Xrcc3 Is Required for Assembly of Rad51 Complexes in Vivo*

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Rad51 is a member of a family of eukaryotic proteins related to the bacterial recombinational repair protein RecA. Rad51 protein localizes to multiple subnuclear foci in Chinese hamster ovary cells. Subnuclear Rad51 foci are induced by ionizing radiation or the DNA cross-linking agent cisplatin. Formation of these foci is likely to reflect assembly of a multimeric form of Rad51 that promotes DNA repair. Formation of damage-induced Rad51 foci does not occur in the Chinese hamster ovary cell line irs1SF, which is sensitive to DNA damaging agents. The Rad51 focus formation defect of irs1SF cells is corrected by a construct that encodes the repair protein Xrcc3. Xrcc3 is a human homolog of Rad51 previously isolated by virtue of its ability to correct the radiation sensitivity of irs1SF cells. Changes in the steady state level of Rad51 protein do not account for the irs1SF defect nor do they account for the appearance of foci following DNA damage. These results suggest that Xrcc3 is required for the assembly or stabilization of a multimeric form of Rad51 during DNA repair. Cell lines defective in two different components of DNA protein kinase formed Rad51 foci in response to damage, indicating DNA protein kinase is not required for damaged-induced mobilization of Rad51.

The Rad51 protein is of particular interest in the study of cellular response to DNA damage because of its structural and functional similarity to the Escherichia coli RecA protein (1). RecA is known to play a central role in the prokaryotic response to DNA damage (2–4). The functional form of RecA and Rad51 is a multimeric helical nucleoprotein filament. RecA nucleoprotein filaments are rapidly assembled on the single-stranded DNA segments that form after various types of DNA damage.

Rad51 is a member of a conserved family of eukaryotic proteins related to RecA. Additional family members have been identified in a wide range of eukaryotes including yeasts and mammals (5). The completion of the Saccharomyces cerevisiae genomic sequence established that four previously identified proteins, Rad51, Rad55, Rad57, and Dmc1, constitute the complete set of RecA relatives in this organism (6–10). Thus far, several mutants defective in Rad51 have been identified in mammals. These include highly conserved human Rad51, HsRad51 (11), murine and human Dmc1 (12, 13), human Xrcc2 (14) and Xrcc3 (15), which were isolated by their ability to complement hamster mutant cells, and finally Rad51B (16), Rad51C (17), Rad51D (18), which were found by data base searching.

S. cerevisiae Rad51 (ScRad51) has been shown to be functionally similar to RecA. Both RecA and ScRad51 promote homology-dependent repair of the DNA double strand breaks induced by ionizing radiation (19, 20). Homology-dependent double strand break repair is an accurate process that usually repairs double strand breaks (DSBs) without generating insertions, deletions, or other chromosomal rearrangements. This accuracy results from the use of an undamaged copy of the broken DNA as a template for the repair process (21). The recruitment of an undamaged copy of the DNA requires strand exchange activity. Like RecA (22) and ScRad51 (23), HsRad51 promotes DNA strand exchange in vitro (24, 25), indicating that the role of Rad51 in recombinational DSB repair is likely to be conserved in mammals. Acquisition of genetic evidence implicating mammalian RAD51 genes in DNA repair has been complicated by the fact that rad51 mutant mice die during early embryonic development (26, 27). However, studies on early mouse embryos suggest that the murine rad51 mutant, like its yeast counterpart, is sensitive to ionizing radiation (26).

In addition to being able to repair DSBs by homology-dependent mechanisms, eukaryotes also have a homology-independent end-joining mechanism (28). In mammalian cells, the homology-independent process is the predominant pathway for the repair of damage-induced double strand breaks. DNA protein kinase, a heterotrimeric protein with both protein kinase and DNA end binding activity, is one of several proteins that promote homology-independent repair of DSBs (29, 30). Mice with the scid mutation are defective in the catalytic subunit of DNA protein kinase (31, 32). The CHO line xrs5 is one of several mutants defective in Ku86 protein, a subunit required for the end binding activity of DNA protein kinase that is encoded by the XRCC5 gene (33–36).

Immunostaining of both yeast and human nuclei with antibodies against RecA homologues has shown that they form visible complexes during meiotic recombination and mitotic DNA repair (37–41).2 Rad51 foci form in mammalian fibroblasts and lymphocytes in response to ionizing and ultraviolet radiation and also to the DNA alkylating agent methylmethane sulfonate (38). The observation that Rad51 foci are found in a variety of cells undergoing DNA repair and recombination has raised the possibility that these foci mark nucleoprotein complexes engaged in recombinational repair.

Here we show that CHO cells form subnuclear Rad51 foci in response to ionizing radiation and treatment with the nucleotide cross-linking agent cisplatin. More importantly, we show that Rad51 focus formation is defective in the radiation-sensi-

1 The abbreviations used are: DSB, double strand breaks; CHO, Chinese hamster ovary; TBS, Tris-buffered saline; Gy, gray.

2 S. Gasior and D. K. Bishop, unpublished data.
tive CHO line iir15SF. Both the Rad51 localization defect and the DNA damage resistance of iir15SF cells are rescued by DNA constructs that encode Xrc3, a protein that is structurally related to Rad51 (42). Based on these and other data we propose that direct interaction between the Rad51 and Xrc3 proteins is required for assembly or stabilization of Rad51 multimers at the sites of DNA damage in preparation for recombinational repair.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—CHO cell lines AA8, iir15SF, PXR3, and CXX3 (42, 43) were kindly provided by Dr. Larry Thompson, and xrs5 and K1 (44) were provided by Dr. Alan Diamond. A mouse scid cell line (designated UC-RCo-1) was originated from fibroblasts grown from the skin of a C3H scid mouse; mouse 4102 cells (a fibrosarcoma cell line from C3H mice provided by Dr. Hans Schreiber) was used as a control. The CHO cell lines AA8, iir15SF, PXR3, and CXX3 were grown using Dulbecco’s modified Eagle’s medium/F-12 (3:1), 10% fetal bovine serum, and 100 units/ml penicillin and 100 μg/ml streptomycin. The CHO cell lines K1 and xrs5 were grown in McCoy’s medium, 10% fetal bovine serum, and penicillin/streptomycin. Mouse cells were grown in Dulbecco’s modified Eagle’s medium/F-12 (3:1), 5% fetal bovine serum, and penicillin/streptomycin or minimal essential medium, 5% fetal bovine serum, and penicillin/streptomycin.

**Irradiation Experiments**—Exponentially growing cultures in 60-cm dishes were treated with x-rays using a Maxitron generator (General Electric) operating at 250 kV and 26 mA with a dose rate of 114 CgY/min. Dishes were returned to the incubator immediately after treatment. For dose-response studies, cells were incubated 3 h after irradiation before being harvested by trypsin treatment and fixed with 1% paraformaldehyde. For time course analysis, cells were irradiated with 900 CgY and then incubated for various periods of time before being harvested and fixed.

**Cisplatin Treatment**—For dose-response experiments, the cultures were washed twice in serum-free medium and then incubated for 1 h in serum-free medium containing varying concentrations of cisplatin (Bristol Laboratories). After the incubation, the dishes were washed twice in serum-free medium and complete medium was added. Cultures were placed at 37 °C for 3 h, at which time a single-cell suspension was obtained with trypsin/EDTA, and the cells were prepared for immunostaining. For time course experiments, cell cultures were washed twice in serum-free medium and incubated for 1 h in serum-free medium containing 10 μM cisplatin. After incubation, cells were washed and incubated in complete medium. At the appropriate times the cells were harvested by trypsin treatment and prepared for immunostaining.

**Immunostaining**—Fixed cells were transferred to slides using a Cytospin 2 (Shandon) centrifuge. Slides were washed twice in Tris-buff- ered saline (TBS) and incubated for 5 h at room temperature in TBS 1% bovine serum albumin. The wash was removed and α-Rad51 antibody in TBS 1% bovine serum albumin was applied. The slides were incubated overnight at 4 °C in a humidified chamber. The slides were washed twice in TBS and secondary antibody (fluorescein-conjugated goat anti-rabbit; Molecular Probes, Inc., Eugene, OR) was applied at a 1:1000 dilution in the dark. The slides were incubated at room temperature in the dark for 1 h. The slides were washed twice with TBS and stained with DAPI (0.1 μg/ml in TBS) for 5 min. The slides are dried and coverslips mounted with Vectashield mounting medium (Vector Laboratories Inc., Burlingame, CA).

For most experiments affinity-purified α-HsRad51 antibody (40) was used. In some experiments, α-HsRad51 serum was used and gave essentially identical results to those obtained with the affinity-purified IgG but produced faint, nonspecific background staining. This faint background was distinguished from the bright, Rad51-specific signal by competition with purified Rad51 protein; the addition of the pure protein eliminated the bright radiation-inducible foci but not the faint background staining pattern.

**Microscopy**—Immunostained samples were examined via standard epifluorescence microscopy using a Zeiss Axioptics III through a 100× objective. Digital images were obtained with a CCD camera (Imagepoint, Photometrics Inc., Tucson, AZ). A single focal plane was documented for each nucleus and, thus, the numbers presented are less than the total number of foci per nucleus. Samples consisted of focus counts for 50 unselected nuclei. The Kruskal-Wallis test was used to determine the statistical significance of observed differences between samples. Images that contain fluorescein and DAPI staining patterns were generated by converting gray-scale images to pseudo color and then merging the patterns electronically using I. P. Lab Spectrum software (Signal Analytics Corp., Vienna, VA).

**Western Analysis**—Samples were prepared for Western analysis (40) using Immunoblot membranes (Millipore Corp., Bedford, MA). Nonspecific binding was prevented by pre-treatment of the blocking solution that was TBS containing 5% dry milk and 0.1% Tween 20. The primary antibody was α-HsRad51 IgG at 0.5 μg/ml, and the secondary antibody was used (goat anti-rabbit peroxidase conjugate, Boehringer Mannheim) at a 1/5000-fold dilution. Signals were detected by chemiluminescence (Renaissance, NEN Life Science Products; Boston, MA). The level of signal detected was shown to be in the linear range of detection. Parallel analysis of a dilution series of protein extract from AA8 cells (data not shown). Measurement of cross-reactivity of α-Rad51 for Xrc3 by Western analysis was performed using pure Rad51 and Xrc3. Xrc3 was expressed in bacterial cells (BL21/pLysS) bearing the plasmid pET20-55 (a generous gift from Dr. Larry Thompson, Lawrence Livermore National Laboratory) and purified on a Ni2+ column (CLON-TECH; Palo Alto, CA).

**Clonogenic Survival Assays**—To measure radiation resistance of cell lines, between 100 and 40,000 cells were plated in 100-mm cell culture dishes. Four h after plating, cells were irradiated as described above and immediately returned to the incubator. 10–12 days later the colonies were fixed and stained with crystal violet. Only colonies of >50 cells were scored as survivors.

**Expression of HsRad51 in CHO Cells**—Constructs containing the HsRad51 gene (pEG915) bearing an N-terminal His6-epitope tag were transiently co-transfected together with a plasmid bearing a major histocompatibility complex class-surface antigen (φ2L-1), a generous gift from Dr. Andrea Sonti) into CHO cell lines PXR3 and iir15SF cells. Transient transfections were performed using TransIT™ reagents (Panvera Corp., Madison, WI) according to manufacturers’ recommendations. Cells were also co-transfected with the vector lacking the RAD51 gene (pEBVHisB, Invitrogen) or with no DNA (in addition to the pH-2L-1 plasmid) in addition, as controls. The response of all transfectants to X-irradiation damage was analyzed in three independent experiments by indirect immunostaining with α-His6 antibody at a 1:2000 dilution (to detect for the plasmid-expressed Rad51) and mouse anti-H-2L monoclonal antibody; secondary antibodies were conjugated with Texas red and fluorescein isothiocyanate (Southern Biotech, Birmingham, AL), respectively. We then searched cytologically for the presence or absence of subnuclear foci. Expression of His6-tagged HsRad51 (41.3-kDa fusion protein versus 37-kDa HsRad51) in transfecteds was confirmed by Western blot analysis with α-His6 antibody and the α-HsRad51 antibody.

**RESULTS**

**Radiation Induces the Formation of Subnuclear Foci in CHO Cells**—Cycling cells of CHO line AA8 were exposed to x-rays and then incubated for various periods of time. After incubation, the cells were fixed and indirectly immunostained with α-HsRad51 antibodies. Examination of stained samples by fluorescence microscopy revealed that the antibody was localized to multiple subnuclear foci (Fig. 1). For simplicity, the subnuclear structures detected in CHO cells by α-HsRad51 stainings are referred to as Rad51 foci. In addition to radiation-induced foci, a small number of foci (typically fewer than 5) were detected in cells that were either untreated or fixed immediately after radiation treatment (Figs. 1 and 2). These results are qualitatively similar to previous observations in human fibroblasts and lymphocytes (38) (45), although the number of foci detected in untreated cells is somewhat less than that reported previously. The maximum response, both in terms of the fraction of the cell population that showed a response and in terms of the number of Rad51 foci present per responding cell, was obtained with 9 Gy of X-irradiation (Fig. 2A). The properties of Rad51 focus induction are most easily described by using a threshold of 5 foci/cell to distinguish cells that show a focus formation response from those that do not. Using this threshold, the fraction of responding cells reached a maximum 6 h after irradiation. The average number of foci per responding cell reached a maximum 1 to 3 h after irradiation and typically remained at this induced level until after 6 h (Fig. 2B, data not
There was substantial heterogeneity in the number of foci detected per nucleus, even under conditions that gave maximum numbers of foci. For example, 40% of AA8 cells contained 5 or fewer foci 6 h after a dose of 9 Gy (Fig. 2B). Preliminary results suggest that the cells that fail to produce Rad51 foci are those in the G1 phase of the cell cycle at the time of treatment. A low Rad51 expression level may be responsible for the failure of G1 CHO cells to produce foci given that a low Rad51 expression level has been reported for human fibroblasts in G1 (46).

**Radiation Promotes Redistribution of Rad51 Protein**—The dramatic change in the pattern of α-Rad51 staining caused by radiation is not associated with a corresponding change in Rad51 steady state protein levels. Western analysis was carried out to determine the steady state level of Rad51 protein before and after irradiation (Fig. 3). Little or no difference in steady state protein levels was observed when treated and untreated samples were compared, suggesting that radiation-induced focus formation is not an indirect consequence of increased protein levels.

**Radiation-induced Rad51 Redistribution Is Defective in the Radiation-sensitive Cell Line irs1SF but Is Rescued by Expressing XRCC3 cDNA**—The CHO line designated irs1SF is a radiation-sensitive derivative of AA8 (47). Radiation did not induce Rad51 focus formation in irs1SF cells (Fig. 2); the number of foci detected in irs1SF after radiation was never significantly different from the number detected in untreated AA8 or irs1SF cells (p = 0.4). Western analysis of steady state Rad51 levels showed the level of Rad51 in irs1SF cells was essentially the same as in AA8, both before and after radiation, indicating that the failure of irs1SF cells to form foci did not result from the failure of these cells to express Rad51 protein (Fig. 3). These results indicate that irs1SF is defective in damage-induced redistribution of Rad51 foci.

Two independent derivatives of irs1SF, designated PXR3 and CXR3, have been described that display near normal radiation resistance (42). These derivatives were obtained by transfection of irs1SF with XRCC3 cDNA expression plasmids. PXR3 and CXR3 cells form Rad51 foci in response to x-rays. The numbers of Rad51 foci detected in these two radiation-resistant derivatives were essentially identical to the numbers detected in parental AA8 (Fig. 2). Western analysis did not reveal any effect of the XRCC3 construct on steady state Rad51 protein levels (Fig. 3). Since Xrcc3 is distantly related to Rad51 (22% identical at the predicted amino acid level), we considered the possibility that the foci detected are assemblies of Xrcc3 protein rather than Rad51 protein. However, Western analysis of the two proteins overexpressed in *E. coli* demonstrated a 10^4-fold lower reactivity of α-Rad51 specific antibody to Xrcc3 as compared with HsRad51 (data not shown). Moreover, our Western blot analysis allowed us to resolve Rad51 and Xrcc3, yet no signal was detected at the position of Xrcc3 in any of our experiments, providing further evidence that the signal detected cytologically is not Xrcc3. Hence, it is highly unlikely that the signal observed in cytological preparations results from cross-reaction of the antibody to Xrcc3. Western analysis also indicated that if the probe cross-reacted with another protein, this protein must have an electrophoretic mobility identical to HsRad51 (note that highly purified HsRad51 is used as a standard on the Western blot shown). Together the results provide strong evidence that Xrcc3 promotes the subnuclear assembly of hamster Rad51 after radiation.

Xrcc3 also promoted subnuclear foci of human Rad51 in CHO cells. As a further demonstration that Xrcc3 specifically influences nuclear distribution of Rad51 after radiation, we performed transient co-transfection experiments on PXR3 and

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3 U. Ear, J. Murley, R. R. Weichselbaum, and D. K. Bishop, unpublished data.
iro1SF cells. Cells were cotransfected with a plasmid bearing a construct that expresses a His 6-tagged derivative of human Rad51 protein, pEG915 (38). This construct was cotransfected with a plasmid expressing a murine major histocompatibility complex cell-surface antigen marker, H-2-Ld (pH-2Ld) to identify transfected cells. As a control, cells were also co-transfected with vector alone (in addition to pH-2-Ld). We observed subnuclear foci only in Rad51-His 6-transfected PXR3 cells that had been irradiated before examination. Parallel control experiments indicated that appearance of foci depended on the Rad51-His6 construct and on irradiation. Most importantly, no focus-containing cells were observed after transfection of iros1SF with Rad51-His6 under the same conditions used for detection of focus-positive PXR3 cells. This observation provides a second line of evidence indicating that Xrcc3 can promote subnuclear assembly of Rad51 after radiation. In these experiments the staining foci detected by the anti-His6 probe were similar in terms of the number of foci/positive staining cells and in terms of the staining insensitivity of foci compared with those detected with anti-Rad51. However, the number of nuclei-containing foci recognized by the anti-His6 antibody was only about 1 in 170 transfected cells. The low frequency of focus-containing cells may have been a consequence of the heterologous expression system. Western analysis using a anti-Rad51 antibodies (not anti-His6 antibodies) indicated that although the steady state level of the His6-tagged protein was the same in PXR3 and iro1SF cells, this level was quite low compared with that of the endogenous hamster protein, suggesting that only a small fraction of transfected cells express the protein encoded by the construct.

The Cross-linking Agent Cisplatin Induced Xrcc3-dependent Rad51 Foci—Xrcc3 promotes resistance to cross-linking agents as evidenced by the ability of cDNA constructs that encode XRC3 to rescue the cisplatin sensitivity of iros1SF cells (42). To implicate Rad51 in the response to cisplatin and to show that Xrcc3 promotes Rad51 focus formation in response to a second type of DNA damage, CHO lines were treated with

![FIG. 3. Western analysis of Rad51 protein levels. Whole cell protein extracts were prepared from the cell lines indicated and subjected to Western blot analysis with a-HsRad51 antibody. 40 μg of total protein was loaded in each lane. The first lane contains 5 ng of purified recombinant HsRad51 protein (a positive control for antibody staining and a molecular weight standard). The next eight lanes contain equal amounts of whole cell protein extracts from the four different cell lines indicated. The extracts were from cells that were unirradiated (–) or irradiated (+) to a dose of 9 Gy 3 h before the time of extract preparation. rad, radiation.]
various amounts of cisplatin and then stained with α-Rad51 antibodies. Cisplatin treatment induced up to 30 Rad51 foci in AA8, PXR3, and CXR3 cells (Fig. 4). In contrast, no induction of foci was detected after treatment of irs1SF cells with cisplatin. Cisplatin-induced Rad51 foci in AA8 cells tended to be fainter than foci induced by x-rays. Significant induction of foci was seen after treatment with 1 mM cisplatin for 1 h, a dose that allows 50–80% of cells to survive (42) (data not shown). Near maximum induction of foci was seen at 10 mM.

DNA Protein Kinase-defective Cell Lines Are Proficient in Formation of Rad51 Foci—To determine if DNA protein kinase is required for Rad51 focus formation, a fibroblast line derived from a scid mouse was examined. The murine fibroblast line designated 4102, a fibrosarcoma line, was used as a control. Although the two cell lines displayed the expected difference in radioresistance in clonogenic survival assays (data not shown), the number of Rad51 foci detected after radiation treatment was not significantly different (p = 0.58, Fig. 5, A and B). We also examined the CHO cell line xrs5, which has a DNA repair defect complemented by XRCC5, a human gene encoding the Ku86 subunit of DNA protein kinase (34, 48). As with the scid cell line, the xrs5 line showed significant induction of Rad51 foci in response to x-rays (p = 0.0001). Unexpectedly, the number of foci detected in xrs5 cells at 9 h after a dose of 9 Gy was significantly greater than the number detected in the radiation-resistant parent line (p = 0.0001, Fig. 5D). Additional work will be required to determine if this difference between K1 and xrs5 depends on Ku86. In summary, induction of Rad51 foci was observed in two different DNA protein kinase-defective cell lines, indicating that focus formation does not depend on fully functional DNA protein kinase.

**DISCUSSION**

Cells of the radiation-sensitive CHO line irs1SF fail to form Rad51 foci in response to radiation. A construct that encodes the XRCC3 gene, a relative of RAD51, restores both radiation resistance (42) and Rad51 focus formation to irs1SF. These results support the view that Rad51 foci reflect the mechanism that promotes cellular resistance to radiation. Given that Rad51, like RecA, functions in vitro by assembling into multimeric nucleoprotein filaments, it is likely that the damage-induced foci are Rad51 multimers assembled at damaged sites. If Rad51 foci represent productive repair intermediates, they might be expected to reach a maximum as the repair capacity of the cell is saturated. This may be the case for cisplatin-induced damage; 10 mM cisplatin is a dose that nearly saturates the repair capacity of the cell and gives near maximum levels of foci (Ref. 42 and data not shown). On the other hand, efficient induction of Rad51 foci by x-rays requires doses that cause at least a 50-fold loss of viability (Ref. 42 and data not shown). It is therefore possible that the repair capacity of the cell must be saturated for x-ray-induced complexes of Rad51 protein to be readily detected by our method. It should be noted, however, that there may be differences in the composition of Rad51-associated protein complexes that are induced by cisplatin and X-irradiation damage that may affect the appearance Rad51 foci.
The results presented here indicate that Xrcc3 promotes Rad51 focus formation in response to damage. It will be of interest to determine if Xrcc3 also promotes formation of the S-phase foci seen in lymphocytes and fibroblasts. It is unclear at present if the few foci we see in untreated CHO cells are equivalent to the S-phase foci seen in other cell types.

The possibility that Rad51 foci seen here correspond to sites of DNA damage is supported by observations made in normal meiotic yeast cells (37). First, ScRad51 and ScDmc1, two RecA homologs known to be required for repair of meiotic DSBs, colocalize with one another in wild type cells as expected if the foci mark sites where multiple recombination proteins are engaged in recombination. Second, the foci formed by RecA homologs appear specifically at the time of meiotic DSB repair. Third, mutational analysis shows that blocking DSB formation blocks the appearance of ScRad51 foci and that blocking DSB repair blocks the disappearance of foci. Although these results support the hypothesis that foci of RecA-like proteins can mark sites of ongoing recombination and repair, it has yet to be established if this is the case for damage-induced Rad51 foci in mammalian nuclei. A very recent study in which ultra-soft x-rays were used to damage subsections of nuclei did not yield evidence for localization of Rad51 foci to damaged regions, raising the possibility that assembly is not specific to damaged sites (57). It is possible however, that some or all of the Rad51 foci seen in these experiments were not induced by radiation but were instead the S-phase foci observed in untreated fibroblasts (38).

Rad51 foci form as a consequence of treatment with cisplatin, a DNA cross-linking agent that is widely used in chemotherapy. Although XRCC3 has been shown to rescue cellular sensitivity to cisplatin, demonstration of a similar function for Rad51 is difficult because the RAD51 gene is essential for viability. However, a role for mammalian Rad51 protein in

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**Fig. 5.** Murine scid fibroblasts and CHO xrs5 cells form Rad51 foci in response to X-irradiation. Cells were treated with x-rays and analyzed as described in the legend of Fig. 1. A, x-ray dose-response analysis of Rad51 focus formation from a normal mouse fibrosarcoma cell line 4102 and a scid mouse-derived fibroblast line. Quantitation is as described in the Fig. 2A legend. B, time course analysis of Rad51 focus formation from 4102 and scid cells. C, dose-response analysis of Rad51 foci from the radiation-resistant CHO parent cell line K1 and the radiation-sensitive derivative xrs5. D, time course analysis of Rad51 focus formation in CHO K1 and xrs5 cell lines.
promoting resistance to cisplatin is expected because such a role has been demonstrated for yeast Rad51 (20). The Rad51 focus assay provides a means of detecting the involvement of mammalian Rad51 in DNA damage responses and has been used to show mammalian Rad51 is involved in the response to ionizing radiation, UV irradiation, and the alkylating agent methylmethane sulfonate (38). Our experiments show that cisplatin induces Xrcc3-dependent Rad51 foci, indicating that mammalian Rad51 also plays a role in the response to this clinically important drug.

The failure of Rad51 to form damage-induced foci in irs1SF cells and the rescue of this defect by XRCC3 suggest that participation of Rad51 in the normal response to DNA damage requires Xrcc3. A mechanism through which Xrcc3 promotes Rad51 function is suggested by recent two-hybrid studies showing that HsRad51 protein and Xrcc3 protein interact with one another directly (49). The results presented here suggest that the Xrcc3-Rad51 interaction is biologically relevant and that Xrcc3 protein promotes the assembly of or stabilizes a higher order Rad51-containing structure required for DNA repair. A related situation applies in yeast where ScRad51 focus formation depends on the RAD52, RAD55, and RAD57 genes (50). These results, together with evidence for corresponding protein-protein interactions (10, 51–54) suggest that, in both yeast and mammalian cells, Rad51 must interact directly with other repair proteins to assemble during DNA repair.

The combination of cytology and genetics used here provides a general method for characterizing the pathway of assembly of DNA repair proteins in vivo. Of particular interest for future studies of mammalian cells are the proteins encoded by the breast cancer genes BRCA1 and BRCA2, whose products were recently shown to interact directly with Rad51 (55, 56).

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REFERENCES

1. Shinohara, A., and Ogawa, T. (1995) _Trends Biochem. Sci._ 20, 378–391
2. Roca, A. I., and Cox, M. M. (1991) _Crit. Rev. Biochem._ 26, 415–456
3. Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K., Lauder, S. D., and Rehrauer, W. M. (1994) _Microb. Res._ 142, 401–465
4. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) _DNA Repair and Mutagenesis._ pp. 435–440, American Society for Microbiology, Washington, D. C.
5. Stassen, N. Y., Logsdon, J. J., Vora, G. J., Offenberg, H. H., Palmer, J. D., and Zolan, M. E. (1997) _Curr. Genet._ 31, 144–157
6. Abouslekhra, A., Chanet, R., Adjiri, A., and Fabre, P. (1992) _Mol. Cell. Biol._ 12, 3224–3234
7. Basile, G., Alter, M., and Mortimer, R. K. (1992) _Mol. Cell. Biol._ 12, 3235–3246
8. Lovett, S. T. (1994) _Gène_ 142, 103–106
9. Bishop, D. K., Park, D., Xu, L., and Kleckner, N. (1992) _Cell_ 69, 439–456
10. Shinohara, A., Ogawa, H., and Ogawa, T. (1992) _Cell_ 69, 457–470
11. Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, K., Ikou, I., and Ogawa, T. (1993) _Nat. Genet._ 4, 239–43
12. Sato, S., Kobayashi, T., Hotta, Y., and Tabata, S. (1995) _DNA Repair_ 1, 24–57
13. Liu, N., Lamerding, J. E., Tebbs, R. S., Schild, D., Tucker, J. D., and Carrano, A. V. (1996) _Environ. Mutagen._ 27, (suppl.) 68