DNA double strand breaks (DSBs) are potentially serious chromosomal lesions. However, cells sometimes deliberately cleave their own DNA to facilitate certain chromosomal processes, and there is much interest in how such self-inflicted breaks are effectively managed. Eukaryotic DSBs occur in the context of chromatin and the RSC chromatin-remodeling ATPase complex has been shown to promote DSB repair at the budding yeast MAT locus DSB, created by the HO endonuclease during mating type switching. We show that the role of RSC at MAT is highly specialized. The Rsc1p subunit of RSC directs nucleosome sliding immediately after DSB creation at both MAT and generally and is required for efficient DNA damage-induced histone H2A phosphorylation and strand resection during repair by homologous recombination. However, the Rsc2p and Rsc7p subunits are additionally required to set up a basal MAT locus structure. This RSC-dependent chromatin structure at MAT ensures accessibility to the HO endonuclease. The RSC complex therefore has chromatin remodeling roles both before and after DSB induction at MAT, promoting both DNA cleavage and subsequent repair.

This work was supported by departmental grants (to N. A. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1–3.

1 To whom correspondence should be addressed: Cardiff School of Biosciences, Cardiff University, Museum Ave., Cardiff CF10 3US, United Kingdom. E-mail: kentn@cardiff.ac.uk.

2 Present address: Medical Research Council Genome Damage and Stability Centre, University of Sussex, Brighton BN1 9QG, United Kingdom.

3 A Jenner Fellow of the Lister Institute of Preventative Medicine.

4 The abbreviations used are: DSB, double strand break; HR, homologous recombination.
experiments, the Sth1p ATPase subunit of RSC can be detected at the MAT DSB almost immediately following HO cleavage (9) but can also be observed to accumulate further over a period of several hours (10). Recruitment of Sth1p is reduced in rsc2 and rsc30 mutants and is also dependent on the integrity of the MRX and Ku complexes (10). Thus, there would seem to be a stepwise and repair factor-interdependent recruitment of RSC activities to the MAT DSB. These results and the observation that mammalian RSC orthologues are also implicated in DSB processing (14) suggest that RSC is a fundamental DSB repair factor.

We have been interested in defining chromatin structures and remodeling events that are general to DSB induction but also those that may represent specializations at the MAT locus. In this work, we have used micrococcal nuclease (MNase) accessibility mapping to analyze nucleosome positioning both before and after DSB induction in yeast in a variety of chromatin remodeling-defective mutants. Here we successfully dissect functional roles for RSC during both general DSB repair and MAT-specific chromatin organization.

EXPERIMENTAL PROCEDURES

*S. cerevisiae* Strains—For chromatin analysis of MAT, we primarily employed JKM179, a MATα strain in which the silent mating type loci have been deleted (15). Deletion of HML and HMR allows hybridization of specific indirect end label probe to both sides of the HO site at the MATα locus. MK205a (16) contains an HO site at +207 bp within the URA3 coding region on Chr V. YFP17 (17) contains an HO site at +117 bp within the LEU2 coding region on Chr III. AMH11 is a MATα strain but with intact *HM* loci and capable of repairing the MAT DSB by homologous recombination/switching (18). All strains express the HO gene, integrated into the ADE3 locus, under control of a galactose-inducible promoter. Full genotypes are given in the supplemental materials. All knock-out mutants were created by replacing entire coding sequences with *KanMX4* using standard techniques, apart from a JKM179 background Δrsc30::KanMX mutant SLY462 (10), which was a gift from S.-E. Lee.

Chromatin Analysis—We used a method for rapid nucleosome digestion in detergent-permeabilized yeast spheroplasts (19, 20) with the following modifications. Yeast cultures were grown in 1% peptone, 1% yeast extract plus either 2% d (+)-raffinose for JKM179 and YFP17 or 1% glycerol, 1% sodium DL-lactate for MK205a and AMH11. Cells were grown at 30 °C to densities of ~2.0 × 10^7 nucleated cells/ml (determined by hemocytometry). Cells were sampled just before (“HO 0 min” samples) and at time points after HO induction by the addition of d (+)-glucose to 2%. Spheroplasts (cells without cell walls) were created by digestion in 1 ml of 10 mg/ml *Arthrobacter luteus* yeast lytic enzyme (20,000 units/g; MP Biomedical) in 1 ml sorbitol, 5 mM β-mercaptoethanol for 4 min at 22 °C. Samples containing 2.0 × 10^8 nucleated cells were digested with MNase (150 and 300 units/ml for experiments such as those shown in Fig. 1A; 150 units/ml for Fig. 1C; and 75, 150, and 300 units/ml for Fig. 4A) at 37 °C for 3 min or processed for purification of deproteinized DNA. Deproteinized DNA samples were digested with 5 units/ml MNase at 22 °C for 10 s to create “DNA” control digests. Purified DNA samples were digested to completion with BspE1, BanII, BamHI, or PstI and separated on agarose gels (1.5% for MAT locus and *ura3::Hocs* blots, 1.2% for *leu2::Hocs* blots) together with medium range markers (GeneFlow). Southern blots of the gels were hybridized to 400-bp indirect end label probes, which abut the relevant restriction sites.

Western Blotting Analyses—JKM179 background strains were grown to midlog phase, treated with 0.2% methyl methanesulfonate for the indicated time, and harvested. Total protein was isolated by lysing cells by glass bead disruption into 20% trichloroacetic acid. Samples were electrophoresed, transferred to nitrocellulose, and analyzed using either H2A phospho-Ser^29^ primary antibody (Abcam) or an antibody that recognizes H2A regardless of phosphorylation state (21), followed by horseradish peroxidase-conjugated anti-rabbit secondary antibody (Pierce). Bands were visualized by enhanced chemiluminescence (Pierce).

DNA Damage Sensitivity Assay—5-Fold serial dilutions of midlog phase cultures of JKM179 background cells were spotted onto media containing no phleomycin or 0.02% phleomycin and incubated at 30 °C for 2 days.

Strand Resection Assay—HO expression was induced in cultures of AMH11 and an isogenic Δrsc1::KanMX mutant by the addition of 1.5% galactose and incubation for 30 min at 30 °C. HO expression was then repressed by the addition of d (+)-glucose to 1.5%. 2.0 × 10^8 nucleated cell samples were harvested for genomic DNA preparation at 0, 30, and 60 min from glucose addition. Aliquots of purified DNA (equivalent to 5.0 × 10^6 nuclei) were digested with BspE1 to generate a known end and then digested with Banl, which has a recognition site 601 bp from the HO site in the MAT-distal region. A DNA sample at the 0 min time point was digested with BspE1 alone to provide a marker for the HO site itself. Samples were denatured in a loading buffer containing 50 mM NaOH, 1 mM EDTA, and 2.5% Ficoll 400 and electrophoresed through a 1.2% agarose alkaline denaturing gel as described in Ref. 22. The gel was neutralized and blotted as for a normal Southern blot. Blots were hybridized with the double-stranded MAT-distal BspE1 end label probe as described for chromatin analysis.

RESULTS

To examine local nucleosome organization at HO-induced DSBs, we used a procedure that involves rapid MNase digestion of chromatin in permeabilized yeast cells followed by indirect end labeling (23), which maps nucleosome cleavage sites over a region of ~2 kb. Because MNase preferentially cleaves chromatin within nucleosomal linker regions, nucleosomes appear as “footprints” with this technology when digestion patterns in chromatin and deproteinized DNA are compared.

Chromatin Structure and Remodeling at the MAT DSB—Fig. 1, A and B, shows analyses of the MAT-proximal and MAT-distal sides of the MATα HO site, respectively, with cells sampled immediately before and 40 min after HO endonuclease induction. Inferred chromatin structures at the MAT locus before and after HO induction are shown superimposed over the MNase digestion patterns in Fig. 1, A and B, and in map form in Fig. 1, C and D. This analysis reveals that the uncleaved...
MATα locus has an unusual chromatin structure. The MATα-proximal side of the HO site (containing the divergent α1 and α2 coding regions) appears to consist of markedly different chromatin environments. First, chromatin MNase cleavage sites with linker periodicity suggest that two relatively positioned nucleosomes occur within the body of the α2 gene (marked 1 and 2); second, the divergent promoter for both α1 and α2 and the 5’ end of the α2 coding region demarcates an area where, although chromatin-specific and deproteinized DNA MNase cleavages are clearly different (suggesting protein/nucleosome binding), there is no obvious simple pattern of nucleosomal protection (marked Disordered region); third, the body of the α1 gene contains a particularly MNase-resistant structure protecting ~500 bp of DNA in chromatin (marked MNase resistant region). In contrast, the MATα-distal side of the HO site consists of a regular pattern of chromatin MNase cleavages with linker periodicity likely to represent an array of translationally positioned nucleosomes (marked A–I). The HO induction by MNase digestion using yeast strain JKM179 and indirect end labeling with the probe indicated. The HO-induced DSB appears as the strong band in HO 40 min samples at 1310 bp. A long exposure of the MNase-resistant region is shown to illustrate that this structure is not significantly remodeled upon DSB induction. The right-hand panel shows an inferred chromatin structure superimposed over the MNase cleavage data from the left-hand panel. The inferred structures are shown in detail in C and D. B, the MATα-distal side of the HO site consists of a tract of well positioned nucleosomes, six of which slide to new positions after DSB creation. Analysis was as for Fig. 1A but with a BspEI-specific probe. The HO-induced DSB shows up as the strong band in HO 40 min samples at 1750 bp. The right-hand panel again shows an inferred chromatin structure superimposed over the MNase cleavage data from the left-hand panel. The inferred structures are shown in detail in C and D. C, schematic map of inferred nucleosome positions and chromatin structures at the yeast MATα locus before HO cleavage based on data from A and B. D, schematic map of changes in chromatin structure upon creation of a DSB by induction of the HO endonuclease based on data from A and B. Changes in nucleosome position and the alteration in extent of MNase sensitivity in the MATα-proximal region are emphasized with arrows.
site itself occurs within the linker region between nucleosome A and the MNase-resistant structure within the α1 gene in a manner that might be expected to promote its accessibility to HO. Whatever the precise nucleosomal organization of the MATα-proximal side of the HO site, Fig. 1A shows that creation of the DSB, by inducing HO, does not significantly affect its structure. A small increase in MNase accessibility is observed immediately flanking the DSB, but the main chromatin features of the MATα-proximal side remain unchanged. This is in contrast to events on the MATα-distal side of the DSB, where numerous sites of MNase cleavage in chromatin in the region flanking the HO site reappear at different locations after introduction of the DSB. Because MNase cleavage sites normally characteristic of deproteinized DNA remain protected after introduction of the DSB (with the exceptions discussed below), these results suggest that the MATα-distal DSB-flanking nucleosomes are repositioned rather than evicted. The alterations in position of chromatin-specific MNase cleavage sites are consistent with the six nucleosomes closest to the DSB (marked A–F) sliding away from the break and taking up new positions. Nucleosomes further away from the break (marked G, H, and I) appear to remain in the same position, showing that this remodeling event is relatively localized to the DSB. Fig. 2 shows a time course experiment of the changes in MATα-distal MNase cleavage after HO induction, and DSB-dependent nucleosome reorganization appears concomitant with HO cleavage. Nucleosome sliding at the MAT DSB therefore precedes the histone eviction event, mediated primarily by the Mre11p-Rad50p-Xrs2p (MRX) complex, which can be shown to occur 60–180 min after HO induction (24). We have also tested both mre11 and rad50 mutant strains and can show that MATα-distal nucleosome remodeling after DSB induction is entirely normal (supplemental Fig. 1). We conclude that this localized remodeling event at the MAT DSB represents a process mechanistically different from MRX-dependent histone eviction.

Nucleosomes Are Also Remodeled at Non-MAT DSBs—In order to test the generality of our observations at MAT, we next examined HO-induced DSBs at two other loci. Fig. 3, A and B, show an MNase accessibility analysis of both sides of an HO site engineered into the URA3 coding region. This HO site does not cleave as efficiently as the HO site within MAT, with only half of the cells in the population showing cleavage even after a 60-min galactose induction. Thus, the galactose-induced cell samples yield MNase cleavage patterns that are a superimposition of HO-cleaved and uncleaved states. Nevertheless, novel chromatin-specific sites of MNase accessibility are observed in the galactose-induced samples con-

![Figure 2](image-url)  
**FIGURE 2.** DSB-dependent nucleosome sliding at the MATα locus occurs concomitantly with break formation. JKM179 yeast cells were sampled before and at 10-min intervals after induction of the HO endonuclease. Chromatin samples were digested with 150 units/ml MNase, and MNase cleavage sites in the MATα-distal region were visualized by indirect end labeling as in Fig. 1B. The region of remodeling shown in Fig. 1, B and D, is marked with a dotted line.

![Figure 3](image-url)  
**FIGURE 3.** DSB formation by HO cleavage is generally accompanied by localized nucleosome remodeling. A, nucleosome sliding occurs on the 5′ side of an HO DSB within the URA3 gene. Indirect end label analysis using yeast strain MK205a with a probe to the BspEI site of ura3::HOcs. A region of altered chromatin MNase accessibility that occurs after DSB creation is indicated with a dotted line to the right of the blot. Inferred nucleosome positions are shown in the supplemental materials. B, nucleosome sliding occurs on the 3′ side of an HO DSB within the URA3 gene. Indirect end label analysis using yeast strain MK205a with a probe to the BamHI site of ura3::HOcs. C, nucleosome sliding occurs on the 3′ side of an HO DSB within the LEU2 gene. Indirect end label analysis using yeast strain YFP17 with a probe to the PstI site of leu2::HOcs.
consistent with localized nucleosome remodeling after DSB formation. Both sides of the HO site at *URA3* are significantly remodeled in a similar manner to the nucleosomes of the *MATα-distal region. Fig. 3C shows an analysis of an HO site within the *LEU2* coding region. Although this HO site cleaves very efficiently, the presence of a Ty retroelement immediately 5′ to *LEU2* precludes analysis of both sides of this DSB. Nevertheless, the LEU2 3′ side of the HO site again shows significant changes in chromatin MNase accessibility on DSB formation consistent with six nucleosomes flanking the HO site moving to new posi-
Rsc1p Is Required for DSB-dependent Nucleosome Remodeling—Next, we used the MATα-distal assay of Fig. 1B to search for potential factors that might influence nucleosome sliding at DSBs. We tested mutants deficient in all of the known yeast chromatin-remodeling ATPase complexes, including isw1, isw2, chd1, arp8 (which affects INO80.com activity), fun30, and rad54. None of these mutants prevented nucleosome sliding at the MAT DSB (data not shown). Yeast deficient in either SWI/SNF or SWR.com also showed normal MAT DSB nucleosome sliding (supplemental Fig. 3). These results are consistent with functional roles for SWI/SNF at a relatively late stage of HR repair (9) and for SWR.com in histone H2A/Htz1p exchange rather than in nucleosome displacement (25). Strikingly, however, mutants affecting the RSC chromatin-remodeling ATPase complex were found to have profound effects on MAT locus nucleosome positioning.

Fig. 4A shows that cells with mutations in the rsc2, rsc7, and rsc30 genes all show evidence of the characteristic DSB-dependent change in MATα-distal chromatin MNase accessibility characteristic of wild-type cells. Thus, the Rsc2p, Rsc7p, and Rsc30p subunits are not required for DSB-dependent nucleosome remodeling. In contrast, HO-cleared chromatin MNase cleavage patterns in a rsc1 mutant are identical to the uncleaved pattern, suggesting that nucleosome sliding is abolished at MATα in these cells. To test the generality of this observation, we also tested DSB-dependent chromatin remodeling at the HO site within the LEU2 locus. Fig. 4B shows that, again, loss of Rsc1p substantially abolishes the change in chromatin MNase accessibility associated with DSB formation at LEU2, whereas loss of Rsc7p does not. We therefore conclude that the Rsc1p subunit of the RSC complex specifically mediates nucleosome remodeling after DSB formation in yeast.

Rsc1p Is Required for Efficient H2A Phosphorylation and Strand Resection during HR—One of the earliest responses to a DNA break is phosphorylation of H2A on Ser129 (21), and this occurs at approximately the same time after a DNA break as our Rsc1p-dependent remodeling event. We therefore examined the rsc1 mutant strain and found that there is a significant reduction in H2A phosphorylation compared with wild type (Fig. 4C). This is consistent with the reduced H2A phosphorylation seen in a strain in which Sth1p is depleted (11). Interestingly, however, H2A phosphorylation appears to be indistinguishable from wild type in an rsc2 mutant strain. These data suggest that the Rsc1p-dependent remodeling event subsequent to DSB formation is specifically required for efficient H2A phosphorylation. A genome-wide role for Rsc1p in DSB repair is confirmed in Fig. 4D, which shows that our Δrsc1::KanMX mutant is highly sensitive to the DSB-inducing drug phleomycin.

The results concerning the MAT locus, shown above, are all demonstrated in yeast based on the strain JKM179 (15), in which the silent mating type loci are deleted. These strains can therefore only repair the MAT DSB via the nonhomologous end joining pathway. As described above, recent analyses suggest that Rsc1p and Rsc2p play a functional role within the HR pathway, and chromatin immunoprecipitation analysis of repair factors in Sth1p-depleted cells suggests that the Mre11p component of the MRX complex and the single-stranded DNA-binding factor RPA no longer associate with the MAT DSB (11). These results predict a defect in MRX-dependent 5’ to 3’ exonuclease-mediated strand resection that occurs in the early stages of HR to create a DNA single strand capable of invading a donor locus (6, 7). We therefore performed an adaptation of a resection assay (22) to measure the formation of strand-resected DNA up to the Banl site within the MATα-distal region that occurs 601 bp from the HO-induced DSB (Fig. 5A). Because restriction endonucleases can only recognize and cleave their cognate binding motifs in double-stranded DNA, loss of cleavage of a restriction enzyme site, such as Banl, within a region of DNA can be used to infer the presence of single-stranded HR repair intermediates (6, 7, 22). HO expression in yeast based on the HR-competent HML, HMR strain AMH11 (18) was induced by galactose induction for 30 min. HO expression was then repressed by the addition of glucose to the culture, which leads to the rapid clearance of HO from the cells by proteosomal destruction (6) and allows repair via HR/switching to proceed (22). Samples of genomic DNA were harvested over a 60-min period, and the presence of cleavable Banl sites was
assayed by indirect end labeling of DNAs separated on alkaline denaturing agarose gels with the BspEI probe used for MAT-distal chromatin structure analysis (Fig. 5B). Strand-resected intermediates are detected as higher molecular weight (BanI-uncleaved) products, similar in size to the HO-induced DSB fragment revealed by probing a DNA sample at time 0 that is not treated with BanI. Fig. 5B shows that strand-resected intermediates are clearly observed in wild-type AMH11 but are completely absent in a Δrsc1::KanMX mutant. Fig. 5C shows that DSB-dependent nucleosome sliding occurs normally in the HML, HMR strain. We therefore conclude that Rsc1p is required for strand resection during HR/switching at the MATα locus.

**RSC Is Also Required for Normal MATα Chromatin Structure**—A close inspection of the chromatin digests before HO cleavage (Fig. 4A) shows significant differences in MNase cleavage pattern from that observed in wild-type cells. Fig. 6A shows a direct comparison of MNase cleavage patterns in wild-type and rsc7 cells at the intact MATα locus mapped from the MATα-distal BspEI site. This comparison shows that both nucleosome positioning within the MATα-distal region and the integrity of the unusual MNase-resistant structure at the MATα-proximal region are both disrupted in rsc7 cells. A comparison of MATα/chromatin in rsc2 and rsc7 cells mapped from the MATα-proximal BanII site, Fig. 6B, shows that the defect is similar in both mutants. Rsc7p is part of a module within RSC, which appears to be involved in transcriptional regulation related to cell wall stress (26). However, we note that loss of Rsc7p is known to affect the overall integrity of the RSC complex (26); thus, we cannot exclude the possibility that the rsc7 defect we observe is relatively nonspecific. We also note that the Rsc2p/Rsc7p-dependent defect in basal MATα chromatin structure appears to propagate into the remodeled state after DSB induction (Fig. 4A). This result may therefore explain a minor rsc2-associated defect in

FIGURE 6. The Rsc2p and Rsc7p components of RSC are required for normal MATα chromatin structure and HO site accessibility. A, indirect end label analysis comparing MATα chromatin structure in wild-type and isogenic Δrsc7::KanMX strains using a probe to the BspEI site as in Fig. 1B. The region of Rsc7p-dependent chromatin in the intact locus is indicated with a dashed line. B, loss of the MATα-proximal MNase-resistant structure in rsc2 or rsc7 mutants does not alter the extent of nucleosome remodeling on DSB induction. Shown are indirect end label analyses of isogenic Δrsc2::KanMX and Δrsc7::KanMX strains as described for Fig. 1A. The region normally resistant to MNase in wild-type cells (compare with Fig. 1A) is indicated with a dashed line. C, HO endonuclease cleavage at MATα is reduced in rsc2 and rsc7 cells. DNA was isolated from isogenic (strain JKM179) wild-type, Δrsc2::KanMX, and Δrsc7::KanMX cells either before or 40 min after HO induction, cleaved with BspEI, and analyzed by Southern blotting using the MAT BspEI end label probe. D, HO endonuclease cleavage at an HO site within the LEU2 locus is normal in rsc2 and rsc7 cells. DNA was isolated from isogenic (strain YFP17) wild-type, Δrsc2::KanMX, and Δrsc7::KanMX cells either before or 40 min after HO induction, cleaved with PstI, and analyzed by Southern blotting using the LEU2 PstI end label probe.
remodeling reported by Shim et al. (11). Nevertheless, we conclude that the presence of either Rsc2p or Rsc7p, but not Rsc1p, is required for normal MATα chromatin structure.

Rsc2p/Rsc7p-dependent MAT Chromatin Structure Is Required for HO Site Accessibility—Although loss of Rsc2p or Rsc7p affects the integrity of the MATα-proximal MNase-resistant structure, Fig. 6B shows that this region of chromatin remains refractory to nucleosome remodeling via the Rsc1p-dependent RSC activity that is still available in the cells. Therefore, the Rsc2p/Rsc7p-dependent chromatin structure at MATα does not appear to have a function in controlling the asymmetry of subsequent remodeling. However, we noted that during chromatin analyses of the rsc2 and rsc7 mutants, the percentage of cells cleaved by the HO nuclease at MATα after galactose induction of HO seemed lower than that normally observed in the other strains. A Southern analysis (Fig. 6C) shows that the proportion of cells with a DSB at MATα after 40 min of HO induction is ~40% lower in rsc2 or rsc7 cells than that observed in wild-type cells. In contrast, loss of Rsc2p or Rsc7p does not similarly affect the cleavage of the LEU2 HO site (Fig. 6D). Our analysis, in Fig. 4B, showed that LEU2 locus chromatin structure is completely normal in a rsc7 mutant (this is also true of a rsc2 strain; data not shown) and therefore provides a control for any effect that loss of RSC-dependent chromatin remodeling activity might have on HO expression. We conclude that the Rsc2p/Rsc7p-dependent chromatin structure at MATα functions to maximize accessibility of the HO site.

DISCUSSION

Chromatin Structure Surrounding the MAT DSB—Our analysis of the HO-induced DSB at MATα provides a detailed picture of the chromatin structures present at both the uncleaved and cleaved loci. The structure we determined for the MATα locus prior to HO induction is entirely consistent with previous work comparing regions of the locus (such as the a1/a2 divergent promoter) with its silent counterpart HML, which showed Sir protein-dependent structures involved in transcriptional repression and occlusion of the HO site (27, 28). Upon induction of the HO endonuclease, we observed a highly localized chromatin remodeling event concomitantly with break formation. Changes in MNase digestion patterns are consistent with six nucleosomes sliding away from the break and taking up new positions on the MATα-distal side of the DSB and a more modest reorganization of an MNase-resistant structure present at the MATα-proximal side of the break. These results are reasonably consistent with recent work, using a technique of quantitative PCR measurement technique of MNase-protected DNA species, which concluded that four nucleosomes are displaced on the MATα-distal side of the DSB and one nucleosome on the MATα-proximal side (11). We note that MNase cleavage sites immediately adjacent to both sides of the DSB, normally detected only in deproteinized DNA, become accessible in the chromatin samples after HO induction. This suggests that this localized chromatin remodeling event, although asymmetrical, renders both DNA ends accessible to incoming repair factors, again consistent with the results of Shim et al. (11).

RSC-dependent Chromatin Remodeling at MAT and Non-MAT DSBs—Indirect end label analysis of chromatin at HO-induced DSBs within the URA3 and LEU2 genes showed alterations in MNase accessibility similar to those observed at MAT. Thus, localized nucleosome displacement also appears to occur generally in a non-MAT chromosomal context. Our reverse genetic analysis, using yeast mutants defective in a variety of DNA repair and chromatin-remodeling factors, confirmed that this process is separate from the histone eviction event mediated by MRX (24) and specifically requires the Rsc1p-dependent activity of the RSC ATPase complex. This result is consistent with recent work showing that depletion of the RSC ATPase subunit Sth1p from cells abolishes nucleosome sliding at the MATα DSB (11). We were also able to show that loss of Rsc1p activity specifically affects the efficiency of C-terminal H2A phosphorylation and abolishes strand resection during the HR/switching repair process at MAT. These results suggest that localized nucleosome sliding at DSBs is important both for creating a critical damage-specific histone mark and for an early step in homologous recombination. RSC is a 15-subunit SWI/SNF-related chromatin-remodeling complex, and the Rsc1p and Rsc2p subunits are homologues, sharing 62% amino acid similarity (29). Both factors contain bromo, bromo-adjacent homology, and AT-hook domains, which may play a role in chromatin binding, and it is possible to purify distinct Rsc1p- and Rsc2p- associated forms of RSC (29). However, genome-wide chromatin immunoprecipitation analysis shows that Rsc1p and Rsc2p co-localize, suggesting that the two forms of RSC could exist in the cell as a single multifunctional molecular entity (30). Fortunately, our nucleosome positioning methodology provides an assay for the activity of chromatin-remodeling enzymes and allowed us to separate Rsc1p and Rsc2p functions at MAT. Although both subunits have been implicated in DSB repair, their functional roles (as described in the Introduction) appear to be different, with Rsc2p directing RSC activity during some aspect of the DNA ligation process during HR (9, 10). Our results indicate that, whatever the precise function of Rsc2p subsequent to DSB formation, it is not mediated via an observable nucleosome remodeling event over the time scale of our assays. Interestingly, however, we were able to demonstrate a chromatin remodeling role for Rsc2p (and Rsc7p), specific to the MATα locus, prior to HO-endonuclease cleavage. Both Rsc2p and Rsc7p are required for normal uncleaved MATα locus nucleosome organization, and this chromatin environment promotes efficient HO-endonuclease cleavage.

Chromatin structure has previously been shown to play a major role regulating the function of the yeast silent mating type loci, with involvements in transcriptional silencing, HO site occlusion, and recombination enhancer control (6). We have now shown that chromatin structure and remodeling is also a key component of MAT locus function, with the RSC complex playing a dual role in promoting both DSB formation and its subsequent repair. RSC orthologues in mammalian cells have been implicated in DSB repair via damage response phosphorylation of histone variant H2AX (14), suggesting that the Rsc1p-mediated DSB repair function we have characterized is conserved from yeast to humans. It will therefore be interesting to test whether RSC orthologues in mammalian cells might play...
an additional role in modulating DSB formation via chromatin structure at locations such as VDJ regions within antibody and T cell receptor genes (3).

Acknowledgments—We are grateful to Viv Perkins, André Furger, Martin Dalziel, and Koichi Ito for comments and Amaris Walker for technical help. We thank N. Sugawara, J. Haber, S-E. Lee, and M. Kupiec for yeast strains.

REFERENCES
1. Jackson, S. P. (2002) Carcinogenesis 23, 687–696
2. Keeney, S., and Neale, M. J. (2006) Biochem. Soc Trans. 34, 523–525
3. Cobb, R. M., Oestreich, K. J., Osipovich, O. A., and Oltz, E. M. (2006) Adv. Immunol. 91, 45–109
4. Costelloe, T., FitzGerald, J., Murphy, N. J., Flaus, A., and Lowndes, N. F. (2006) Exp. Cell Res. 312, 2677–2686
5. Osley, M. A., and Shen, X. (2006) Trends Genet. 22, 671–677
6. Haber, J. E. (1998) Annu. Rev. Genet. 32, 561–599
7. Pâques, F., and Haber, J. E. (1999) Microbiol. Mol. Biol. Rev. 63, 349–404
8. Foster, E. F., and Downs, J. A. (2005) FEBS J. 272, 3231–3240
9. Chai, B., Huang, J., Cairns, B. R., and Laurent, B. C. (2005) Genes Dev. 19, 1656–1661
10. Shim, E. Y., Ma, J. L., Oum, J. H., Yanez, Y., and Lee, S. E. (2005) Mol. Cell Biol. 25, 3934–3944
11. Shim, E. Y., Hong, S. I., Oum, J. H., Yanez, Y., Zhang, Y., and Lee, S. E. (2007) Mol. Cell Biol. 27, 1602–1613
12. Neely, K. E., and Workman, J. L. (2002) Biochim. Biophys. Acta 1603, 19–29
13. Huang, J., Hsu, J. M., and Laurent, B. C. (2004) Mol. Cell 13, 739–750
14. Park, J. H., Park, E. J., Lee, H. S., Kim, S. J., Hur, S. K., Imbalzano, A. N., and Kwon, J. (2006) EMBO J. 25, 3986–3997
15. Lee, S. E., Moore, J. K., Holmes, A., Umezlu, K., Kolodner, R. D., and Haber, J. E. (1998) Cell 94, 399–409
16. Aylon, Y., Liefshitz, B., Bitan-Banin, G., and Kupiec, M. (2003) Mol. Cell Biol. 23, 1403–1417
17. Pâques, F., Leung, W. Y., and Haber, J. E. (1998) Mol. Cell Biol. 18, 2045–2054
18. Holmes, A. M., and Haber, J. E. (1999) Cell 96, 415–424
19. Kent, N. A., and Mellor, J. (1995) Nucleic Acids Res. 23, 3786–3787
20. Fazzio, T., and Tsukiyama, T. (2003) Mol. Cell 12, 1333–1340
21. Downs, J. A., Lowndes, N. F., and Jackson, S. P. (2000) Nature 408, 1001–1004
22. White, C. L., and Haber, J. E. (1990) EMBO J. 9, 663–673
23. Wu, C. (1980) Nature 286, 854–860
24. Tsukuda, T., Fleming, A. B., Nickoloff, J. A., and Osley, M. (2005) Nature 438, 379–383
25. Papamichos-Chronakis, M., Krebs, J. E., and Peterson, C. L. (2006) Genes Dev. 20, 2437–2449
26. Wilson, B., Erdjument-Bromage, H., Tempst, P., and Cairns, B. R. (2006) Genetics 172, 795–809
27. Nasmyth, K. A. (1982) Cell 30, 567–578
28. Weiss, K., and Simpson, R. T. (1998) Mol. Cell. Biol. 18, 5392–5403
29. Cairns, B. R., Schlichter, A., Erdjument-Bromage, H., Tempst, P., Kornberg, R. D., and Winston, F. (1999) Mol. Cell 4, 715–723
30. Ng, H. H., Robert, F., Young, R. A., and Struhl, K. (2002) Genes Dev. 16, 806–819