LETTERS

Chromatin remodelling at a DNA double-strand break site in *Saccharomyces cerevisiae*

Toyoko Tsukuda[1], Alastair B. Fleming[1], Jac A. Nickoloff[1] & Mary Ann Osley[1]

The repair of DNA double-strand breaks (DSBs) is crucial for maintaining genome stability. Eukaryotic cells repair DSBs by both non-homologous end joining and homologous recombination. How chromatin structure is altered in response to DSBs and how such alterations influence DSB repair processes are important issues. In vertebrates, phosphorylation of the histone variant H2A.X occurs rapidly after DSB formation[1], spreads over megabase chromatin domains, and is required for stable accumulation of repair proteins at damage foci[2]. In *Saccharomyces cerevisiae*, phosphorylation of the two principal H2A species is also signalled by DSB formation, which spreads ~40 kb in either direction from the DSB[3]. Here we show that near a DSB phosphorylation of H2A is followed by loss of histones H2B and H3 and increased sensitivity of chromatin to digestion by micrococcal nuclease; however, phosphorylation of H2A and nucleosome loss occur independently. The DNA damage sensor MRX[4] is required for histone loss, which also depends on INO80, a nucleosome remodelling complex[5]. The repair protein Rad51 (ref. 6) shows delayed recruitment to DSBs in the absence of histone loss, suggesting that MRX-dependent nucleosome remodelling regulates the accessibility of factors directly involved in DNA repair by homologous recombination. Thus, MRX may regulate two pathways of chromatin changes: nucleosome displacement for efficient recruitment of homologous recombination proteins; and phosphorylation of H2A, which modulates checkpoint responses to DNA damage[2].

To elucidate the chromatin pathways leading to DSB repair in *S. cerevisiae*, here we have used a *MATα* haploid strain that lacks *HMR* and *HML* donor sequences and carries a galactose-inducible homothallic switching endonuclease gene (*HO*)[7]. In this strain, HO endonuclease introduces a DSB at *MAT* that can be repaired only by non-homologous end joining, although the principal proteins involved in homologous recombination are recruited to the break site[6]. We analysed chromatin structure over a region of 12–20 kb encompassing the DSB by chromatin immunoprecipitation (ChIP) followed by real-time polymerase chain reaction (PCR), which provides a sensitive measurement of the kinetics and spatial distribution of chromatin changes and the recruitment of repair proteins around the break site.

Budding yeast H2A is phosphorylated on Ser 129 by the ATM and ATR homologues Tel1 and Mec1, respectively[8]. In agreement with a previous report[3], we found that phosphorylated H2A (γ-H2A) accumulated rapidly and extensively on either side of the DSB, and that the amount of γ-H2A was less near the DSB than at a position 6-kb distant (Fig. 1a and Supplementary Fig. 3a). This latter result suggested that nucleosome integrity is lost near the DSB. The nucleosome consists of 146 bp of DNA wrapped about twice around a histone octamer comprising a $(H3–H4)_2$ tetramer and two H2A–H2B dimers. To determine whether nucleosome stability was altered at the DSB, we did ChIP in strains expressing either Flag–H2B or Flag–H3. The quantities of both histones decreased 60–90 min after HO induction and were reduced threefold by 120 min (Fig. 1a). The comparable loss of both histones suggests that whole nucleosomes were displaced from chromatin near the DSB. Although no histone loss occurred in the first 30 min of DSB induction, quantities of γ-H2A were about fourfold less near the DSB than at distal sites at this time (Fig. 1a). This difference may reflect phosphatase activity near the DSB or enhanced phosphorylation at distal sites.

To confirm that nucleosomes were remodelled near the DSB, we analysed the sensitivity of *MATα* chromatin to micrococcal nuclease (MNase; Fig. 1b). Before DSB induction, a strong MNase ladder reflects positioned nucleosomes[9]. We found that after DSB formation the nucleosome ladder became progressively less organized with time (Fig. 1b and Supplementary Figs 1 and 2). The alteration in the nucleosome pattern closely paralleled histone depletion, indicating that nucleosome integrity is compromised around the DSB.

Histone eviction during transcription often depends on histone-modifying or ATP-dependent nucleosome remodelling factors[10–12]. Because the accumulation of γ-H2A temporally preceded histone loss, we first examined its role in nucleosome displacement by eliminating the MRX (Mre11–Rad50–Xrs2) complex. Phosphorylation of H2A.X by ATM in vertebrates requires the homologous MRN (Mre11–Rad50–Nbs1) complex[13]. MRX is one of the earliest factors recruited to yeast DSBs, and it regulates the ATM homologue Tel1 (ref. 14). In *mre11Δ*, γ-H2A was not abolished at the DSB, although its overall amounts were reduced 2–3-fold (Supplementary Fig. 3c). Histone loss was significantly impeded (Fig. 2a), however, and Flag–H2B was present 3 h after DSB induction (data not shown). In addition, the nucleosome ladder at *MATα* did not change over this time period, indicating that the chromatin structure remained intact (Fig. 2b and Supplementary Figs 1 and 2).

Because these data suggested that phosphorylation of H2A and histone loss might not be directly coupled, we examined histone occupancy in an H2A mutant lacking the Ser 129 phosphorylation site (*hta1*/*hta2-S129**; Fig. 2a). Although γ-H2A could not be detected at the DSB in this mutant (data not shown), Flag–H3 was lost to the same extent as in a wild-type strain. Thus, histone eviction depends on MRX but not on γ-H2A.

Next, we determined whether an ATP-dependent nucleosome remodelling activity was required for histone loss. We focused on INO80 (a complex named for the Ino80 protein) because an *ino80Δ* mutant shows sensitivity to agents that cause DSBs, INO80 can move nucleosomes *in vitro*[5,15], and the Ino80 protein is enriched at *MAT* after DSB induction[16–18]. Because the *ino80Δ* mutation causes inviability in our strain background, we used an *arp8Δ* mutant, which is defective in the INO80 ATPase and chromatin remodelling activities and is sensitive to DNA damage[15]. In *arp8Δ*, Flag–H3 and Flag–H2B were retained at the DSB for 2 h, and were evicted only 3 h after break formation (Fig. 3a and Supplementary Fig. 3b). Consistent with postponed histone eviction, there was a similar delay in nucleosome

[1]Department of Molecular Genetics and Microbiology, University of New Mexico School of Medicine, 915 Camino de Salud Albuquerque, New Mexico 87131, USA.

disruption (Fig. 3c and Supplementary Figs 1 and 2). Thus, *arp8Δ* significantly slows but does not eliminate histone loss. Together, these results indicate that INO80-dependent chromatin remodelling increases the kinetics of nucleosome displacement at a DSB and that this activity depends on MRX but not γ-H2A.

In wild-type cells, histone loss begins ~60 min after DSB induction, which temporally coincides with the 5′ to 3′ resection of DNA ends[19]. Moreover, an *mre11Δ* mutant, which is defective in histone eviction, is also defective in end processing[7]. We tested whether the slower rate of histone eviction in *arp8Δ* results from a slower rate of DSB formation or end processing, and found that both cleavage and resection were similar in wild-type and *arp8Δ* (Supplementary Fig. 4), in contrast to a previous report[17]. This finding also indicates that nucleosomes can associate *in vivo* with single-stranded DNA, as observed *in vitro*[20], and that nucleases can resect DNA that is nucleosomal. Thus, single-stranded DNA is necessary but not sufficient for histone eviction, and the INO80 ATPase is required for chromatin remodelling at the DSB.

Because Ino80 physically associates with γ-H2A and γ-H2A recruits Ino80 to the *MAT* DSB[16–18], our finding of γ-H2A-independent histone displacement was puzzling. To address this issue, we measured Ino80 association with *MATα* before DSB induction and found that it was present even in the absence of the break (Fig. 3b, −Gal). As shown by DNA microarray studies, this pool of Ino80 probably contributes to *MATα* transcription[21], and we confirmed that *MATα1* and *MATα2* transcript levels are 2–3-fold lower in *arp8Δ*

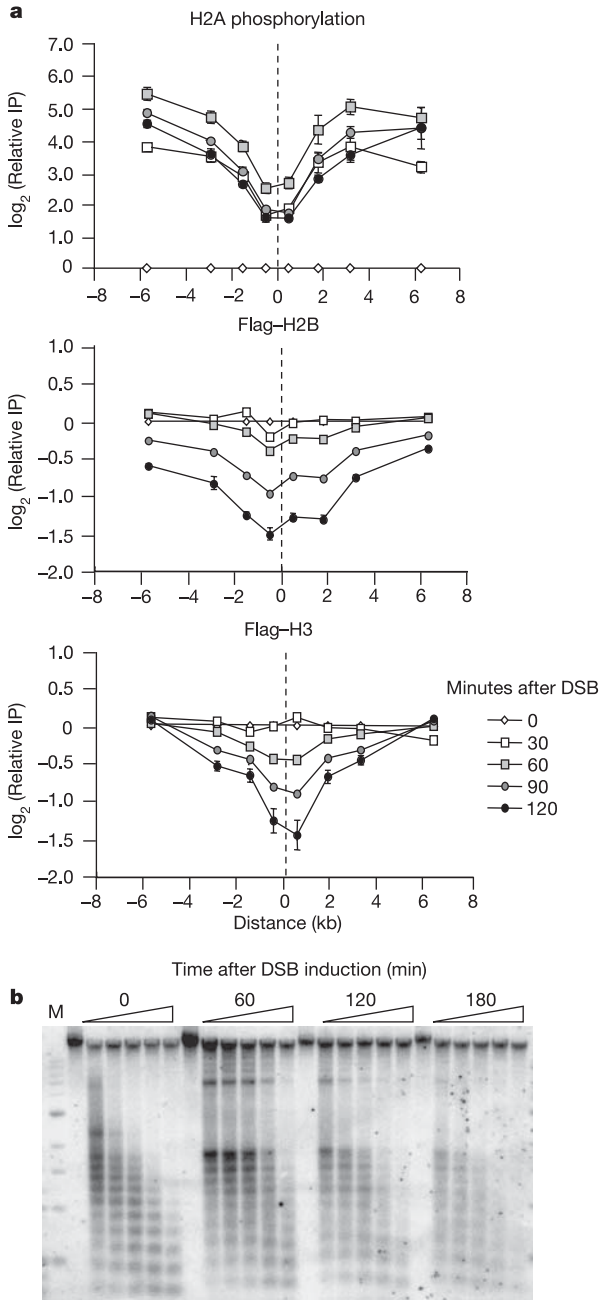

Figure 1 | Chromatin changes at the *MATα* DSB. **a**, A DSB was induced at *MATα* in strains expressing Flag–H2B or Flag–H3, and ChIP was done with antibodies against γ-H2A or Flag. DNA was analysed by real-time PCR using primers corresponding to sequences on the left (−) or the right (+) side of the DSB (0), and results were normalized to the ratio of immunoprecipitation (IP) to input DNA at time 0. Data are the mean ± s.e.m. **b**, Nuclei were prepared after DSB induction, and chromatin was digested with MNase and subjected to Southern blot analysis using a probe for *MATα* DNA. The triangles denote increasing times of MNase digestion. M indicates a 1-kb DNA ladder.

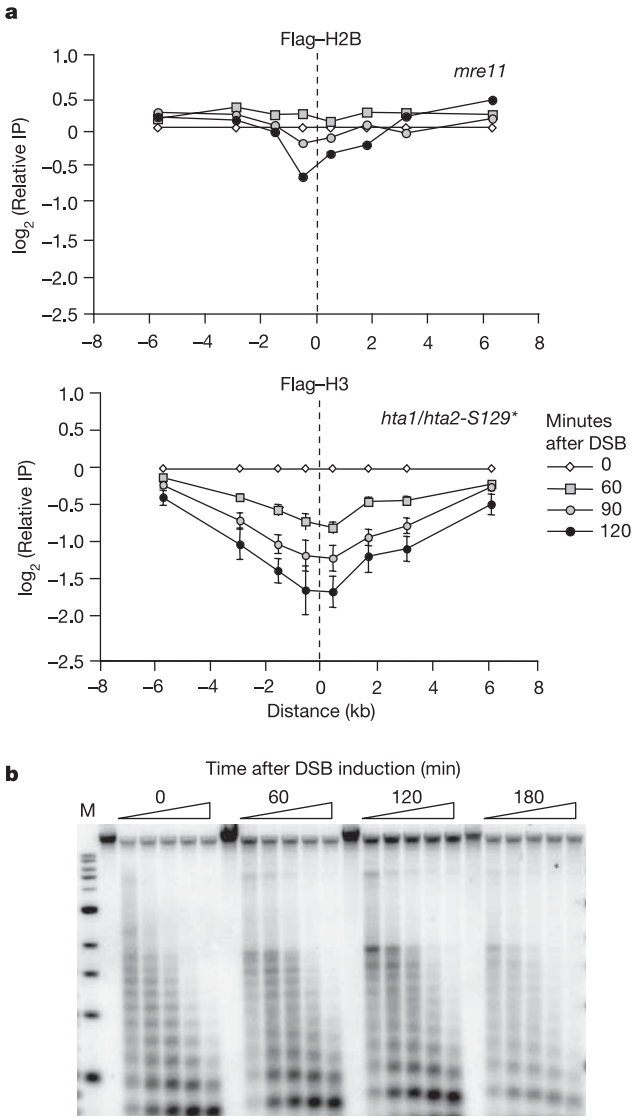

Figure 2 | MRX is involved in histone loss at the *MATα* DSB. **a**, A DSB was induced at *MATα*, and ChIP was done with antibodies against Flag in an *mre11::Kan-MX* strain expressing Flag–H2B and an *hta1/hta2-S129** strain expressing Flag–H3. DNA was analysed by real-time PCR on both the left (−) and the right (+) side of the DSB (0). Data are the mean ± s.e.m. **b**, MNase analysis was done on nuclei isolated from the *mre11::Kan-MX* strain by Southern blot analysis as described in Fig. 1b.

(data not shown). After DSB induction (Fig. 3b, +Gal), Ino80 preferentially accumulated on the right side of the DSB and, in contrast to the pre-existing pool of Ino80, this association required γ-H2A[16,17] (Supplementary Fig. 5). In addition, 2 h after break induction ~50% of the pre-existing Ino80 pool was lost at a point 0.5 kb from the left side of the DSB, a region corresponding to the *MATα* promoter (Fig. 3b, +Gal). Together, these data suggest that a pre-existing γ-H2A-independent pool of Ino80 related to *MATα* transcription is responsible for histone displacement, whereas a newly recruited pool performs another function. Ino80 binds to both DNA and histones[5,15], and notably DNA sequences to the right of the *MAT* DSB initially participate in strand invasion during homologous recombination, suggesting that the newly recruited Ino80 has a role in this process.

The INO80 complex is associated with the repair of DSBs by homologous recombination[17] (Supplementary Fig. 6), indicating that histone depletion might affect the recruitment of homologous recombination proteins to the *MAT* DSB. HO-generated DSBs are stable for almost 60 min (ref. 19), and the recruitment of homologous recombination proteins such as Rad51 and Rad54 is delayed until the broken ends are processed to 3′ single-stranded tails[6]. Replication protein A (RPA) binds first, spreads along the DNA and is subsequently displaced by Rad51 (refs 22, 23). We found that RPA was efficiently recruited to the DSB in both wild-type and *arp8Δ* cells, with slightly faster kinetics in *arp8Δ* (Fig. 4a). In both strains, RPA eventually spread 6.3 kb from the DSB, confirming that strand resection occurs normally in *arp8Δ*. Recruitment and spreading of Rad51 were delayed in *arp8Δ* (Fig. 4a), however, and correlated precisely with the delay in histone eviction in this mutant. In *mre11Δ* there was a pronounced delay in the binding of both RPA and Rad51 (Fig. 4a), consistent with the delayed resection of DNA ends[7,24] (data not shown) and extended period of nucleosome retention in this strain. In marked contrast, recruitment of Rad51 in the *hta1/hta2-S129** mutant was indistinguishable from that in wild type (data not shown), supporting the observation that histone displacement proceeds with normal timing in the absence of γ-H2A. Together, these data suggest that nucleosome eviction controls the rate at which Rad51 displaces RPA from resected DNA during repair by homologous recombination, and that this displacement depends on MRX and the INO80 complex.

The above results indicate that a temporal sequence of events alters chromatin at DSBs. γ-H2A first spreads over a large domain around the break. Nucleosomes are then displaced near the DSB through the remodelling activity of the INO80 complex, which increases the kinetics of histone loss. Nucleosome loss at *MAT* requires MRX, one of the earliest factors to be recruited to DSBs *in vivo*. We propose that MRX regulates nucleosome displacement through its role in two pathways (Fig. 4b). The first pathway involves the MRX-dependent resection of DNA ends. When strand resection is prevented, or significantly delayed as in *mre11Δ*, nucleosome displacement cannot occur efficiently. Resection itself is not sufficient for nucleosome loss, however, and MRX also functions in a second pathway that controls

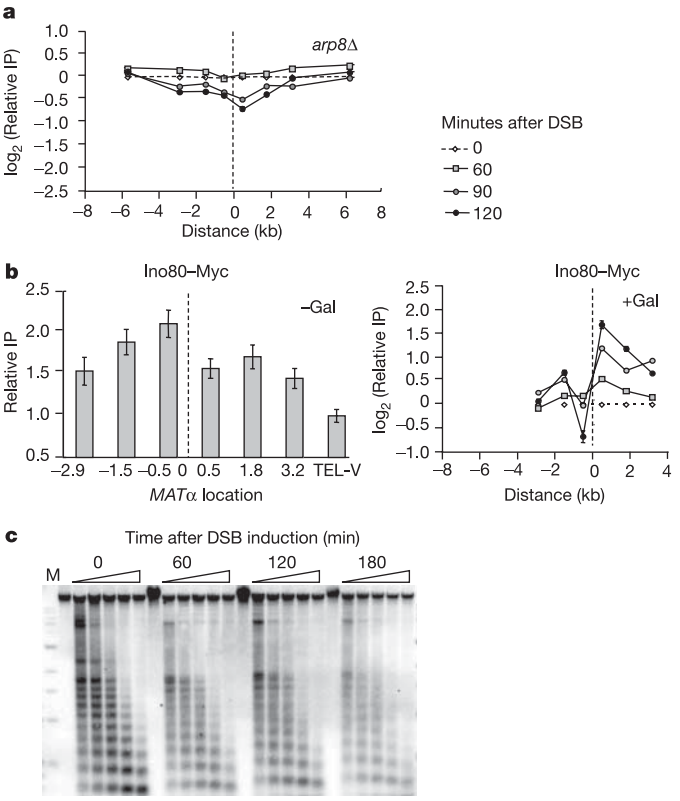

Figure 3 | The INO80 complex is required for histone eviction at the *MATα* DSB. a, A DSB was induced at *MATα* in an *arp8::Kan-MX* mutant expressing Flag–H3, and ChIP was done with antibodies against Flag. Precipitated DNA was quantified as described in Fig. 1a. **b**, ChIP was done with antibodies against Myc in a wild-type strain that contained Ino80–Myc before (−Gal) and after (+Gal) DSB induction. Ino80–Myc association was normalized to histone H3 occupancy. **c**, MNase digestion was done on nuclei isolated from an *arp8::Kan-MX* mutant as described in Fig. 1b. Data in **a** and **b** are the mean ± s.e.m.

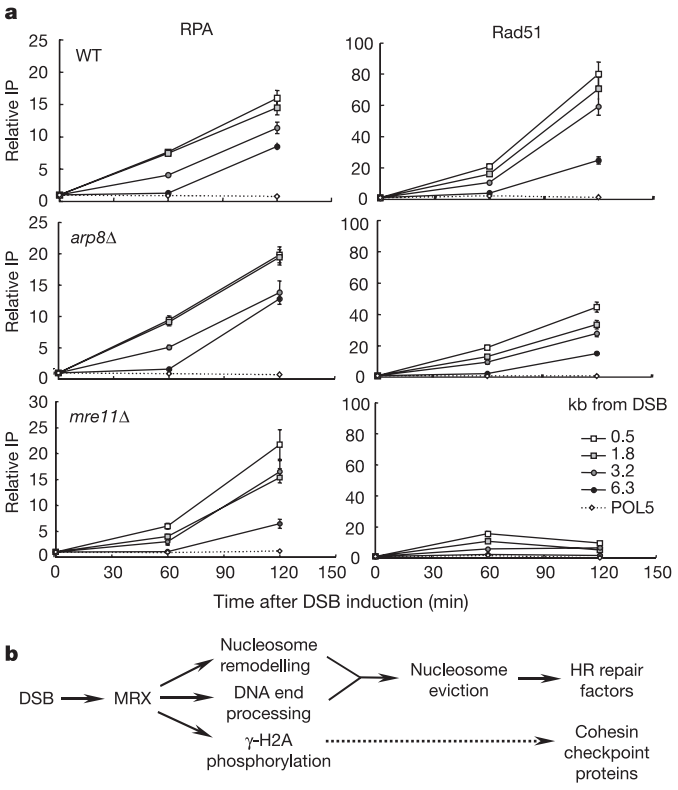

Figure 4 | MRX and INO80 are required for recruiting Rad51 to the *MATα* DSB. a, ChIP was done in wild type, *arp8Δ* or *mre11Δ* with antibodies against RPA (left) or Rad51 (right) after DSB induction at *MATα*. DNA on the right side of the DSB was analysed by real-time PCR. Data are the mean ± s.e.m.. **b**, MRX controls chromatin remodelling at DSBs. MRX is recruited to DSBs, where it regulates DNA end processing and Tel1-dependent phosphorylation of H2A. MRX regulates nucleosome remodelling through INO80, leading to nucleosome eviction and the efficient recruitment of proteins involved in homologous recombination. Phosphorylation of H2A is independent of nucleosome displacement and controls the accumulation of checkpoint proteins, as well as cohesin, at DSBs.

INO80-dependent nucleosome remodelling. These two MRX-dependent pathways converge to promote nucleosome eviction. This convergence of two pathways could account for the more severe defect in nucleosome displacement observed in *mre11Δ* as compared with *arp8Δ*, although we cannot rule out the possibility that another nucleosome remodelling factor shares a redundant role with INO80 (ref. 25).

Histone displacement occurs in a region of the *MATα* locus that includes the promoter and *α1* and *α2* coding regions, raising the possibility that transcription itself is involved in nucleosome loss through INO80-dependent chromatin remodelling. At highly transcribed genes, an increase in RNA polymerase II correlates with a decrease in histone occupancy[11,12]. Our preliminary data indicate that *MATα1* transcripts transiently accumulate on DSB induction, coinciding with an increase in RNA polymerase II and a loss of nucleosomes at the *α1* TATA site; these effects are significantly reduced in *arp8Δ* (T.T. and A.B.F., unpublished data). Thus, the DSB might signal through MRX to the *MAT* transcription machinery, which would then use the remodelling activity of INO80 to displace histones. This pathway might reflect a mechanism that couples transcription-dependent chromatin remodelling to DSB repair in active promoter regions.

INO80-promoted nucleosome displacement regulates the recruitment of proteins with direct roles in homologous recombination. Rad51 does not efficiently replace RPA in the absence of nucleosome loss, probably owing to the delayed recruitment of Rad52 (T.T., unpublished data), which cooperates with Rad51 to displace RPA[6,26]. In agreement with the idea that INO80 has a role in repair by homologous recombination, non-homologous end joining is relatively unaffected in *arp8Δ*, whereas *arp8Δ* and homologous recombination mutants show similar sensitivities to DNA damaging agents[17] (Supplementary Fig. 6) and *arp8Δ* shows a 2–3-fold decrease in allelic recombination (S. Krishna and J.A.N., unpublished data). In addition to nucleosome displacement, the cellular response to DNA damage also requires the formation of γ-H2A. Both forms of chromatin remodelling are ultimately dependent on MRX but are independent of one another, comprising two parallel pathways of chromatin change that have different but complementary roles in the DSB repair response (Fig. 4b). MRX-dependent nucleosome remodelling by INO80 is required for efficient recruitment of homologous recombination proteins, whereas MRX-dependent H2A phosphorylation through Tel1 mediates the accumulation of cohesin and checkpoint proteins at DSB sites[14,24,27].

METHODS

ChIP and PCR analysis. We fixed 50 ml of cells with 1% formaldehyde for either 60 min or 15 min (histones) and lysed them in FA buffer[28]. Immunoprecipitation was done with 5 absorbance units at 600 nm ($A_{600\,nm}$ units) for histones and 20 $A_{600\,nm}$ units for all other proteins. We used 5 μl of antibodies against Myc (9E10, Upstate Biotechnology), 45 μl of agarose beads conjugated to antibodies against Flag (M2, Sigma), 5 μl of antibodies against H2A.X phosphorylated on Ser 139 (Upstate Biotechnology), and 2 μl or 4 μl of antibodies against RPA or Rad51 (a gift from W.-D. Heyer). Extracted DNA was analysed by real-time PCR using a SYBR Green master mix (ABI) in an 7000 sequence detection system (ABI). DNA primers were designed to span a region of 12–20 kb around the HO cut site at *MATα*, as well as a region of the *POL5* gene; primer sequences are available from the authors on request.

Dissociation curve analysis of the amplified DNA melting temperature showed that the each primer set gave a single and specific product. The immunoprecipitation data were normalized to the *POL5* gene, where DSB does not occur, to correct for experimental variation and loss of DNA at the DSB site[6]. Most experiments were repeated at least twice and, in each experiment, PCR reactions were done in triplicate. The relative immunoprecipitation value represents the ratio of immunoprecipitated DNA to *POL5* input DNA after HO induction normalized to the ratio of immunoprecipitated DNA to *POL5* input DNA before HO induction. Standard errors were calculated by using error analysis for more than one measurement[29]. This analysis finds the largest error source by combining standard errors of two measurements, in this case, measurements of immunoprecipitated DNA and input DNA.

Micrococcal nuclease digestion. We collected 1-litre cultures just before and at 60-min intervals after DSB induction. Nuclei were prepared under conditions maintaining HO induction (plus 2% galactose), and 1.5 units of MNase (Worthington) were added to 0.25 ml of nuclei as described[30]. Samples were removed at 1-, 2-, 4-, 8- and 16-min intervals, and DNA was purified and separated by electrophoresis on a 1.25% agarose-TBE gel. DNA was blotted to Genescreen (Dupont-NEN) and hybridized to an 800-bp *MATα* fragment that was ~200 bp from the right side of the HO-induced DSB. Radioactive images were captured on a Model 860 Phosphoimager (Storm) and band intensities were quantified using ImageQuant TL software (Molecular Dynamics).

Received 23 November 2004; accepted 22 August 2005.

1. Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S. & Bonner, W. M. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.* **273,** 5858–5868 (1998).
2. Arkady, C. *et al.* Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. *Nature Cell Biol.* **5,** 675–679 (2003).
3. Shroff, R. *et al.* Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. *Curr. Biol.* **14,** 1703–1711 (2004).
4. Usui, T., Ogawa, H. & Petrini, J. H. A DNA damage response pathway controlled by Tel1 and the Mre11 complex. *Mol. Cell* **7,** 1255–1266 (2001).
5. Shen, X., Mizuguchi, G., Hamiche, A. & Wu, C. A chromatin remodelling complex involved in transcription and DNA processing. *Nature* **406,** 541–544 (2000).
6. Sugawara, N., Wang, X. & Haber, J. E. *In vivo* roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. *Mol. Cell* **12,** 209–219 (2003).
7. Lee, S. E. *et al. Saccharomyces* Ku70, Mre11/Rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* **94,** 399–409 (1998).
8. Downs, J. A., Lowndes, N. F. & Jackson, S. P. A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* **408,** 1001–1004 (2000).
9. Weiss, K. & Simpson, R. T. High-resolution structural analysis of chromatin at specific loci: *Saccharomyces* cerevisiae silent mating type locus *HMLα*. *Mol. Cell. Biol.* **18,** 5392–5403 (1998).
10. Reinke, H. & Horz, W. Histones are first hyperacetylated and then lose contact with the activated *PHO5* promoter. *Mol. Cell* **11,** 1599–1607 (2003).
11. Kristjuhan, A. & Svejstrup, J. Q. Evidence for distinct mechanisms facilitating transcript elongation through chromatin *in vivo*. *EMBO J.* **23,** 4243–4252 (2004).
12. Schwabish, M. A. & Struhl, K. Evidence for eviction and rapid deposition of histones upon transcriptional elongation by RNA polymerase II. *Mol. Cell. Biol.* **24,** 10111–10117 (2004).
13. Uziel, T. *et al.* Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J.* **22,** 5612–5621 (2003).
14. Lisby, M., Barlow, J. H., Burgess, R. C. & Rothstein, R. Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell* **118,** 699–713 (2004).
15. Shen, X., Ranallo, R., Choi, E. & Wu, C. Involvement of actin-related proteins in ATP-dependent chromatin remodeling. *Mol. Cell* **12,** 147–155 (2003).
16. Morrison, A. J. *et al.* INO80 and γ-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. *Cell* **119,** 767–775 (2004).
17. van Attikum, H., Fritsch, O., Hohn, B. & Gasser, S. M. Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. *Cell* **119,** 777–788 (2004).
18. Downs, J. A. *et al.* Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. *Mol. Cell* **16,** 979–990 (2004).
19. Frank-Vaillant, M. & Marcand, S. Transient stability of DNA ends allows nonhomologous end joining to precede homologous recombination. *Mol. Cell* **10,** 1189–1199 (2002).
20. Palter, K. B., Foe, V. E. & Alberts, B. M. Evidence for the formation of nucleosome-like histone complexes on single-stranded DNA. *Cell* **18,** 451–467 (1979).
21. Mizuguchi, G. *et al.* ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* **303,** 343–348 (2004).
22. Wang, X. & Haber, J. E. Role of *Saccharomyces* single-stranded DNA-binding protein RPA in the strand invasion step of double-strand break repair. *PLoS Biol.* **2,** 0104–0112 (2004).
23. Kantake, N., Sugiyama, T., Kolodner, R. D. & Kowalczykowski, S. C. The recombination-deficient mutant RPA (*rfa1-t11*) is displaced slowly from single-stranded DNA by Rad51 protein. *J. Biol. Chem.* **278,** 23410–23417 (2003).
24. Unal, E. *et al.* DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. *Mol. Cell* **16,** 991–1002 (2004).
25. Chai, B., Huang, J., Cairns, B. & Laurent, B. C. Distinct roles for the Rsc and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair. *Genes Dev.* **19,** 1656–1661 (2005).
26. Miyazaki, T., Bressan, D. A., Shinohara, M., Haber, J. E. & Shinohara, A. *In vivo* assembly and disassembly of Rad51 and Rad52 complexes during double-strand break repair. *EMBO J.* **23,** 939–949 (2004).
27. Nakamura, T. M., Du, L. L., Redon, C. & Russell, P. Histone H2A phosphorylation controls Crb2 recruitment at DNA breaks, maintains checkpoint arrest, and influences DNA repair in fission yeast. *Mol. Cell. Biol.* **24,** 6215–6230 (2004).

28. Kuo, M. H. & Allis, C. D. *In vivo* cross-linking and immunoprecipitation for studying dynamic protein:DNA associations in a chromatin environment. *Methods* **19,** 425–433 (1999).
29. Lichten, W. *Data and Error Analysis in the Introductory Physics Laboratory* (Allyn and Bacon, Newton, MA, 1988).
30. Fleming, A. B. & Pennings, S. Antagonistic remodelling by Swi-Snf and Tup1-Ssn6 of an extensive chromatin region forms the background for *FLO1* gene regulation. *EMBO J.* **20,** 5219–5231 (2001).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank J. Haber, X. Shen, J. Downs, M. Christman and V. Zakian for strains or plasmids; W.-D. Heyer for antibodies against RPA and Rad51; C. Hillyer and N. Clark for technical assistance; and C.-F. Kao for comments. This work was supported by grants from the NIH. (to M.A.O. and to J.A.N.).

Author Information Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to M.A.O. (mosley@salud.unm.edu).