FAST TRACK

Xrcc3 Is Recruited to DNA Double Strand Breaks Early and Independent of Rad51

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Abstract   Rad51-mediated homologous recombination (HR) is essential for maintenance of genome integrity. The Xrcc3 protein functions in HR DNA repair, and studies suggest it has multiple roles at different stages in this pathway. Defects in vertebrate *XRCC3* result in elevated levels of spontaneous and DNA damage-induced chromosomal abnormalities, as well as increased sensitivity to DNA damaging agents. Formation of DNA damaged-induced nuclear Rad51 foci requires Xrcc3 and the other Rad51 paralog proteins (Rad51B, Rad51C, Rad51D, Xrcc2), thus supporting a model in which an early function of Xrcc3 involves promoting assembly of active Rad51 repair complexes. However, it is not known whether Xrcc3 or other Rad51 paralog proteins accumulate at DNA breaks, and if they do whether their stable association with breaks requires Rad51. Here we report for the first time that Xrcc3 forms distinct foci in human cells and that nuclear Xrcc3 begins to localize at sites of DNA damage within 10 min after radiation treatment. RNAi-mediated knock down of Rad51 has no effect on the DNA damage-induced localization of Xrcc3 to DNA breaks. Our data are consistent with a model in which Xrcc3 associates directly with DNA breaks independent of Rad51, and subsequently facilitates formation of the Rad51 nucleoprotein filament. J. Cell. Biochem. 93: 429–436, 2004.   © 2004 Wiley-Liss, Inc.

Key words: XRCC3; Rad51; Rad51 paralogs; double strand breaks; homologous recombination

HR is an important pathway in mammalian cells for the repair of DNA double-strand breaks (DSBs) that result from exposure to exogenous DNA damaging agents as well as during normal metabolic processes such as DNA replication and meiotic chromosomal alignment [Pierce et al., 1999; Thompson and Schild, 2001]. HR is essential for the maintenance of genome integrity, as defects in this pathway have been shown to result in chromosomal abnormalities that correlate with a number of cancers [Thompson and Schild, 2002]. In this pathway,

the ends of a DSB are processed by endonucleases to produce a ssDNA tail onto which the Rad51 recombinase is loaded to form a nucleoprotein filament. Rad51 provides the central activity of HR by catalyzing strand exchange between the damaged DNA and an undamaged homologous chromosome, most frequently a sister chromatid, resulting in the formation of cross-over structures referred to as Holliday junctions [Sung et al., 2003; West, 2003]. Genetic evidence demonstrates that successful initiation and completion of HR depends on the function of a group of structurally related proteins referred to as Rad51 paralogs; Rad51B, Rad51C, Rad51D, Xrcc2, and Xrcc3 [Tebbs et al., 1995; Liu et al., 1998; Morrison and Takeda, 2000; Takata et al., 2001; Thompson and Schild, 2001; Yoshihara et al., 2004]. For example, knock-out of each of the paralog genes in chicken DT40 cells resulted in increased sensitivity to DNA damaging agents and elevated levels of chromosomal abnormalities [Takata et al., 2001]. Specific protein–protein interactions between Rad51 paralog proteins have been demonstrated [Schild et al., 2000] and several complexes have been identified

[Kurumizaka et al., 2001; Masson et al., 2001a,b; Sigurdsson et al., 2001; Kurumizaka et al., 2002; Wiese et al., 2002].

Recent studies of Xrcc3 suggest that it has a remarkably diverse set of functions and acts both early and late in the HR pathway. Xrcc3 has been shown to play an active role in DNA damage-induced replication fork slowing, a function that occurs early in the HR pathway [Henry-Mowatt et al., 2004]. Defects in fork slowing in both hamster and chicken $XRCC3^{-/-}$ cells were corrected by either introduction of a human $XRCC3$ cDNA or by inclusion of purified human Rad51C–Xrcc3 complex in a permeabilized cell replication assay [Henry-Mowatt et al., 2004]. Several lines of work also suggest that Xrcc3 functions during the late stages of HR. For example, defective processing of recombination intermediates is observed in both hamster and $Arabidopsis$ $XRCC3^{-/-}$ cells [Bleuyard and White, 2004; Brenneman et al., 2004], and recent studies suggest that human Xrcc3 plays a direct role in Holliday junction resolution [Liu et al., 2004].

Another early function for Xrcc3 likely involves recruitment of Rad51 to the sites of DSBs. Many proteins directly involved in the catalysis of HR appear in distinct nuclear structures termed foci [Haaf et al., 1995; Liu and Maizels, 2000; Essers et al., 2002; Tan et al., 2004] and their accumulation at sites of DNA damage suggests that these are active centers of DNA repair [Raderschall et al., 1999; Tashiro et al., 2000; Aten et al., 2004]. To date, only Rad51 has been studied regarding the requirement of other factors for its appearance within DNA damaged-induced foci. For example, in hamster, chicken, and human cell lines, formation of damage-induced nuclear Rad51 foci requires Xrcc3 [Bishop et al., 1998; Takata et al., 2001; Yoshihara et al., 2004]. Studies using a chicken cell line show that formation of Rad51 foci also requires the other Rad51 paralog proteins [Takata et al., 2001]. Despite their requirement at this early step in the HR pathway, it has not been determined whether any of the five Rad51 paralog proteins accumulate at the site of a DNA break. In the present study, using a combination of immunostaining, transient expression of fluorescent fusion proteins and RNA interference, we show that Xrcc3 forms discrete nuclear foci that localize to DSBs, and that this occurs independent of Rad51.

MATERIALS AND METHODS

Cell Lines and Transfections

HEK293 cells were obtained from ATCC and were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% Pen/Strep. In preparation for transfection, cells were maintained in DMEM plus 10%FBS. Chinese hamster ovarian (CHO) irs1SF cells ($XRCC3^{-/-}$) were maintained in DMEM supplemented with 10% FBS, 1% Pen/Strep, and 1% non-essential amino acids. The $RAD51$-GFP plasmid was made by inserting the $HsRAD51$ gene into the SalI-AgeI sites in the multicloning site of pEGFP-N1 (Clontech, Palo Alto, CA). The $RAD51SM$-GFP (SM = silent mutations) was made by introducing several silent base changes in the siRNA targeted region of the wild type $RAD51$ sequence. Transfection of both a Rad51-specific siRNA duplex (Qiagen, Studio City, CA) and plasmids was performed using a lipid transfection method (Lipofectamine 2000, Invitrogen, San Diego, CA). A control siRNA against lamin A/C and a 3′-fluorescein labeled control siRNA with no matches in human genome (Qiagen, cat# 1022079) showed no effect on Rad51 or Xrcc3 focus formation, or protein levels as determined by Western blots, when transfected into HEK293 cells (data not shown). When used together the siRNA and plasmid were co-transfected.

Antibodies

Primary antibodies used for immunofluorescence staining were mouse anti-phospho-histone H2AX biotin conjugate (clone JBW301, Upstate Biotechnology, Lake Placid, NY), mouse anti-Rad51 (clone 3C10, Upstate Biotechnology), mouse anti-Xrcc3 (10F1/6, Novus Biologicals, Inc., Littleton, CO) and all were diluted 1:500. Alexa 488 and Alexa 555 (Molecular Probes, Eugene, OR), and Immunopure Streptavidin Rhodamine Conjugate (Pierce, Rockford, IL) secondary antibodies were diluted 1:1,000. All dilutions were in PBS containing 1% BSA. DNA was counterstained with Vectashield Fluorescent Mounting Media containing DAPI (4′,6′-diamidino-2-phenylindole; Vector Laboratories, Burlingame, CA).

Immunostaining and Confocal Microscopy

Cells were grown on coverslips in a 6 well dish. For fixation, media was aspirated off and cells were washed once with PBS and immersed in

100% methanol at $-20°C$ for 5 min. Cells were blocked in PBS containing 4% BSA overnight at $4°C$. After blocking, cells were washed five times for 5 min each with PBS. Incubation with both primary and secondary antibodies was performed in 6 well dishes for 1 h at $37°C$ in a humid environment using a slide warmer (Fisher, Medford, MA). Cells were washed five times for 5 min each in PBS after incubation with both primary and secondary antibodies. Coverslips were mounted using Vectashield with DAPI and sealed with polyurethane (nail polish) then stored in the dark at $4°C$. Visualization of immunostains was performed by confocal microscopy using a Leica TCS SP2 AOBS instrument and image processing was performed using the accompanying Leica Confocal Software TCS SP2.

Damage-Induced DNA Double Strand Breaks

Cells were exposed to 10 Gy ionizing radiation (IR) (^{137}Cs) using a Gammacell 40 (MDS Nordion Ottawa, Ont., Canada). After exposure, cells were allowed to recover at $37°C$ (5% CO_2) for the indicated times. Cells were then methanol fixed and prepared for immunostaining.

Western Blotting

HEK293 cells were transfected with the appropriate transgene and/or siRNA as indicated (Fig. 2A). Cells were harvested 20 h post transfection washed with PBS and lysed with RIPA buffer (25 mM Tris pH 7.4, 0.5% triton X-100, 0.5% sodium deoxycholate, 0.05% sodium dodecyl sulfate, 0.05 mM EDTA pH 7.0, 75 mM NaCl) and total protein was determined with BCA Protein Assay Kit (Pierce). Acrylamide mini-gels (10%) were run with 80 µg of total protein in each lane and transferred to PVDF membranes overnight at 200 mV in transfer buffer (192 mM glycine, 25 mM Tris, 20% methanol). Membranes were incubated in blocking buffer (10 mM Tris-HCl pH 8.0, 300 mM NaCl, 0.025% Tween 20) containing 15% instant nonfat dry milk for 45 min. Rad51 primary antibodies (Oncogene #PC130) were added (1:3,000) in blocking buffer containing 2% instant nonfat dry milk for 1 h and membranes were washed five times for 5 min each in blocking buffer. Peroxidase conjugated anti-rabbit secondary antibodies (Pierce #31462) were added (1:12,000) for 1 h and membranes washed as above. Membranes were incubated with LumiGLO chemiluminescent substrate (KPL #54-61-01) for 1 min, exposed to X-ray film, and developed (Kodak 2000A XOMAT processor).

RESULTS

Formation of Xrcc3 Foci Is Independent of Rad51

Immunostaining of HEK293 cells shows the presence of Rad51 in both the cytoplasm and nucleus (Fig. 1A–C), consistent with previous results [Yoshikawa et al., 2000]. Exposure to IR (10 Gy) results in an increase in both the number and size of nuclear Rad51 foci (Fig. 1, compare panels B and E). At the 1 h time point shown in Figure 1 some of the nuclear Rad51 has begun to co-localize with γH2AX (Fig. 1D–F), a phosphorylated histone variant (H2AX) that serves as a marker for sites of DSBs [Rogakou et al., 1998]. Our data in Figure 1 also reveal for the first time the presence of Xrcc3 foci. We find that discrete, small Xrcc3 foci are present in both the cytoplasm and nucleus in the absence of DNA damage (Fig. 1M–O). Similar to Rad51, following exposure to IR nuclear Xrcc3 foci are larger, greater in number, and begin to localize to DNA break sites within 1 h (Fig. 1P–R). Therefore, it appears that exposure to IR results in the redistribution of nuclear Xrcc3 to sites of DNA damage, and that Rad51 and Xrcc3 accumulate at the sites of DNA breaks.

Previous studies have shown that Xrcc3 is required for formation of Rad51 nuclear foci upon DNA damage [Bishop et al., 1998; Takata et al., 2001; Yoshihara et al., 2004]. Our results above provide the first evidence that this function of Xrcc3 is likely to occur directly at the site of the DNA break. This, together with the fact that Xrcc3 interacts directly with Rad51 [Schild et al., 2000], raises the question of whether stable association of Xrcc3 with DNA break sites requires the presence of Rad51. Therefore, we asked whether the DNA damage-induced localization of Xrcc3 to the sites of DSBs is dependent on Rad51. HEK293 cells transfected with a Rad51-specific siRNA showed a significant reduction in the amount of Rad51 prior to treatment of cells with IR (Fig. 1G–I) and eliminated greater than 90% of the damage-induced nuclear Rad51 foci (Fig. 1J–L). However, treatment of cells with Rad51 siRNA had no visible effect on the Xrcc3 staining pattern either before or after DNA damage. Xrcc3 still formed small pre-damage foci in both the
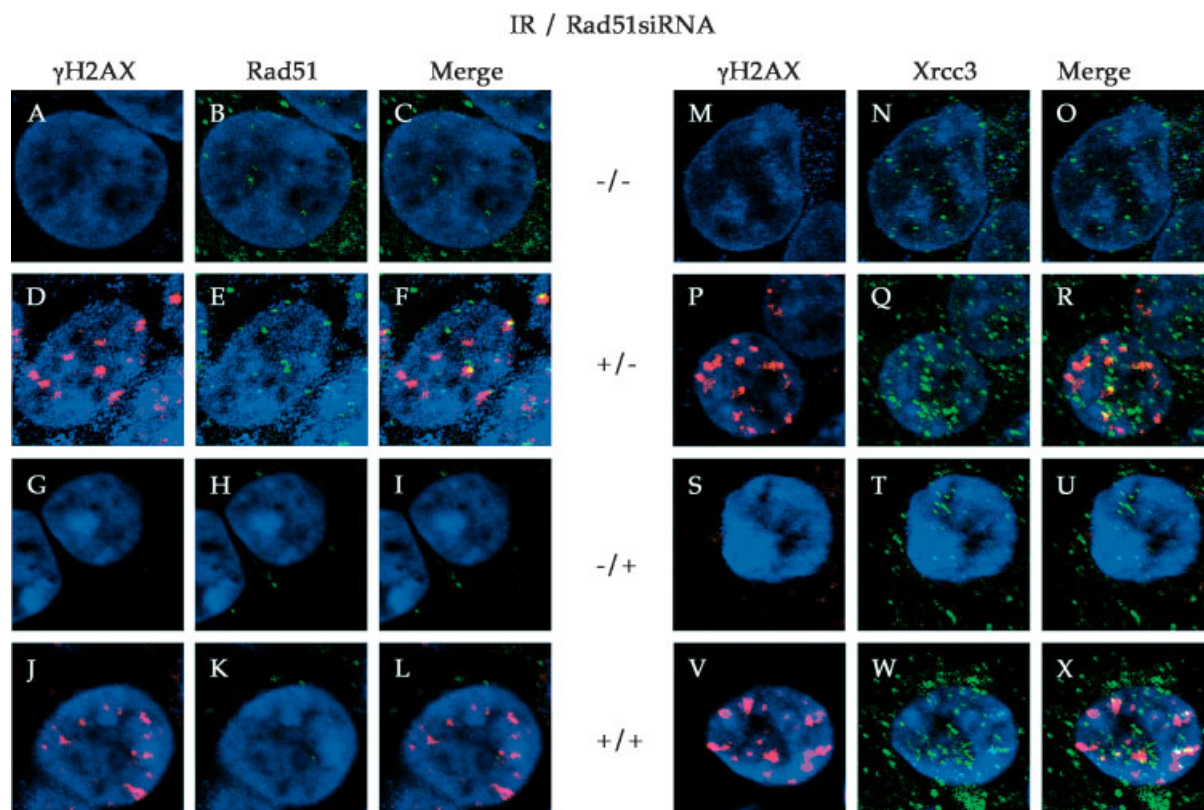
IR / Rad51siRNA

Fig. 1. Localization of Xrcc3 to DNA double strand breaks does not require Rad51. HEK293 cells were immunostained for γH2AX (red) and either Rad51 (green) or Xrcc3 (green). Nuclei are stained with DAPI (blue). Where indicated, cells were exposed to 10 Gy ionizing radiation (IR+), and were fixed 60 min after exposure. Cells treated with Rad51-specific siRNA (Rad51 siRNA+) were transfected 16 h prior to IR exposure. Cells in each panel are typical of >90% of the total observed in a minimum of three independent experiments.

cytoplasm and nucleus (Fig. 1S–U). Following treatment with IR, nuclear Xrcc3 foci increased in both number and size, and showed a partial localization to DSBs similar to that seen in the presence of Rad51 (Fig. 1V–X). As expected, treatment of cells with Rad51-specific siRNA prohibited resolution of DSBs and resulted in cell death (data not shown).

The specificity of the Rad51 siRNA was confirmed using a *RAD51-GFP* transgene carrying silent mutations (*RAD51SM-GFP*) rendering it immune to the action of the siRNA. Western blot analysis shows that co-transfection of the siRNA and Rad51SM-GFP depletes the cells of ≥90% of the endogenous Rad51 while expression of Rad51SM-GFP is maintained (Fig. 2A). The absence of Rad51 foci in siRNA treated HEK293 cells was rescued by co-transfection with *RAD51SM-GFP* which forms DNA damage-induced nuclear foci similar to those seen with the endogeneous protein (Figs. 2B and 1J–L). We confirmed the functional relevance of the Rad51SM-GFP protein in

the early stages of HR by recapitulating previous results using the Xrcc3-deficient irs1SF hamster cell line [Bishop et al., 1998]. Expression of Rad51SM-GFP in irs1SF cells resulted in formation of no damage-induced Rad51 foci (Fig. 2C). However, co-expression with Hs*XRCC3* cDNA resulted in recovery of Rad51 focus formation following exposure to IR (Fig. 2C). The fact that Xrcc3 localizes to DSBs independent of Rad51 prompted us to look into the timing of DNA damage-induced Xrcc3 and Rad51 focus formation.

Rad51 and Xrcc3 Localize to Sites of DNA Breaks Within 10 Min Following Exposure to γ-Irradiation

If Xrcc3 is required for damage-induced Rad51 focus formation, but not the converse, then Xrcc3 may be localized to the site of a DSB prior to Rad51 in order to facilitate recruitment of Rad51. To observe the timing of Rad51 and Xrcc3 focus formation, HEK293 cells were fixed at 10, 30, and 60 min following exposure to 10 Gy
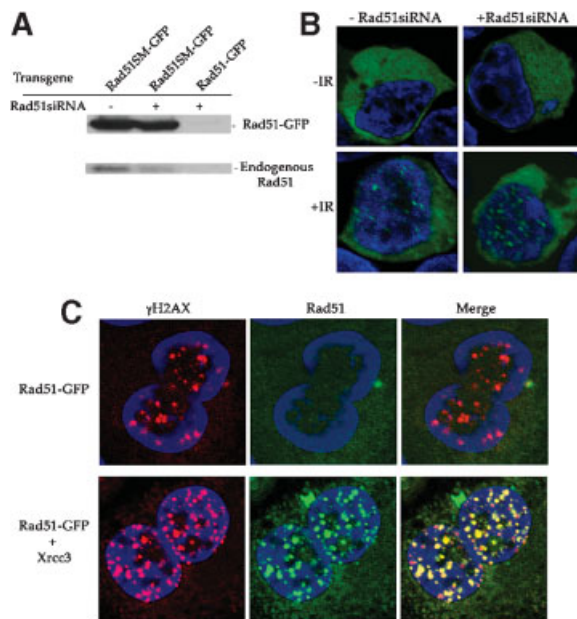
Fig. 2. RNAi-mediated knock-down of Rad51 and rescue using a Rad51–GFP fusion protein. **A**: Western blot showing expression of both endogeneous Rad51 and the Rad51SM–GFP protein (**lane 1**), specific RNAi-mediated knock-down of endogeneous Rad51 but not the fusion protein carrying silent mutations in the targeted region of the Rad51 sequence, Rad51SM–GFP (**lane 2**) and RNAi knock-down of both endogeneous Rad51 and a Rad51–GFP fusion expressed from a wild type *RAD51-GFP* gene (**lane 3**). **B**: HEK293 cells transfected with a plasmid carrying the *RAD51SM-GFP* transgene were co-transfected with a Rad51-specific siRNAi duplex and treated with 10 Gy IRas indicated. Cells exposed to radiation were incubated for 60 min before fixation. **C**: *XRCC3⁻/⁻* Chinese hamster ovary cells (irs1SF) were transfected with a plasmid carrying the *RAD51SM-GFP* transgene or co-transfected with this plasmid and another carrying the wild type *HsXRCC3* gene. Cells were exposed to 10 Gy IR and grown for 60 min before fixation.

Fig. 3. Rad51 and Xrcc3 localize to DNA double strand breaks within 10 min following exposure to IR. HEK293 cells were fixed 10, 30, and 60 min following exposure to 10 Gy IR. Cells were immunostained for γH2AX (red) and Rad51 (green) in the left three panels, and for γH2AX (red) and Xrcc3 (green) in the right three panels. Profiles at the right of each image indicate the fluorescence intensity of the green and red channels (measured independently) along the yellow line in the corresponding image.

IR, and immunostained for endogenous Rad51 and Xrcc3. We find that both Xrcc3 and Rad51 nuclear foci begin to appear within 10 min after treatment with IR, and these foci show a partial co-localization with DSBs as indicated by γH2AX foci (Fig. 3). The incidence of co-localization for several γH2AX foci (red) and either Rad51 or Xrcc3 foci (green) is displayed in the profiles to the right of each image in Figure 3. The fluorescence intensity (y-axis) is plotted against the position of the focus along the yellow line in the image (x-axis). The only other report of Rad51 focus formation at early times after damage (<60 min) noted that approximately 1% of human fibroblast cells (line Hs68) showed DNA damage-induced nuclear Rad51 foci 10 min after exposure to 9 Gy γ-irradiation [Haaf et al., 1995]. However,
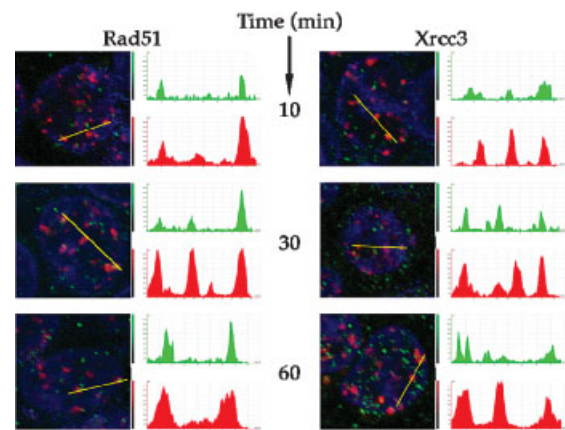
we find that over 80% of HEK293 cells show an increase in nuclear Rad51 foci 10 min after exposure to 10 Gy γ-irradiation. The importance of our results lies in the fact that we observe a partial co-localization of these foci specifically to the site of DNA breaks and that both Rad51 and Xrcc3 begin to associate with breaks as early as 10 min post DNA damage. Under the conditions used we cannot determine whether both Rad51 and Xrcc3 are associated together at any particular γH2AX focus, but this method is currently under development.

DISCUSSION

The data presented here provide new insights into a developing model regarding the dynamics of recombinational repair proteins [Essers et al., 2002; Yu et al., 2003; Aten et al., 2004] and the function of Rad51 paralogs. We provide the first demonstration of DNA damage-inducible redistribution of Xrcc3 within the cell and formation of Xrcc3 nuclear foci that localize to the sites of DSBs. Additionally, we show that both Xrcc3 and Rad51 appear at the sites of breaks within 10 min following exposure to IR. Importantly, we demonstrate that formation of DNA break-associated nuclear Xrcc3 foci occurs independent of Rad51. Within the context of other studies regarding the function of Rad51 paralog proteins and the dynamics of recombinational
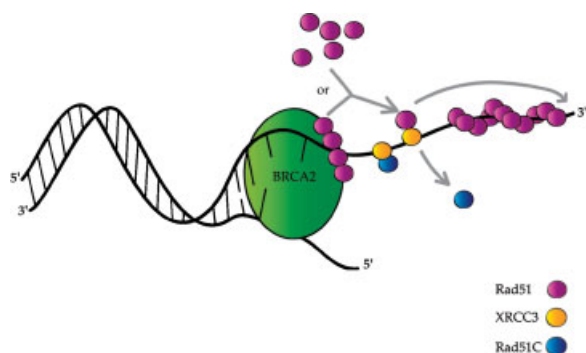
Fig. 4. Model for an early function of Xrcc3 in the homologous recombination pathway. Given that our data shows Xrcc3 focus formation at the site of a DNA break 10 min after exposure to IR, it is likely that at least one function of Xrcc3 occurs directly at the site of the break. The Rad51C/Xrcc3 heterodimer binds single-strand DNA [Masson et al., 2001a] at the break and through direct interactions between Xrcc3 and Rad51, mediates the formation of the Rad51 nucleoprotein filament. Rad51 that forms foci within 10 min after DNA damage is likely derived from a mobile pool of Rad51 [Yu et al., 2003] whereas BRCA2-bound Rad51 [Yu et al., 2003] may contribute to formation of Rad51 foci at later times.

repair proteins following DNA damage, our data supports the following model (Fig. 4) for the action of Xrcc3 early in the HR pathway.

The fact that formation of DNA damage-induced Rad51 nuclear foci requires the presence of Xrcc3 [Bishop et al., 1998; Takata et al., 2001; Yoshihara et al., 2004], and that we now show Xrcc3 beginning to localize with DNA breaks within 10 min following exposure to DNA damage, strongly suggests that Xrcc3 acts specifically at the site of a DNA break to assist in the formation of Rad51 foci, most likely by promoting assembly of the Rad51 nucleoprotein filament. Yu et al. [2003] recently reported that approximately 20% of nuclear Rad51 is sequestered in an immobile complex by virtue of its association with BRCA2, and that it is this pool of Rad51 that is selectively mobilized following replication arrest. However, this mobilization is preceded by a 60–75 min delay. We find that DNA break-associated Rad51 foci form within the first 10 min following DNA damage, suggesting that the Rad51 in these early foci is derived from a pool other than that associated with BRCA2, perhaps either the fraction that is immobile due to Rad51 self-association or the approximate 60% of Rad51 that constitutes the mobile fraction [Yu et al., 2003]. Because Xrcc3 forms nuclear foci at the sites of breaks independent of Rad51 we propose that specific protein–protein interactions between Xrcc3

and Rad51 at the DNA break site are directly involved in formation of a Rad51 nucleoprotein filament. This function of Xrcc3 would not necessarily require that it arrives earlier than Rad51 at a DNA break site. We find that both proteins are associated with DNA breaks within 10 min after IR treatment, but earlier measurements will be needed to provide more detail about the kinetics of the association of repair proteins with DNA breaks. Whether the BRCA2-associated Rad51 is delivered directly to the site of damage, or upon damage BRCA2 releases bound Rad51 into the mobile pool, specific association of Rad51 with Xrcc3 would again promote assembly of Rad51 into nucleo-protein filaments. Although we currently have no information on the cellular localization of Rad51C either before or after DNA damage, we have included Rad51C in the model (Fig. 4) because Xrcc3 forms a specific heterodimeric complex with this protein [Masson et al., 2001b; Wiese et al., 2002]. The Rad51C/Xrcc3 dimer associates with single-stranded but not double-stranded DNA [Masson et al., 2001a], and therefore may be able to rapidly associate with the single-stranded regions that appear early at a break. Recent data shows that the stability of the Rad51C/Xrcc3 dimer is regulated by ATP binding and hydrolysis by Xrcc3 [Yamada et al., 2004]. Given that Rad51 and each of the five Rad51 paralog proteins has an ATP binding site, regulation of the variety of protein–protein interactions required for the establishment of active repair complexes early in the HR pathway may be mediated by specific ATP binding and turnover events. In fact, recent studies by Shim et al. [2004] suggest that Xrcc2 acts as an NTP exchange factor by stimulating ATP processing by Rad51 in a Rad51D-dependent manner.

We consistently find a larger number of Xrcc3 foci relative to Rad51 foci in the nucleus as well as the cytoplasm both before and after exposure to DNA damage. Although we currently do not understand the relationship between these differences in number and localization, further studies are designed to address this issue. While much remains to be discovered about the dynamics and function of recombinational repair proteins, work in this study provides important new information about the DNA damage-induced redistribution and site of action of the Xrcc3 protein early in the homologous recombinational DNA repair pathway.

ACKNOWLEDGMENTS

REFERENCES

Aten JA, Stap J, Krawczyk PM, van Oven CH, Hoebe RA, Essers J, Kanaar R. 2004. Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. Science 303:92–95.

Bishop DK, Ear U, Bhattacharyya A, Calderone C, Beckett M, Weichselbaum RR, Shinohara A. 1998. Xrcc3 is required for assembly of Rad51 complexes in vivo. J Biol Chem 273:21482–21488.

Bleuyard J-Y, White CI. 2004. The Arabidopsis homologue of Xrcc3 plays an essential role in meiosis. EMBO J 23:439–449.

Brenneman MA, Wagener BM, Miller CA, Allen C, Nickoloff JA. 2004. XRCC3 controls the fidelity of homologous recombination: Roles for XRCC3 in late stages of recombination. Mol Cell 10:387–395.

Essers J, Houtsmuller AB, van Veelen L, Paulusma C, Nigg AL, Pastink A, Vermeulen W, Hoeijmakers JHJ, Kanaar R. 2002. Nuclear dynamics of RAD52 group homologous recombination proteins in response to DNA damage. EMBO J 21:2030–2037.

Haaf T, Golub EI, Reddy G, Readdin CM, Ward DC. 1995. Nuclear foci of mammalian Rad51 recombination protein in somatic cells after DNA damage and its localization in synaptonemal complexes. Proc Natl Acad Sci USA 92:2298–2302.

Henry-Mowatt J, Jackson D, Masson JY, Johnson PA, Clements CM, Benson FE, Thompson LH, Takeda S, West SC, Caldecott KW. 2004. XRCC3 and Rad51 modulate replication fork progression on damaged vertebrate chromosomes. Mol Cell 11:1109–1117.

Kurumizaka H, Ikawa S, Nakada M, Eda K, Kagawa W, Takata M, Takeda S, Yokoyama S, Shibata T. 2001. Homologous-pairing activity of the human DNA-repair proteins Xrcc3 · Rad51C. Proc Natl Acad Sci USA 98:5538–5543.

Kurumizaka H, Ikawa S, Nakada M, Enomoto R, Kagawa W, Kinebuchi T, Yamazoe M, Yokoyama S, Shibata T. 2002. Homologous pairing and ring and filament structure formation activities of the human Xrcc2 · Rad51D complex. J Biol Chem 277:14315–14320.

Liu Y, Maizels N. 2000. Coordinated response of mammalian Rad51 and Rad52 to DNA damage. EMBO Rep 1:85–90.

Liu N, Lamerdin JE, Tebbs RS, Schild D, Tucker JD, Shen RM, Brookman KW, Siciliano MJ, Walter CA, Fan W, Narayana LS, Zhou Z-Q, Adamson AW, Sorenson KJ, Chen DJ, Jones NJ, Thompson LH. 1998. XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. Mol Cell 1:783–793.

Liu Y, Masson JY, Shah R, O'Regan P, West SC. 2004. RAD51C is required for holliday junction processing in mammalian cells. Science 303:243–246.

Masson JY, Stasiak AZ, Stasiak A, Benson FE, West SC. 2001a. Complex formation by the human RAD51C and XRCC3 recombination repair proteins. Proc Natl Acad Sci USA 98:8440–8446.

Masson JY, Tarsounas MC, Stasiak AZ, Stasiak A, Shah R, McIlwraith MJ, Benson FE, West SC. 2001b. Identification and purification of two distinct complexes containing the five RAD51 paralogs. Genes Dev 15:3296–3307.

Morrison C, Takeda S. 2000. Genetic analysis of homologous DNA recombination in vertebrate somatic cells. Int J Biochem Cell Biol 32:817–831.

Pierce AJ, Johnson RD, Thompson LH, Jasin M. 1999. XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev 13:2633–2638.

Raderschall E, Golub EI, Haaf T. 1999. Nuclear foci of mammalian recombination proteins are located at single-stranded DNA regions formed after DNA damage. Proc Natl Acad Sci USA 96:1921–1926.

Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. 1998. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 273:5858–5868.

Schild D, Lio YC, Collins DW, Tsomondo T, Chen DJ. 2000. Evidence for simultaneous protein interactions between human Rad51 paralogs. J Biol Chem 275:16443–16449.

Shim KS, Schmutte C, Tombline G, Fishel R. 2004. hXRCC2 enhances ADP/ATP processing and strand exchange by hRAD51. J Biol Chem 279:30385–30394.

Sigurdsson S, Van Komen S, Bussen W, Schild D, Albala JS, Sung P. 2001. Mediator function of the human Rad51B–Rad51C complex in Rad51/RPA-catalyzed DNA strand exchange. Genes Dev 15:3308–3318.

Sung P, Krejci L, Van Komen S, Sehorn MG. 2003. Rad51 recombinase and recombination mediators. J Biol Chem 278:42729–42732.

Takata M, Sasaki MS, Tachiiri S, Fukushima T, Sonoda E, Schild D, Thompson LH, Takeda S. 2001. Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs. Mol Cell Biol 21:2858–2866.

Tan TL, Essers J, Citterio E, Swagemakers SM, de Wit J, Benson FE, Hoeijmakers JH, Kanaar R. 2004. Mouse Rad54 affects DNA conformation and DNA-damage-induced Rad51 foci formation. Curr Biol 9:325–328.

Tashiro S, Walter J, Shinohara A, Kamada N, Cremer T. 2000. Rad51 accumulation at sites of DNA damage and in postreplicative chromatin. J Cell Biol 150:283–292.

Tebbs RS, Zhao Y, Tucker JD, Scheerer JB, Siciliano MJ, Hwang M, Liu N, Legerski RJ, Thompson LH. 1995. Correction of chromosomal instability and sensitivity to diverse mutagens by a cloned cDNA of the XRCC3 DNA repair gene. Proc Natl Acad Sci USA 92:6354–6358.

Thompson LH, Schild D. 2001. Homologous recombinational repair of DNA ensures mammalian chromosome stability. Mutat Res 477:131–153.

Thompson LH, Schild D. 2002. Recombinational DNA repair and human disease. Mutat Res 509:49–78.

West SC. 2003. Molecular views of recombination proteins and their control. Nat Rev Mol Cell Biol 4:435–445.

Wiese C, Collins DW, Albala JS, Thompson LH, Kronenberg A, Schild D. 2002. Interactions involving the Rad51

paralogs Rad51C and XRCC3 in human cells. Nucleic Acids Res 30:1001–1008.

Yamada NA, Hinz JM, Kopf VL, Segalle KD, Thompson LH. 2004. XRCC3 ATPase activity is required for normal XRCC3–Rad51C complex dynamics and homologous recombination. J Biol Chem 279:23250–23254.

Yoshihara T, Ishada M, Kinomura A, Katsura M, Takanori T, Tashiro S, Toshimasa A, Miyagawa K. 2004. XRCC3 deficiency results in a defect in recombination and increased endoreduplication in human cells. EMBO J 23:670–680.

Yoshikawa K, Ogawa T, Baer R, Hemmi H, Honda K, Yamauchi A, Inamoto T, Ko K, Yazumi S, Motoda H, Kodama H, Noguchi S, Gazdar AF, Yamaaoka Y, Takahashi R. 2000. Abnormal expression of BRCA1 and BRCA1-interactive DNA-repair proteins in breast carcinomas. Int J Cancer 88:28–36.

Yu DS, Sonoda E, Takeda S, Huang C, Pelligrini L, Blundell T, Venkitaraman A. 2003. Dynamic control of Rad51 recombinase by self-association and interaction with BRCA2. Mol Cell 12:1029–1041.