

UC Irvine

UC Irvine Previously Published Works

Title

Independent and sequential recruitment of NHEJ and HR factors to DNA damage sites in mammalian cells.

Permalink

<https://escholarship.org/uc/item/9br6c8mj>

Journal

The Journal of cell biology, 170(3)

ISSN

0021-9525

Authors

Kim, Jong-Soo
Krasieva, Tatiana B
Kurumizaka, Hitoshi
[et al.](#)

Publication Date

2005-08-01

DOI

10.1083/jcb.200411083

Peer reviewed

Independent and sequential recruitment of NHEJ and HR factors to DNA damage sites in mammalian cells

Jong-Soo Kim,¹ Tatiana B. Krasieva,² Hitoshi Kurumizaka,³ David J. Chen,⁴ A. Malcolm R. Taylor,⁵ and Kyoko Yokomori¹

¹Department of Biological Chemistry, School of Medicine, and ²Beckman Laser Institute, Department of Surgery, Laser Microbeam and Medical Program, University of California, Irvine, Irvine, CA 92697

³Department of Electrical Engineering and Bioscience, School of Science and Engineering, Waseda University, Tokyo 169-8555, Japan

⁴Molecular Radiation Biology Division, Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, TX 75390

⁵Cancer Research UK Institute for Cancer Studies, University of Birmingham, Edgbaston, Birmingham B15 2TT, England, UK

Damage recognition by repair/checkpoint factors is the critical first step of the DNA damage response. DNA double strand breaks (DSBs) activate checkpoint signaling and are repaired by nonhomologous end-joining (NHEJ) and homologous recombination (HR) pathways. However, *in vivo* kinetics of the individual factor responses and the mechanism of pathway choice are not well understood. We report cell cycle and time course analyses of checkpoint activation by ataxia-telangiectasia mutated and damage site re-

ruitment of the repair factors in response to laser-induced DSBs. We found that MRN acts as a DNA damage marker, continuously localizing at unrepaired damage sites. Damage recognition by NHEJ factors precedes that of HR factors. HR factor recruitment is not influenced by NHEJ factor assembly and occurs throughout interphase. Damage site retention of NHEJ factors is transient, whereas HR factors persist at unrepaired lesions, revealing unique roles of the two pathways in mammalian cells.

Introduction

DNA double strand breaks (DSBs) that are induced by endogenous and exogenous causes have deleterious effects on genome stability. To ensure efficient repair, DSBs activate checkpoint signaling to halt the cell cycle and inhibit DNA replication (Shiloh, 2003). Ataxia-telangiectasia (A-T) mutated (A-TM), a member of the PI3 kinase-related protein kinases (PIKKs; Abraham, 2004), is a major DSB signal transducer and is critical for activating the G1/S, S, and G2/M checkpoints. A-TM phosphorylates several target proteins that are critical for checkpoint signaling, such as Chk2 (Ahn et al., 2000). DSBs are repaired by two major pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR), which require distinct sets of factors (Lieber et al., 2003; West, 2003). DNA-dependent protein kinase catalytic subunit (DNA-PKcs)

and Ku are recruited to DNA ends to initiate the NHEJ cascade, which is followed by recruitment of the XRCC4–ligase IV complex. Rad51 and replication protein A (RPA) are essential factors for the HR pathway in vertebrate cells and are recruited to single-stranded DNA (ssDNA) regions at broken DNA ends to catalyze invasion of ssDNA into the homologous DNA template (Sung, 1994; Baumann et al., 1996). The trimeric complex Mre11–Rad50–Nbs1 (MRN) functions at an early stage of HR (Paull and Gellert, 1998) as well as in A-TM-mediated checkpoint activation in mammalian cells (Carson et al., 2003; Uziel et al., 2003; Costanzo et al., 2004; Horejsi et al., 2004; Lee and Paull, 2004, 2005). These checkpoint and repair factors all contribute to protecting the genome against DSBs. However, the *in vivo* kinetics and cell cycle specificity of damage response by these factors and the relationship between the two DSB repair pathways are not well understood.

Previously, we used 532 nm of second harmonic pulsed Q-switched Nd:YAG (yttrium-aluminum garnet) laser microbeam irradiation (“laser scissors”) to induce multiple DNA breaks at defined regions in the cell nucleus. We demonstrated the immediate recruitment of several DSB factors, including MRN, and the sister chromatid cohesion factor cohesin to the site of damage (Kim et al., 2002). Cohesin recruitment was recently confirmed by chromatin cross-linking and immunoprecipitation analysis of endonuclease cut sites in yeast (Ström

Correspondence to Kyoko Yokomori: kyokomor@uci.edu

Jong-Soo Kim's present address is Neuroscience Research Institute, Gachon Medical School, Namdong-gu, Incheon 405-760, Korea.

Abbreviations used in this paper: ad, after damage; A-T, ataxia-telangiectasia; A-TLD, A-T-like disorder; A-TM, A-T mutated; A-TR, A-T and Rad3 related; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSB, DNA double strand break; HR, homologous recombination; IR, ionizing radiation; IRIF, IR-induced foci; Nd:YAG, neodymium/yttrium-aluminum garnet; NHEJ, non-homologous end joining; MRN, Mre11–Rad50–Nbs1; PARP-1, poly(ADP-ribose) polymerase 1; PIKK, PI3 kinase-related protein kinase; RPA, replication protein A; ssDNA, single-stranded DNA.

The online version of this article contains supplemental material.

et al., 2004; Ünal et al., 2004). By using the laser system, we report a cell cycle and time course analysis of checkpoint signaling and DSB factor assembly at the damage sites. Our results demonstrate different timing and durations of damage recognition by MRN, NHEJ, and HR factors, providing new insight into the unique roles of MRN and the two repair pathways in mammalian cells.

Results and discussion

Laser-induced damage causes checkpoint activation and cell cycle delay in human cells

Chk2 was phosphorylated at the site of damage, which is consistent with the immediate recruitment of A-TM and A-TM and Rad3 related (A-TR) to the laser-induced damage site (Fig. 1 A). Phosphorylation of Chk2 occurred in A-TM-inactive A-T cells (Fig. 1 B). This is a result of the redundant function of other PIKKs such as A-TR (Shiloh, 2003), which was suppressed by further treatment with wortmannin (Fig. 1 B). Chk2 was phosphorylated immediately at the damage sites in both G1 and S/G2 phases (Fig. 1 C). Chk2 phosphorylation was observed as foci throughout the nucleus by 2 h postdamage induction, which was particularly prominent in G1 phase. Thus, Chk2 phosphorylation initially occurs at the damage sites and subsequently spreads to the nucleus. Similar spreading was observed with UV laser-induced DSB damage (Lukas et al., 2003). The results demonstrate that Nd:YAG laser-induced damage evokes a checkpoint response and that checkpoint signaling initially occurs at the damage sites and is followed by dissemination throughout the nucleus.

Consistent with the presence of DSBs, H2AX phosphorylation (γ H2AX) was observed at the damage site immediately after damage induction (Fig. 2 A). γ H2AX persisted even after 24 h after damage (ad), indicating the presence of unrepaired damage in the irradiated region. Concomitantly, a significant cell cycle delay was observed (Fig. 2 B). Although the adjacent undamaged cells continued to divide, damaged cells remained in interphase for >40 h, and some eventually entered mitosis (Fig. 2 B, Caffeine [−]). When cells were treated with caffeine, however, all the damaged cells underwent and successfully completed mitosis within the first 18 h in a manner similar to the adjacent undamaged cells (Fig. 2 B, Caffeine [+]). These results demonstrate that although some DSBs are sustained, laser-induced damage is not lethal and causes checkpoint-dependent cell cycle delay.

Immediate and independent recruitment of MRN and NHEJ factors in G1 and S/G2 phases

We observed that Mre11 and Ku were recruited immediately to the damage sites (Fig. 3; Kim et al., 2002). Neither Ku nor Mre11 was depleted by laser-induced DSBs because comparable amounts of Ku and Mre11 were detected at two successively induced damage sites (Fig. 3 A).

Mre11 was recruited to damage sites in both G1 and S/G2 phases (Fig. 3 B), as were its partners Rad50 and Nbs1 (not

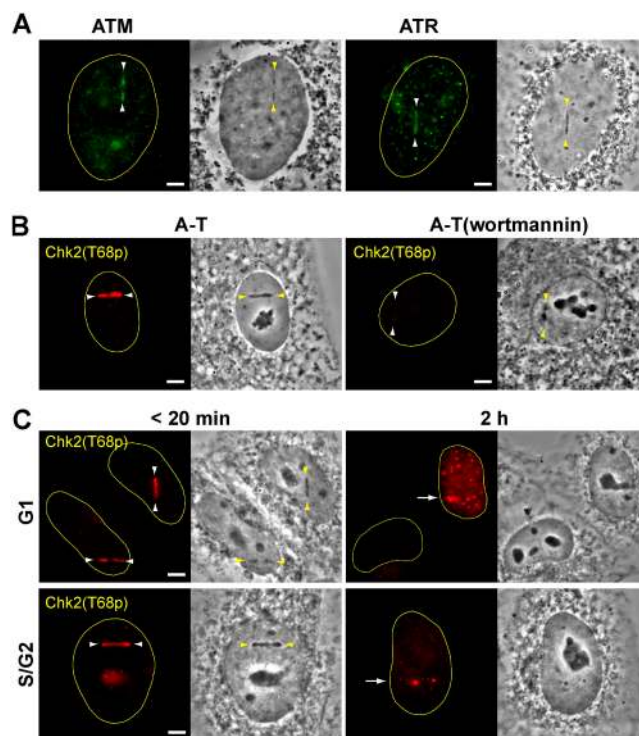


Figure 1. A-TM-mediated checkpoint signaling. (A) Recruitment of A-TM and A-TR to damage sites. Cells were fixed within 20 min ad and were stained. Damage sites are indicated by arrowheads. (B) Chk2 phosphorylation detected by anti-Chk2 (T68P) antibody in A-T cells in the absence or presence of wortmannin. (C) Chk2 phosphorylation in G1 and S/G2 phases. Cells damaged at G1 or S/G2 were fixed at the indicated time points ad for immunostaining. Arrowheads and arrows indicate damage sites. Bars, 5 μ m.

depicted). Similarly, the NHEJ factors Ku, DNA-PKcs, and ligase IV immediately clustered to damage sites in both G1 and S/G2 phases, supporting the notion that NHEJ is active in both phases (Fig. 3 B and Fig. S1 A, available at <http://www.jcb.org/cgi/content/full/jcb.200411083/DC1>; Rothkamm et al., 2003). Despite similar timing, recruitment of MRN and Ku were independent of each other (Fig. 3 C). Mutation of Mre11 or Nbs1 did not affect the localization of Ku. Conversely, Mre11 recruitment was intact in Ku knockout cells.

Although Ku binding to the endonuclease-induced damage site was demonstrated *in vivo* by chromatin immunoprecipitation analysis (Martin et al., 1999), the localization of NHEJ factors at ionizing radiation (IR)-induced damage sites has not been cytologically detected (Jakob et al., 2002; Lisby et al., 2004). This was thought to be caused by subdetectable amounts of recruited NHEJ factors (Jakob et al., 2002; Lisby et al., 2004). The number of DNA breaks that are induced by the Nd:YAG laser should be substantial and are likely comparable with those induced by a UV laser (in combination with halogenated nucleotides). This is based on γ H2AX staining and DNA end labeling by terminal deoxynucleotidyltransferase in both systems (Fig. 2 A; Paull et al., 2000; Kim et al., 2002; Lukas et al., 2003; Bradshaw et al., 2005). A conservative estimate of UV laser energy was reported to be >80 Gy (Paull et al., 2000), which was estimated to cause \sim 2,800 breaks per nucleus

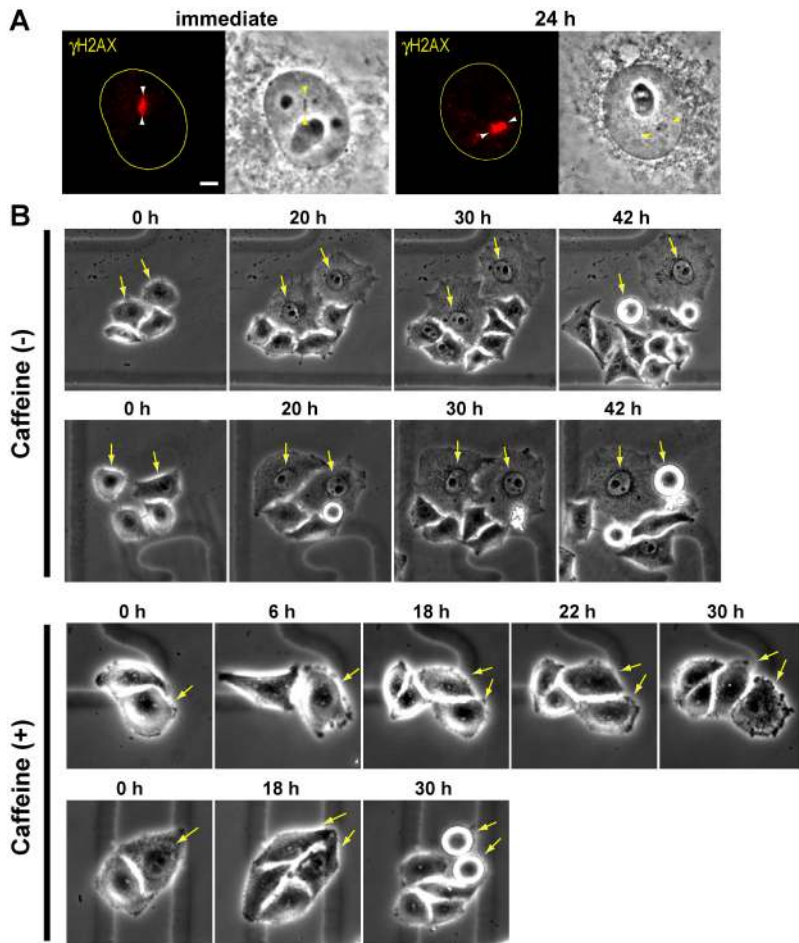


Figure 2. **Long-term effect of laser damage.** (A) γ H2AX staining of laser-induced damage immediately and 24 h ad. Bar, 5 μ m. Arrowheads indicate damage sites. (B) Caffeine-sensitive cell cycle delay after laser damage. Laser-damaged cells (arrows) on gridded coverslips were photographed at the times indicated.

(Bradshaw et al., 2005). Thus, clustering of these breaks to one small region in the nucleus appears to allow detection of the weak immunofluorescent signals of NHEJ factors at the individual DNA ends.

NHEJ factor assembly at the damage sites is transient

Interestingly, Ku was no longer found at the damage sites at 8 h ad, whereas Mre11 persisted (Fig. 3 D). The retention time of Ku was found to be between 4 and 6 h (not depicted). Thus, Ku leaves damage sites before the completion of DNA repair. A similar observation was made with DNA-PKcs (not depicted). For comparison, recruitment of the DNA break sensor poly(ADP-ribose) polymerase 1 (PARP-1) was examined. Binding of PARP-1 to DNA breaks triggers auto-(ADP-ribosylation), resulting in its dissociation from the lesion (Lindahl et al., 1995). PARP-1 accumulated at the damage site immediately after damage but became undetectable by 2 h ad, revealing the factor-specific kinetics of damage site recognition.

Delayed but persistent HR factor recruitment is not affected by NHEJ factor assembly and occurs throughout interphase

In S/G2 phase, the HR factor Rad51 was recruited to damage sites, although its recruitment was distinctly delayed (Fig. 4).

Within 20 min ad, the accumulation of Mre11 was observed as a continuous line corresponding to the area of laser ablation, with no evidence of Rad51 accumulation. By 2 h, however, Rad51 was clearly visible as discontinuous speckles along the damaged area that colocalized with Mre11. Rad51 recruitment was first observed between 30 min and 1 h ad (Fig. 5 A). A similar delay of Rad52 focus formation was observed in response to γ irradiation in yeast (Lisby et al., 2004). The IR-induced foci (IRIF) of MRN and Rad51 were shown to be nonoverlapping and mutually exclusive (Maser et al., 1997). Because laser-irradiated regions contain multiple DNA breaks, MRN and Rad51 possibly bind to different DNA ends in different stages of processing, which is similar to the overlapping signals of MRN and Rad52 that are observed at IR-induced damage sites in *Saccharomyces cerevisiae* (Lisby et al., 2004). The morphological changes of the damage site are likely caused by partial repair and damage clustering that is similar to what was observed with localized α particle irradiation (Aten et al., 2004). A few bright fluorescent signals of Mre11 and Rad51 remained at the damage sites at 24 h ad, indicating that unrepaired DSB lesions are persistently marked by MRN and Rad51 in addition to γ H2AX (Figs. 2 A and 4). This is in marked contrast to the transient retention of Ku (Fig. 3 D).

It was thought that competition occurs between the NHEJ and HR pathways, in which DNA end binding of Ku precludes

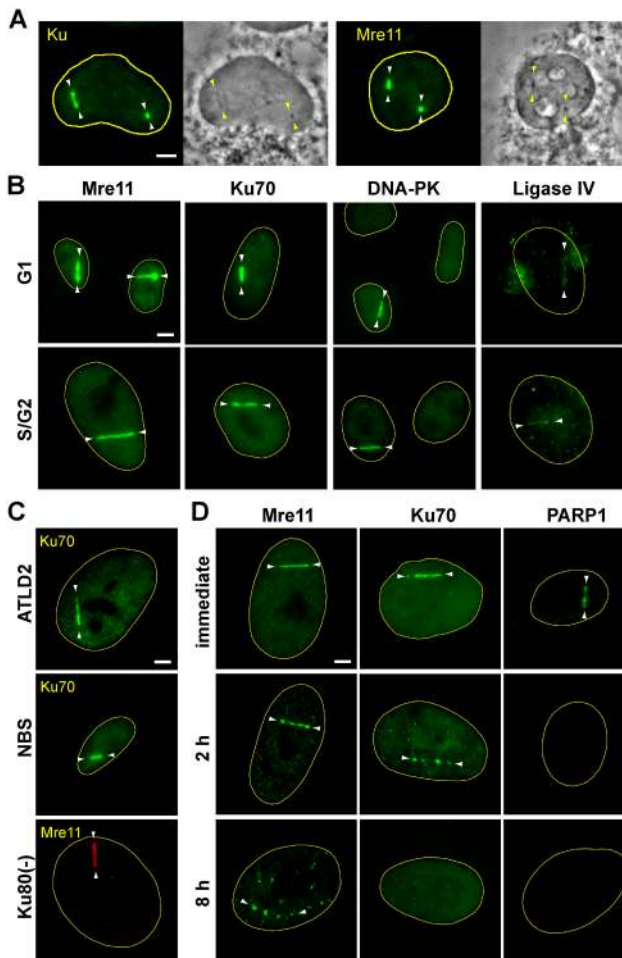


Figure 3. Damage site association of MRN, NHEJ, and HR factors. (A) Clustering of Ku and Mre11 to two sequentially induced damage sites. Damage was induced 15 min apart, and cells were immediately fixed after the second damage. The damage on the left was induced first. (B) Recruitment of MRN and NHEJ factors in G1 and S/G2 phases. G1 and S/G2 phase cells were damaged and fixed within the first 20 min ad for staining. (C) Recruitment of Ku and Mre11 in A-TLD2, Nijmegen breakage syndrome (NBS), and Ku80 knockout MEF (Ku80^{-/-}) cells fixed within 20 min ad. (D) A time course analysis of Mre11, Ku, and PARP-1 localization at the damage sites. Cells were fixed at indicated time points ad and were stained. Arrowheads indicate damage sites. Bars, 5 μ m.

the assembly of HR factors at damage sites (Van Dyck et al., 1999; Fukushima et al., 2001; Pierce et al., 2001; Allen et al., 2002; Karanjawala et al., 2002). However, the delay of Rad51 recruitment is not caused by competition with NHEJ factor assembly because the timing of Rad51 recruitment does not change in either Ku- or DNA-PKcs-deficient cells (Fig. 5 A). Because RPA recruitment is also delayed (Fig. S1 B and not depicted), DNA end processing to produce sufficient ssDNA may be the rate-limiting step for HR factor assembly in vivo. The timing of MRN, Ku, and Rad51 recruitment is unaffected by wortmannin (Fig. S1 C).

Because HR prefers the use of sister chromatids as templates in vertebrate cells, HR repair is considered to be the main pathway for postreplicative repair during late S/G2 phase (Takata et al., 1998; Sonoda et al., 1999; Rothkamm et al., 2003). Surprisingly, however, Rad51 accumulated at the laser-

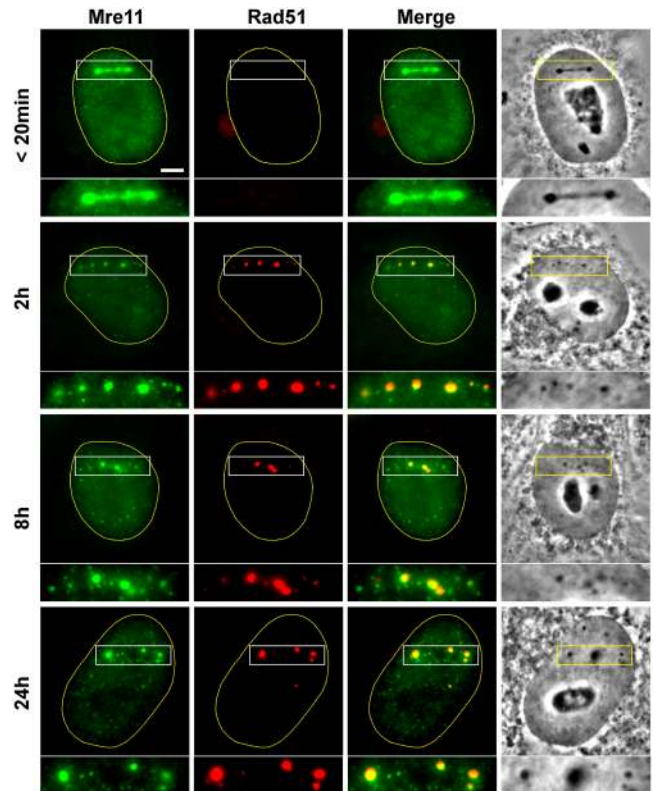


Figure 4. A time course analysis of Mre11 and Rad51 clustering at the damage sites. S/G2 cells were fixed at the indicated time points ad. Light microscope images of the damaged cells are also shown. Damage sites typically appear as a dark line under the light microscope as a result of changes in the refractive index (Berns et al., 1998). Damage sites (boxes) under high magnification are shown below. Bar, 5 μ m.

induced damage sites in G1 phase with kinetics similar to those in S/G2 phase (Fig. 5 B, Fig. S1 D, and Fig. S2 B, available at <http://www.jcb.org/cgi/content/full/jcb.200411083/DC1>) even though the expression level of Rad51 protein was low in G1 phase (Fig. 5 C). A similar observation was made with RPA (Fig. S1 B). The results indicate that Rad51 and RPA are capable of detecting the processed ssDNA ends at DSB sites throughout interphase in human cells. Interestingly, a similar G1 phase recruitment of RPA, but not Rad51, was observed in yeast (Lisby et al., 2004). The presence of dense, laser-induced DSBs may explain the observed Rad51 signal in G1 phase, which was not previously detected as IRIF (Tashiro et al., 2000; Lisby et al., 2004). Curiously, several studies using lasers suggested that IRIF formation involves more than initial damage site factor recruitment. For example, although Nbs1 is required for IRIF formation by Mre11–Rad50 (Carney et al., 1998), it is not required for recruitment of Mre11–Rad50 to laser-induced damage sites (Kim et al., 2002). H2AX is required for Nbs1 focus formation (Celeste et al., 2002) but is dispensable for the recruitment of Nbs1 to UV/BrdU-induced damage sites (Celeste et al., 2003). Thus, although S/G2-specific Rad51 IRIF may reflect ongoing HR, Rad51 may nonetheless associate with ssDNA at damage sites without initiating the actual strand invasion process until the homologous templates are available.

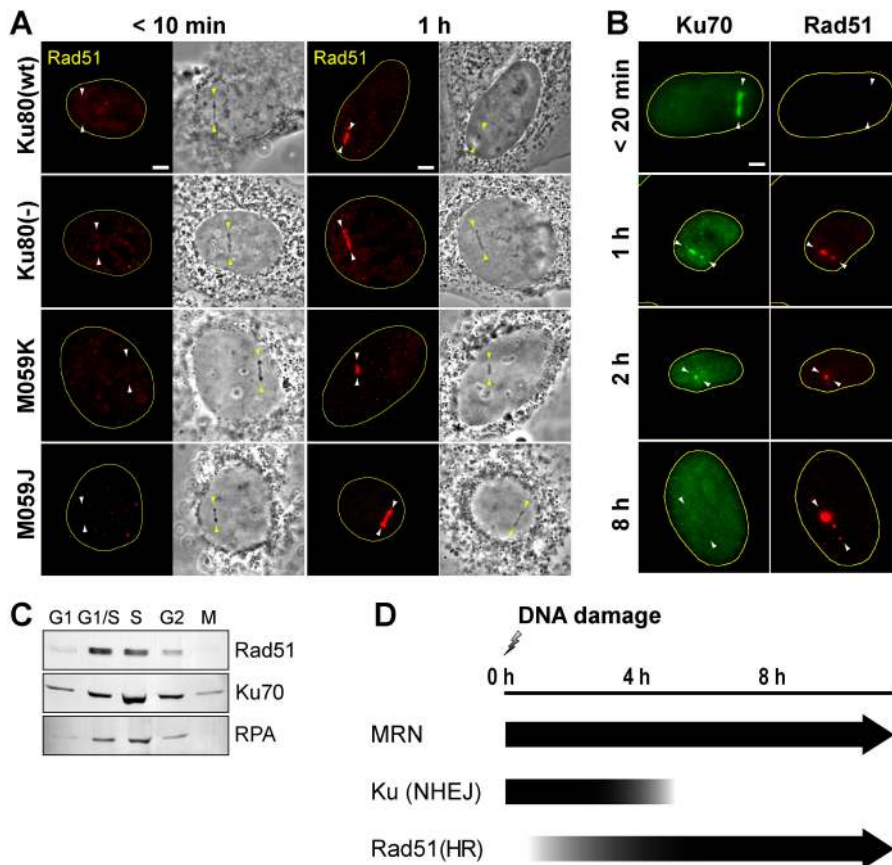


Figure 5. Recruitment of Rad51 in NHEJ mutant and G1 phase cells. (A) The timing of Rad51 recruitment is not affected by NHEJ factor mutations. Ku80(-) and DNA-PKcs(-) (M059J) cells were fixed at indicated time points and stained. The wild-type cells Ku80(wt) and M059K are controls. (B) Damage site clustering of Ku and Rad51 in G1 phase. G1 HeLa cells were damaged and fixed at indicated time points. Arrowheads indicate damage sites. Bars, 5 μ m. (C) Low level expression of Rad51 and RPA in G1 phase. Equal amounts of nuclear extracts from HeLa cells synchronized at G1, G1/S, S, G2, and M were analyzed by Western blot. (D) A summary of damage site recruitment of MRN, NHEJ, and HR factors. Damage was induced at 0 h (lightning bolt).

Our findings demonstrate the distinct kinetics of DNA damage recognition by different DSB repair factors *in vivo*. Our results reveal the unique behavior of MRN, which is different from both NHEJ and HR factors, as a DNA damage marker constitutively associated with unrepaired lesions (Fig. 5 D). We found that Ku localization at damage sites is transient, which is in contrast to the prolonged retention of Rad51. The distinct timing of recruitment between NHEJ and HR factors clearly demonstrates that NHEJ factor assembly precedes that of HR factors even in S/G2 phases. This is consistent with previous reports suggesting that NHEJ repair precedes HR repair (Delacote et al., 2002; Frank-Vaillant and Marcand, 2002). Thus, the results suggest that NHEJ and HR are not two competing parallel pathways. Rather, NHEJ serves as an immediate early repair pathway, whereas HR factors make a more prolonged attempt to repair persistent DNA lesions. These partially overlapping but complementary roles of the two pathways could explain the compensatory (originally interpreted as “competition”) and cooperative functions of NHEJ and HR in the maintenance of genome integrity (Takata et al., 1998; Fukushima et al., 2001; Pierce et al., 2001; Delacote et al., 2002; Couëdel et al., 2004; Mills et al., 2004).

Although confined to a small area ($\sim 0.01\%$ of the nuclear volume), one must be cautious because cellular responses to such extensive Nd:YAG laser-induced damage may differ from those after more physiologically relevant low dose IR, as the observed protein clustering may be specific to high density DSBs. However, there is a strong similarity of laser-evoked re-

sponses (both checkpoint activation and repair factor recruitment) to the known and/or predicted responses to DSB damage by conventional methods without lethality. This, along with the fact that different factors exhibit distinct timing and duration of damage site clustering, strongly argue that this type of laser system is useful for defining a temporal framework of DNA damage recognition and a nuclear-wide response of DSB factors in mammalian cells.

Materials and methods

Cell culture and synchronization

Wild-type human fibroblast IMR-90 cells and isogenic human glioma cell lines lacking (M059J) or expressing (M059K) DNA-PKcs were obtained from the American Type Culture Collection. Mre11 mutant fibroblast A-T-like disorder (A-TLD) 2 cells were derived from an A-TLD patient (Stewart et al., 1999). The human A-T fibroblast cell line (GM02052D) was obtained from Coriell Cell Repositories. HeLa and other human cells were cultured as previously described (Kim et al., 2002) or according to the suppliers' instructions. Ku80 knockout and wild-type mouse embryonic fibroblasts (MEFs) were cultured as described previously (Bailey et al., 1999). Cells were grown on etched grid coverslips (Bellco Biotechnology) that were attached to the bottom of preholed tissue culture plates.

HeLa cells were synchronized to S and G2 phases by a double thymidine block (Gregson et al., 2001). For G1 phase, cells in mitosis after a single thymidine block were continuously monitored to confirm their division and were subjected to laser microirradiation 5 h after M phase identification (Fig. S2 B).

PIKK inhibition

Cells were preincubated with 200 μ M wortmannin (AG Scientific, Inc.) 1 h before DNA damage induction. Wortmannin treatment was maintained until the cells were fixed. For caffeine treatment, cells that were re-

leased from double thymidine block were preincubated with or without 2 mM caffeine (Sigma-Aldrich) for 1 h before damage induction (Blasina et al., 1999).

Laser microirradiation

Laser damage was induced as previously described (Kim et al., 2002). In brief, 532 nm of the second harmonic of a pulsed Nd:YAG laser beam (~2–3 μ J/pulse energy after objective; ~4–6 ns pulse duration; 7.5 Hz; just above the estimated threshold values of optical breakdown in water [Venugopalan et al., 2002]) was focused through a 100 \times Ph3 Neofluar oil-immersion objective (NA1.3; Carl Zeiss MicroImaging, Inc.) in a confocal system (model LSM410; Carl Zeiss MicroImaging, Inc.). Unlike a UV laser, the Nd:YAG laser can be focused to a 200–300 nm spot, which is calculated to affect a minimum region of ~24 kb of a 30-nm chromatin fiber with a packing ratio of 40. Scattering should be minimum because 532 nm (visible green) is not absorbed by the cell. An EM study confirmed that the damage is structurally confined to the focused area (Berns et al., 1998). Damage is most likely caused by ionization (optical breakdown) of the medium (water) and plasma formation rather than heat because of the extremely short duration of the pulse (5 ns; Berns et al., 1998; Venugopalan et al., 2002). The expansion of cavitation bubbles that was caused by plasma generated transient photomechanical pressure, which resulted in breakage of molecular bonding and created numerous DNA breaks in the confined area. At least three independent experiments for each antibody staining were performed. Five to seven cells were damaged in one plate, and three to four plates were subjected to laser damage in each experiment. For the data presented, 100% of cells exhibited the same staining patterns with a given antibody.

Immunofluorescent staining

After laser microirradiation, cells were fixed in 4% PFA (10 min at 4°C) and stained with antibodies. The staining procedure was described previously (Kim et al., 2002). Imaging was performed on a microscope (model IX81; Olympus) coupled with a CCD camera (model FV II; Olympus) using a 100 \times Ph3 UPlanFl oil objective (NA 1.3; Olympus).

Antibodies

Rabbit pAb against the human Rad51 protein was raised against the bacterially expressed protein. Commercial antibodies that were used include mouse mAbs specific for Mre11, Rad50, and A-TM (GeneTex), Ku70 (Novus Biologicals), PARP-1 (Trevigen), DNA-PKcs (Abcam Limited), rabbit pAbs specific for hMre11 (Oncogene Research Products), ligase IV and RPA (Chemicon International), phospho-Chk2 (Thr68; Cell Signaling Technology), and pAb and mAb anti-Nbs1 (Novus Biologicals and BD Biosciences, respectively). The specificity of antibodies against A-TM was confirmed (Fig. S2 A). Goat anti-A-TR pAb was from Santa Cruz Biotechnology, Inc. Donkey anti-mouse IgG and anti-goat IgG conjugated with AlexaFluor488 and goat anti-rabbit IgG conjugated with AlexaFluor546 were from Molecular Probes. Cy3-conjugated goat anti-rabbit IgG was from Jackson ImmunoResearch Laboratories.

Online supplemental material

Supplemental figures provide additional immunostaining data. Fig. S1 shows colocalization of Ku and Mre11 at the damage site; RPA recruitment in G1 and S/G2 phases; the lack of effect of wortmannin treatment on Ku, Mre11, and Rad51 recruitment; and colocalization of Mre11 and Rad51 at the damage site in G1 phase cells. Fig. S2 shows the specificity of the anti-A-TM antibody by the absence of A-T cell staining and shows the distinction of cell cycle stages by proliferating cell nuclear antigen staining. Online supplemental material is available at <http://www.jcb.org/cgi/content/full/jcb.200411083/DC1>.

We would like to thank Drs. Shunichi Takeda, Stephen West, Stephen Jackson, and Michael Lieber for helpful comments on the manuscript.

This work was supported in part by the Bioarchitect Research Program (RIKEN) and grants-in-aid from the Japan Society for the Promotion of Science and the Ministry of Education, Culture, Sports, Science, and Technology of Japan to H. Kurumizaka, and by grants from the National Institutes of Health (CA50519 to D.J. Chen and CA100710 to K. Yokomori) and the Department of Defense Breast Cancer Research Program (DAMD17-03-1-0436 to K. Yokomori). K. Yokomori was a Leukemia and Lymphoma Society Scholar.

Submitted: 15 November 2004

Accepted: 21 June 2005

References

- Abraham, R.T. 2004. PI 3-kinase related kinases: 'big' players in stress-induced signaling pathways. *DNA Repair (Amst.)* 3:883–887.
- Ahn, J.Y., J.K. Schwarz, H. Piwnica-Worms, and C.E. Canman. 2000. Threonine 68 phosphorylation by ataxia telangiectasia mutated is required for efficient activation of Chk2 in response to ionizing radiation. *Cancer Res.* 60:5934–5936.
- Allen, C., A. Kurimasa, M.A. Brenneman, D.J. Chen, and J.A. Nickoloff. 2002. DNA-dependent protein kinase suppresses double-strand break-induced and spontaneous homologous recombination. *Proc. Natl. Acad. Sci. USA.* 99:3758–3763.
- Aten, J.A., J. Stap, P.M. Krawczyk, C.H. van Oven, R.A. Hoebe, J. Essers, and R. Kanaar. 2004. Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. *Science.* 303:92–95.
- Bailey, S.M., J. Meyne, D.J. Chen, A. Kurimasa, G.C. Li, B.E. Lehnert, and E.H. Goodwin. 1999. DNA double-strand break repair proteins are required to cap the ends of mammalian chromosomes. *Proc. Natl. Acad. Sci. USA.* 96:14899–14904.
- Baumann, P., F.E. Benson, and S.C. West. 1996. Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. *Cell.* 87:757–766.
- Berns, M.W., Y. Tadir, H. Liang, and B. Tromberg. 1998. Laser scissors and tweezers. *Methods Cell Biol.* 55:71–98.
- Blasina, A., B.D. Price, G.A. Turenne, and C.H. McGowan. 1999. Caffeine inhibits the checkpoint kinase ATM. *Curr. Biol.* 9:1135–1138.
- Bradshaw, P.S., D.J. Stavropoulos, and M.S. Meyn. 2005. Human telomeric protein TRF2 associates with genomic double-strand breaks as an early response to DNA damage. *Nat. Genet.* 37:193–197.
- Carney, J.P., R.S. Maser, H. Olivares, E.M. Davis, M. Le Beau, J.R. Yates III, L. Hays, W.F. Morgan, and J.H. Petrini. 1998. The hMre11/hRad50 protein complex and Nijmegen breakage syndrome: linkage of double-strand break repair to the cellular DNA damage response. *Cell.* 93:477–486.
- Carson, C.T., R.A. Schwartz, T.H. Stracker, C.E. Lilley, D.V. Lee, and M.D. Weitzman. 2003. The Mre11 complex is required for ATM activation and the G2/M checkpoint. *EMBO J.* 22:6610–6620.
- Celeste, A., S. Petersen, P.J. Romanienko, O. Fernandez-Capetillo, H.T. Chen, O.A. Sedelnikova, B. Reina-San-Martin, V. Coppola, E. Meffre, and M.J. Difilippantonio, et al. 2002. Genomic instability in mice lacking histone H2AX. *Science.* 296:922–927.
- Celeste, A., O. Fernandez-Capetillo, M.J. Kruhlak, D.R. Pilch, D.W. Staudt, A. Lee, R.F. Bonner, W.M. Bonner, and A. Nussenzweig. 2003. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. *Nat. Cell Biol.* 5:675–679.
- Costanzo, V., T. Paull, M. Gottesman, and J. Gautier. 2004. Mre11 assembles linear DNA fragments into DNA damage signaling complexes. *PLoS Biol.* 10.1371/journal.pbio.0020110.
- Couédel, C., K.D. Mills, M. Barchi, L. Shen, A. Olshen, R.D. Johnson, A. Nussenzweig, J. Essers, R. Kanaar, G.C. Li, et al. 2004. Collaboration of homologous recombination and nonhomologous end-joining factors for the survival and integrity of mice and cells. *Genes Dev.* 18:1293–1304.
- Delacote, F., M. Han, T.D. Stamato, M. Jasin, and B.S. Lopez. 2002. An *xrcc4* defect or Wortmannin stimulates homologous recombination specifically induced by double-strand breaks in mammalian cells. *Nucleic Acids Res.* 30:3454–3463.
- Frank-Vaillant, M., and S. Marcand. 2002. Transient stability of DNA ends allows nonhomologous end joining to precede homologous recombination. *Mol. Cell.* 10:1189–1199.
- Fukushima, T., M. Takata, C. Morrison, R. Araki, A. Fujimori, M. Abe, K. Tatsumi, M. Jasin, P.K. Dhar, E. Sonoda, et al. 2001. Genetic analysis of the DNA-dependent protein kinase reveals an inhibitory role of Ku in late S–G2 phase DNA double-strand break repair. *J. Biol. Chem.* 276:44413–44418.
- Gregson, H.C., J.A. Schmiesing, J.-S. Kim, T. Kobayashi, S. Zhou, and K. Yokomori. 2001. A potential role for human cohesin in mitotic spindle aster assembly. *J. Biol. Chem.* 276:47575–47582.
- Horejsi, Z., J. Falck, C.J. Bakkenist, M.B. Kastan, J. Lukas, and J. Bartek. 2004. Distinct functional domains of Nbs1 modulate the timing and magnitude of ATM activation after low doses of ionizing radiation. *Oncogene.* 23:3122–3127.
- Jakob, B., M. Scholz, and G. Taucher-Scholz. 2002. Characterization of CDKN1A (p21) binding to sites of heavy-ion-induced damage: colocalization with proteins involved in DNA repair. *Int. J. Radiat. Biol.* 78:75–88.
- Karanjawa, Z.E., N. Adachi, R.A. Irvine, E.K. Oha, D. Shibata, K. Schwarz, C.-L. Hsieh, and M.R. Lieber. 2002. The embryonic lethality in DNA ligase IV-deficient mice is rescued by deletion of Ku: implications for unifying the heterogeneous phenotypes of NHEJ mutants. *DNA Repair*

(Amst.). 1:1017–1026.

- Kim, J.-S., T.B. Krasieva, V.J. LaMorte, A.M.R. Taylor, and K. Yokomori. 2002. Specific recruitment of human cohesin to laser-induced DNA damage. *J. Biol. Chem.* 277:45149–45153.
- Lee, J.H., and T.T. Paull. 2004. Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. *Science.* 304:93–96.
- Lee, J.H., and T.T. Paull. 2005. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science.* 308:551–554.
- Lieber, M.R., Y. Ma, U. Pannicke, and K. Schwarz. 2003. Mechanism and regulation of human non-homologous DNA end-joining. *Nat. Rev. Mol. Cell Biol.* 4:712–720.
- Lindahl, T., M.S. Satoh, G.G. Poirier, and A. Klungland. 1995. Post-translational modification of poly(ADP-ribose) polymerase induced by DNA strand breaks. *Trends Biochem. Sci.* 20:405–411.
- Lisby, M., J.H. Barlow, R.C. Burgess, and R. Rothstein. 2004. Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell.* 118:699–713.
- Lukas, C., J. Falck, J. Bartkova, J. Bartek, and J. Lukas. 2003. Distinct spatiotemporal dynamics of mammalian checkpoint regulators induced by DNA damage. *Nat. Cell Biol.* 5:255–260.
- Martin, S.G., T. Laroche, N. Suka, M. Grunstein, and S.M. Gasser. 1999. Relocalization of telomeric Ku and SIR proteins in response to DNA strand breaks in yeast. *Cell.* 97:621–633.
- Maser, R.S., K.J. Monsen, B.E. Nelms, and J.H. Petrini. 1997. hMre11 and hRad50 nuclear foci are induced during the normal cellular response to DNA double-strand breaks. *Mol. Cell. Biol.* 17:6087–6096.
- Mills, K.D., D.O. Ferguson, J. Essers, M. Eckersdorff, R. Kanaar, and F.W. Alt. 2004. Rad54 and DNA ligase IV cooperate to maintain mammalian chromatid stability. *Genes Dev.* 18:1283–1292.
- Paull, T.T., and M. Gellert. 1998. The 3' to 5' exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks. *Mol. Cell.* 1:969–979.
- Paull, T.T., E.P. Rogakou, V. Yamazaki, C.U. Kirchgessner, M. Gellert, and W.M. Bonner. 2000. A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. *Curr. Biol.* 10:886–895.
- Pierce, A.J., P. Hu, M. Han, N. Ellis, and M. Jasin. 2001. Ku DNA end-binding protein modulates homologous repair of double-strand breaks in mammalian cells. *Genes Dev.* 15:3237–3242.
- Rothkamm, K., I. Kruger, L.H. Thompson, and M. Lobrich. 2003. Pathways of DNA double-strand break repair during the mammalian cell cycle. *Mol. Cell. Biol.* 23:5706–5715.
- Shiloh, Y. 2003. ATM and related protein kinases: safeguarding genome integrity. *Nat. Rev. Cancer.* 3:155–168.
- Sonoda, E., M.S. Sasaki, C. Morrison, Y. Yamaguchi-Iwai, M. Takata, and S. Takeda. 1999. Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells. *Mol. Cell. Biol.* 19:5166–5169.
- Stewart, G.S., R.S. Maser, T. Stankovic, D.A. Bressan, M.I. Kaplan, N.G.J. Jaspers, A. Raams, P.J. Byrd, J.H. Petrini, and A.M.R. Taylor. 1999. The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell.* 99:577–587.
- Ström, L., H.B. Lindroos, K. Shirahige, and C. Sjögren. 2004. Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. *Mol. Cell.* 16:1003–1015.
- Sung, P. 1994. Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. *Science.* 265:1241–1243.
- Takata, M., M.S. Sasaki, E. Sonoda, C. Morrison, M. Hashimoto, H. Utsumi, Y. Yamaguchi-Iwai, A. Shinohara, and S. Takeda. 1998. Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. *EMBO J.* 17:5497–5508.
- Tashiro, S., J. Walter, A. Shinohara, N. Kamada, and T. Cremer. 2000. Rad51 accumulation at sites of DNA damage and in postreplicative chromatin. *J. Cell Biol.* 150:283–291.
- Ünal, E., A. Arbel-Eden, U. Sattler, R. Shroff, M. Lichten, J.E. Haber, and D. Koshland. 2004. DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Mol. Cell.* 16:991–1002.
- Uziel, T., Y. Lerenthal, L. Moyal, Y. Andegeko, L. Mittelman, and Y. Shiloh. 2003. Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J.* 22:5612–5621.
- Van Dyck, E., A.Z. Stasiak, A. Stasiak, and S.C. West. 1999. Binding of double-strand breaks in DNA by human Rad52 protein. *Nature.* 398:728–731.
- Venugopalan, V., A. Guerra III, K. Nahen, and A. Vogel. 2002. Role of laser-induced plasma formation in pulsed cellular microsurgery and micro-manipulation. *Phys. Rev. Lett.* 10.1103/PhysRevLett.88.078103.
- West, S.C. 2003. Molecular views of recombination proteins and their control. *Nat. Rev. Mol. Cell Biol.* 4:435–445.