that meiotic DNA–DNA interactions (homologous pairing and recombination) may have evolved from somatic recombinational repair systems (3). Immunocytochemical localization on mouse pachytene spermatocytes revealed that the MmRad51 protein is highly enriched in the SC structure. In addition, MmRad51 protein is also present in numerous nucleoplasmic domains, akin to the foci seen in somatic cells. We presently have no direct experimental evidence as to the function of mammalian Rad51 protein homologs during meiosis. Since most of the MmRad51 protein is associated with synapsed regions of meiotic prophase chromosomes, it is reasonable to assume that mammalian Rad51 proteins play a role in the repair of meiotic double-strand breaks and strand-exchange reactions. This provides direct evidence for a functional role of the proteinaceous SC structure in meiotic recombination in higher eukaryotes.

Note Added in Proof. Bishop (25) has recently reported the immunolocalization of ScRad51 protein to multiple complexes in yeast meiotic nuclei.

Since Western blots have not shown a net increase in HsRad51 protein in irradiated cells, we conclude that DNA damage affects its nuclear distribution.

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