Involvement of Sir2/4 in Silencing of DNA Breakage and Recombination on Mouse YACs during Yeast Meiosis

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Submitted July 15, 2004; Revised November 29, 2004; Accepted December 21, 2004
Monitoring Editor: Douglas Koshland

Yeast artificial chromosomes (YACs) that contain human DNA backbone undergo DNA double-strand breaks (DSBs) and recombination during yeast meiosis at rates similar to the yeast native chromosomes. Surprisingly, YACs containing DNA covering a recombination hot spot in the mouse major histocompatibility complex class III region do not show meiotic DSBs and undergo meiotic recombination at reduced levels. Moreover, segregation of these YACs during meiosis is seriously compromised. In meiotic yeast cells carrying the mutations *sir2* or *sir4*, but not *sir3*, these YACs show DSBs, suggesting that a unique chromatin structure of the YACs, involving Sir2 and Sir4, protects the YACs from the meiotic recombination machinery. We speculate that the paucity of DSBs and recombination events on these YACs during yeast meiosis may reflect the refractory nature of the corresponding region in the mouse genome.

INTRODUCTION

DNA double-strand breaks (DSBs) appear to be one of the hallmarks of meiosis in budding yeast (Keeney, 2001) as well as in fission yeast (Cervantes *et al.*, 2000; Zenvirth and Simchen, 2000) and in mouse (Mahadevaiah *et al.*, 2001; Zenvirth *et al.*, 2003). Meiotic DSBs occur preferentially in regions that have a high tendency to undergo recombination, the so-called recombination hot spots, at least in budding yeast. A DSB in one of the four chromatids initiates the process of meiotic recombination; resection of the DSBs, to form 3' single-strand overhangs, was found both in yeast (Sun *et al.*, 1991) and in mouse spermatocytes (Zenvirth *et al.*, 2003). Meiotic DSBs are repaired by interaction with homologous nonsister chromatids, thus forming recombinant molecules and chiasmata, which hold together the two homologous chromosomes and facilitate their proper segregation.

The universality of meiotic DSBs and their repair by homologous recombination is further enhanced by the findings that yeast artificial chromosomes (YACs), consisting largely of DNA from human cells, also undergo breakage during meiosis in yeast (Klein *et al.*, 1996a, 1996b), as well as meiotic recombination (Sears *et al.*, 1992, 1994) at rates characteristic to yeast rather than to meiosis in humans. Moreover, YACs containing DNA from recombination hot spots in the human genome undergo 2–3 times more double-strand breakage in yeast meiosis than YACs containing DNA from cold spots (Klein *et al.*, 1996b), indicating that important features of the recombination machinery are conserved and shared between humans and yeast.

Here we report on mouse-DNA YACs that, unlike all other YACs previously examined, do not undergo meiotic

DSBs during yeast meiosis. We also find that recombination on these YACs is severely compromised and that their segregation in meiosis is very distorted. We show that silencing of meiotic DSBs on these YACs is relieved by mutations in two *SIR* genes, suggesting that the YACs assume a chromatin configuration that is inaccessible to the meiotic-DSB forming proteins. As the two refractory YACs contain DNA from a recombination hot spot in the MHC class III region of mouse chromosome 17 (Snoek *et al.*, 1998), we try to speculate on the behavior of this region during meiosis in the mouse.

MATERIALS AND METHODS

YACs and Strains of *Saccharomyces cerevisiae*

YAC99 (200 kb) and YAC100 (450 kb) contain DNA from the MHC class III region on chromosome 17 of the mouse (Snoek *et al.*, 1996, 1998). YAC109 (150 kb), YAC35 (370 kb), and YAC328 (500 kb) contain DNA from mouse chromosome 2. The five YACs were obtained by M. Snoek by selection from the YACs at Genethon (Paris), using microsatellite markers located in the desired region. The YACs were derived from plasmid pYAC4 (Burke and Olson, 1991) and carry the yeast markers *URA3* (at the end of the long arm) and *TRP1* (on the short arm, near the centromere), unless otherwise indicated in the text. The YACs were originally generated in yeast strains derivatives of AB1380 *MATa*, *ura3*, *trp1*, *ade2-l*, *his5*, *lys2*, *ile*[–], *thr*[–], *can1* (Burke and Olson, 1991) and were transferred by *Kar1*[–] matings (Hugerat *et al.*, 1994; Spencer *et al.*, 1994) to haploids of SK1 genetic background. For examination of meiotic DSBs, the final recipients of the YACs were of the following genotypes: Strain 2850: *MATa*, *ho::hisG*, *ura3*, *lys2*, *trp1*, *rad50S::ura3*, *can-1*; Strain 2851: *MATa*, *ho::hisG*, *ura3*, *lys2*, *trp1*, *rad50S::ura3*, *cyh2*; and Strain 2860: *MATa*, *ho::LYS2*, *ura3Δ*, *lys2*, *leu2*, *rad50S::ura3*, *cyh2*. The YAC-carrying haploids were mated with SK1 haploids of the opposite mating type, carrying the *rad50S* mutation, either with the same YAC or without a YAC.

To test the effect of *sir* mutations on meiotic DSBs, *sir* gene disruptions were introduced by one-step transformation with the following plasmid-derived fragments: *HindIII* fragment of plasmid pJH540 for disrupting *SIR2* (Ivy *et al.*, 1986), *SalI-XhoII* fragment of plasmid pKL12 for disrupting *SIR3* (Stone *et al.*, 1991) and *SmaI-PvuII* fragment of pBe200 for disrupting *SIR4* (Ivy *et al.*, 1986). The *sir* deletions were verified by PCR analysis and by their phenotypic expression (these haploid strains did not mate and started sporulation.)

For the genetic analysis of meiotic recombination, end-markers on the YACs had to be replaced by other markers. Thus on one YAC, *TRP1* was replaced by *ADE2* by one-step transformation with a *NotI-SacI* fragment of plasmid pPHH2 (Hugerat and Simchen, 1993), and on another, homologous

This article was published online ahead of print in *MBC in Press* (<http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E04-07-0592>) on January 12, 2005.

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YAC, *URA3* was replaced by *LEU2*, by transformation with the *HindIII-PvuII* fragment of plasmid pPH3. The latter was obtained by inserting the *LEU2* *SalI-XhoI* fragment (2.3 kb) from YEp13, into the *SalI* site of pBR322. These two replacements provided us with two homologous YACs, between which recombination could easily be examined by tetrad analysis. One YAC was marked *ADE2-URA3* and the other *TRP1-LEU2*. The two YACs were transferred sequentially by *Kar1⁻* matings (Hugerat and Simchen, 1993) to strain NE29 (*MAT α , ura3, lys2, leu2, trp1, ade2, his4, can1*), which was later mated to NE30 (*MAT α , ura3, lys2, leu2, trp1, ade2, HIS4*), to form the diploids that were sporulated and of which tetrads were dissected.

Media, Growth, and Sporulation Conditions

Yeast cultures were grown on rich (YEPD), on synthetic defined (SD) medium (Sherman, 1991), or on SPO plates (Kassir and Simchen, 1991). To keep the YACs from getting lost, cells were grown on SD plates that lacked the nutrients for which the markers on the YACs provided (e.g., for a strain containing a YAC marked *LEU2-URA3* the medium lacked leucine and uracil).

For determination and mapping of meiotic DSBs (Zenvirth *et al.*, 1992), cells were grown overnight in liquid YEPD resuspended in YEPA at dilution 1:400, and grown with vigorous agitation to cell density of $1-2 \times 10^7$ /ml and then washed in water and resuspended at the same cell density in liquid SPM.

For tetrad analysis of YAC segregation and recombination, cells were sporulated on plates; first they were grown for 2–3 d on YEPD plates and then the culture was spread on SPO plates (Kassir and Simchen, 1991).

Identification of Meiotic DSBs in Sporulating Cultures

Chromosome-length DNA was prepared from samples withdrawn immediately (time 0) and 6 h after transfer to SPM sporulation medium and analyzed by pulsed-field gel electrophoresis (PFGE) in a CHEF^{DR} apparatus (Bio-Rad, Richmond, CA), as described earlier (Zenvirth *et al.*, 1992). Southern blots were hybridized to a series of ³²P-labeled probes and the autoradiograms compared.

Probes used in this work were prepared from digested DNA fragments or PCR products: pBR322: marks all YACs by hybridizing to YAC ends; B2: repetitive sequences specific to the mouse genome, used to identify mouse DNA in YACs. Fragment of gene *YCR48w* was used to test meiotic DSBs on yeast chromosome III. The probes were labeled by random priming protocol (Roche, Mannheim, Germany) with [α -³²P]dCTP.

RESULTS

Absence of DSBs on Two Mouse DNA YACs

Unlike more than thirty YACs with human or plant DNA previously examined during yeast meiosis (Klein *et al.*, 1996a, 1996b; and unpublished results), yeast strains carrying mouse DNA YAC99 (~200 kb) or YAC100 (~450 kb) did not show any signs of DSBs during meiosis. The experimental procedure by which these YACs were examined was as follows. Each of the YACs was transferred from the strain in which the YAC library was generated, AB1380 (Burke and Olson, 1991), to a *rad50S MAT α* strain (2580), by two-stage *Kar1⁻* mating (Hugerat *et al.*, 1994; Spencer *et al.*, 1994). The *rad50S MAT α* strains with the YACs were mated to a *rad50S MAT α* strain, to form diploids, in which meiotic DSBs could be easily examined. The diploid strains were grown in YEPA medium and sporulated in SPM, as described in *Materials and Methods*. Cell samples were collected at times 0 and 6 h after the initiation of meiosis (transfer from YEPA to SPM), from which intact, chromosome-size DNA molecules were prepared (Zenvirth *et al.*, 1992). These DNA samples were run on a PFGE apparatus, the gels were blotted onto membranes, and these were hybridized to mouse-DNA specific ³²P-labeled probe, B2 (Figure 1A); similar results were obtained when pBR322 DNA was used as a probe (unpublished data). Three “trivial” explanations for the absence of meiotic DSBs on the MHC class III YACs were considered: 1) The strains carrying YAC99 or YAC100 did not undergo meiosis and therefore did not initiate the recombination process. We know that due to homozygosity of the *rad50S* mutation, these cells cannot complete meiosis and only aberrant asci are formed. Such cells were observed in the cultures undergoing sporulation, but it was not clear whether meiotic DSBs were formed in these cells (on the

Figure 1. Absence of meiotic DSBs on YACs containing mouse MHC class III sequences, in *rad50S* strains. Analysis of two yeast strains containing two different YACs, YAC99 (200 kb) and YAC100 (450 kb). Chromosome-size DNA was extracted from samples after 0 and 6 h in meiosis, separated by PFGE, and blotted. The blots were Southern-hybridized to mouse-DNA probe B2 (A) and to yeast chromosome-III-specific probe *YCR48w* (B).

“native” yeast chromosome). To check whether early events of meiosis and recombination had taken place, we stripped off the blotted membranes from the B2 radioactive label and rehybridized the blots with another ³²P-labeled probe, specific for yeast chromosome III. As seen in Figure 1B, the native yeast chromosome did undergo meiotic breakage in the same DNA samples in which the YACs remained intact. Therefore it appears that silencing of double-strand breakage on these mouse YACs is specific to the alien chromosomes. 2) Another possibility that we considered was that YACs carrying mouse DNA did not show meiotic DSBs because they differ in a fundamental way from native yeast chromosomes. Such a difference was not found with human DNA YACs, however. Nevertheless, we checked the behavior, during yeast meiosis, of several additional YACs that contain DNA from chromosome 2 of the mouse (Figure 2 and unpublished data). Figure 2 represents an experiment, in which meiotic DSBs were examined in four yeast strains, three with YACs containing DNA from chromosome 2 and a fourth strain with YAC100, from the MHC class III region. The former three YACs underwent meiotic breakage, like the yeast chromosomes, whereas the latter did not. We conclude that silencing of meiotic DSBs occurs only on the YACs containing DNA from this region, YAC99 and YAC100, and not on other mouse DNA YACs. 3) Another possible reason why the two MHC YACs were silenced with respect to meiotic DSBs could be that they lacked the DNA sequence that are normally associated with the main meiotic DSBs in yeast, the CoHR motif (Blumental-Perry *et al.*, 2000). (However, a recent study by Haring *et al.* (2004) has questioned the association of the defined CoHR with some DSBs). We checked for the presence of CoHR in the sequence containing the genes *G7e*, *G7a*, and *G7c* of the MHC class III region, which are found in YAC99 and YAC100 (van Kooij *et al.*, 2001), both forward and reverse DNA strands, using the program ProfileGap (Blumental-Perry *et al.*, 2000). Five high-quality matches to CoHR were found (Figure 3); thus, the absence of DSBs on the MHC YACs could not be explained by lack of the CoHR motif. We conclude that these, mouse MHC class III YACs, are refractory to meiotic DSBs despite containing the sequence motifs that are often found near or at the breaks.

We have also considered the possibility that the MHC class III YACs undergo DSBs only very late in meiosis,

Figure 2. Accumulation of meiotic DSBs on YACs containing DNA sequences from mouse chromosome 2, compared with absence of meiotic DSBs on YAC containing mouse MHC class III sequences. Analysis of yeast strains containing YACs with inserts of different mouse DNA sequences, YAC328 (500 kb), YAC35 (370 kb), YAC109 (150 kb), and YAC100 (450 kb). Chromosome-size DNA was extracted from cell samples undergoing meiosis, after 0 and 6 h, separated by PFGE, and blotted onto membranes. The membranes were hybridized to mouse-DNA specific probe B2.

because it is known that late-occurring DSBs might not be detected in *rad50S* strains (Borde *et al.*, 2000). We therefore examined DSBs during meiosis in a *Rad*⁺ strain that contain a pair of YAC100. No obvious breaks were observed on the YACs (using pBR322 DNA as a probe), whereas transient meiotic DSBs were clearly seen on chromosome III (unpublished data).

Meiotic Recombination of the MHC Class III YACs Is Severely Compromised

As the two YACs that contained DNA of the mouse MHC class III region were refractory to meiotic double-strand breakage in yeast cells, we wanted also to see whether these chromosomes undergo recombination during yeast meiosis. Human-DNA YACs have been shown to recombine readily during yeast meiosis, at high frequencies, comparable to recombination along native yeast chromosomes (Sears *et al.*, 1992). To study meiotic recombination on YACs, one needs to construct diploid strains with pairs of homologous YACs

that differ from each other at markers in at least two loci. The original YACs carry the marker *TRP1* on the short arm, near the centromere, and *URA3* at the end of the long arm. For each YAC in the original strains, *TRP1* was replaced by *ADE2* by transformation, as described in *Materials and Methods*, thus resulting in a YAC with the markers *ADE2-URA3*. In parallel to this transformation, the original strain (with YAC *TRP1-URA3*) was also subject to replacement of *URA3* by the marker *LEU2*, thus resulting in a YAC with the markers *TRP1-LEU2*. The two YACs were each transferred by two-stage *Kar1*[−] mating (Hugerat *et al.*, 1994) to the same haploid strain, NE29, from which *MATa/MATα* diploids were derived by mating to strain NE30; each diploid was homozygous for the mutations *ade2*, *ura3*, *trp1*, *leu2*, *lys2*, and carried the two homologous YACs, marked *ADE2-URA3* and *TRP1-LEU2*. The diploids did not carry any *rad* mutation and underwent normal meiosis with high efficiency. On tetrad dissection, good spore germination was observed (>90%). These YAC manipulations and strain constructions were done separately for YAC99, YAC100, and YAC109, the latter being a ~150-kb YAC with DNA from mouse chromosome 2. No significant rearrangements or aberrations of these YACs were observed during these manipulations or during vegetative growth of the yeast cells (chromosomes were examined by pulsed-field gel electrophoresis at each stage of the genetic manipulation). Similar strains were previously constructed in our lab and analyzed (Table 1), with YAC pairs from the pseudoautosomal region (PAR) of human chromosome X, YAC18ED5 (~190 kb) carrying DNA from the recombination hot spot in PAR, and YACy-WXD4932 (~100 kb) from a recombination colder spot in the same region (Klein *et al.*, 1996b).

The results of tetrad analysis of asci dissected from all five strains are shown in Table 1. The first part of the table contains data on the segregation patterns of the YACs (Table IA). Meiotic segregation of YAC99 and YAC100 is very distorted. Table IB contain the different types of aberrant segregation as describe earlier (Sears *et al.*, 1992). About half the cells undergoing meiosis had lost one or both YACs or have shown to contain three YACs instead of two. This YAC instability could be at least partly premeiotic. For YAC109, only ~20% of meioses showed YAC loss or duplication and similar levels of YAC instability were found with the human DNA YACs, 18ED5 and WXD4932. In the genetic analysis, the three mouse YACs and the cold-spot human YAC also showed high frequencies of meiotic nondisjunction, especially in meiosis I. Recombination analysis of the YACs is shown in Table IC, which only contains data from tetrads that showed normal segregation of the YACs (i.e., the four spore colonies containing one YAC each, with markers on both YAC ends segregating 2:2). Here one can see that in very few cases YAC99 and YAC100 underwent recombina-

Figure 3. CoHR profile matches on YAC99 and YAC100. Five High-quality CoHR-profile-hits (1–5) found on an 80-kb sequence from the MHC class III region (sequence AF397035). Both the forward and reverse DNA strands were checked for the presence of sequences similar to the preferred DSB-associated CoHR (Blumental-Perry *et al.*, 2000), using the program ProfileGap from the GCG package. CoHR2, 4 and 5 are present in both, YAC99 and YAC100. CoHR3 is present only in YAC99, which may also contain CoHR1 (CoHR1 and 3 are absent on YAC100).

Table 1. Segregation of five YAC pairs among dissected tetrads

A. Normal vs. aberrant segregation of YACs					
YAC (size in kb)	99 (200)	100 (450)	109 (150)	18ED5 (190)	WXD4932 (100)
Total number of tetrads	205	191	219	216	247
Normal segregation	42 (20%)	39 (20.5%)	120 (55%)	184 (85.2%)	155 (63%)
Aberrant segregation	163 (80%)	152 (79.5%)	99 (45%)	32 (14.8%)	92 (37%)
B. Types of aberrant segregation ^a					
Nondisjunction meiosis I	12	18	13	2	10
Nondisjunction meiosis II	4	4	6	3	7
Precocious sister separation	1	1	0	4	1
Chromatid loss	8	2	3	3	3
Premeiotic gene conversion	27	18	22	4	21
YAC loss	38	63	43	10	4
YAC duplication	46	43	7	6	42
Other	27	3	5	0	4
C. Recombination of YAC markers among tetrads with normally segregating YACs					
PD	30 (70%)	33 (85%)	59 (49%)	33 (18%)	69 (44%)
TT	6 (15%)	5 (13%)	57 (48%)	122 (66%)	79 (51%)
NPD	6 (15%)	1 (2%)	4 (3%)	29 (16%)	7 (5%)
Total	42 (100%)	39 (100%)	120 (100%)	184 (100%)	155 (100%)

Premeiotic gene-conversion events were assigned to tetrads of which YAC markers on one end segregated 2:2, whereas the markers on the other end segregated 4:0. PD, parental ditype; TT, tetratype; NPD, non-parental ditype.

^aThe different segregation types were scored as described by Sears *et al.* (1992).

tion events that lead to tetratype segregation (TT), presumably one event per meiosis. The given proportion of these events is obviously an overestimate for the frequency of recombination, as only one fifth of the meioses showed normal segregation of the YACs, out of which this proportion was calculated. None of the other tetrads (80%, showing abnormal segregation and instability of YACs) contained evidence of recombinant YACs. Unexpectedly, the diploid with YAC99 had six tetrads that showed nonparental ditype segregation (NPD) of the YACs, the same frequency as TT. NPD usually indicates two independent exchange events in meiosis and its frequency is considerably lower than TT, which results from a single exchange, as seen for YAC109 and yWXD4932. Even when numerous exchanges occur between the two markers, and almost all segregation patterns (PD, TT, NPD) involve more than one exchange, like in YAC18ED5, the ratio NPD:TT only reaches 1:4. A plausible explanation for the six NPD tetrads of YAC99 is that they represent cases of reciprocal recombination between the two YACs in the mitotic division that preceded the meiosis at question, or in G1 of meiosis, before DNA replication.

We conclude that in parallel to the absence of meiotic DSBs on the MHC class III YACs, these YACs recombine very rarely in meiosis and their segregation is also seriously compromised. Meiotic segregation of a pair of homologous chromosomes clearly depends on the occurrence of at least one recombination event (and chiasma) between them. Therefore it is not surprising that these YACs show aberrant segregations. However, the genetic analysis (tetrad analysis, in Table 1) shows that these YACs are also very unstable, both in meiosis and in the preceding divisions. To evaluate instability of these YACs during mitotic divisions and under conditions leading to meiosis/sporulation, we tested the loss of YAC markers in vegetatively grown cells, on YEPD plates, and in the mitotic cell divisions before meiosis, on SPO plates. Diploid cells that contained two homologues

YACs (with different end markers) were spread on these two types of plates and then transferred to YEPD plates at times 0 and 8 h to form single colonies. The latter were replica plated onto differential media, to determine the presence of the YAC markers. A colony that did not grow on the two media indicative of markers at the ends of a given YAC was considered as having arisen from a cell that had lost that particular YAC. The percentage of YAC loss was calculated by dividing the number of such colonies by the total number of colonies (Table 2). From these data it appears that the two mouse YACs that carry MHC III DNA are somewhat unstable during mitotic divisions, compared with the other YACs, especially during the few divisions before meiosis (on SPO plates). Thus the instability of YAC99 and YAC100 observed in the analysis of tetrads (Table 1) might be an extension of the (milder) instability observed already during mitotic divisions (Table 2).

Mutations in SIR Genes Release Meiotic DSB Silencing

The best-documented case of chromosomal regions in yeast that are refractory to double-strand breakage are the silent mating-type cassettes, *HML* and *HMR* (Haber, 1998). Although these cassettes contain DNA sequences that are also present in the mating-type locus, *MAT*, in which the *Ho* endonuclease efficiently generates DSBs, the silent cassettes are normally devoid of such breaks, remain inaccessible to *Ho*, and may participate in mating-type interconversion only as donors of genetic information. *HMR* and *HML* are protected from *HO*-induced breaks by their unique chromatin structure, consisting of the Sir proteins. Mutants with mutations in *SIR* genes show mating-type switching in the silent cassettes, which may (in these mutants) also serve as recipients of genetic information, following the occurrence of DSBs in the cassettes (Klar *et al.*, 1981; Nasmyth, 1982). There is also a case of Sir2-mediated silencing of meiotic recombination in the array of ribosomal DNA genes of *S.*

Table 2. Frequencies of cells that lost a YAC (in percent) during mitotic growth (on YEPD) and during premeiotic growth (on SPO medium)

	YAC99		YAC100		YAC109		YAC18ED5		YACWDX4932	
	YEPD	SPO	YEPD	SPO	YEPD	SPO	YEPD	SPO	YEPD	SPO
0 h	2.5	2.5	5	5	2	2	0	0	0	0
8 h	4.5	10.75	7	8	2	2.5	2	2.5	1	1

Times of transfer of cells from YEPD or SPO plates are given on the left margin. Each value in the table is based on 110–205 colonies derived from cells transferred at time 0 or 161–530 colonies derived from cells transferred at 8 h.

cerevisiae (Gottlieb and Esposito, 1989). We asked whether the mouse MHC class III YACs were also protected from meiotic DSBs by assuming a Sir-based chromatin structure. To examine this possibility, we introduced the mutations *sir2Δ*, *sir3Δ*, or *sir4Δ* into the *rad50S* haploid strains carrying YAC99 or YAC100. Such strains enter meiosis as haploids because a *sir* mutation allows the expression of the silent cassettes *HMLα* and *HMRα*. Figure 4 presents experiments in which meiosis was induced in *Sir⁻ rad50S* haploids, carrying YAC100. DNA was gently extracted at times 0 and 6 h after the induction of meiosis, DNA samples were run on PFGE, blotted onto membranes, and probed with ³²P-labeled YAC-specific probe pBR322. Meiotic DSBs can be seen at 6 h in the *sir2Δ* and *sir4Δ* strains but not in the *sir3Δ* strain. The DSBs on the YACs, however, could not be accurately mapped because their DNA was not sequenced. Stripping the membranes of the YAC-specific ³²P label and rehybridization with yeast chromosome III-specific probe has shown that in the *sir3Δ* strain, as well as in the others, meiosis was initiated and native yeast chromosomes underwent DNA breakage (unpublished data). Interestingly, the pattern of breakage of the YACs in the *sir2Δ* strain was different from that found in *sir4Δ* (Figure 4). This could be due to the different roles of the two proteins in chromatin structure (Gasser and Cockell, 2001; Luo *et al.*, 2002): binding of Sir2, a histone deacetylase, to the chromatin depends on the presence of Sir4, whereas the latter can be present even without Sir2. We conclude that Sir 2 and Sir4 proteins, but not Sir3, take part in making the MHC class III YACs inaccessible to DSBs during yeast meiosis.

Figure 4. Effect of *sir* mutations on meiotic DSBs on MHC class III YAC. Analysis of three double mutant haploid strains that contain YAC100: *sir2Δ rad50S*, *sir3Δ rad50S*, *sir4Δ rad50S*. Haploids carrying *sir* mutations initiate meiosis, like *MATα/MATα* diploids. Chromosome-size DNA was extracted from cell samples after 0 and 6 h in sporulation medium, separated by PFGE, blotted, and hybridized to a YAC-specific probe (pBR322 DNA).

DISCUSSION

DSBs in DNA initiate the process of meiotic recombination, which leads to the formation of chiasmata that hold together pairs of homologous chromosomes, thus assuring their proper segregation in meiosis I. As meiotic DSBs were found to occur in budding yeast (for review Keeney, 2001), in fission yeast (Cervantes *et al.*, 2000; Zenvirth and Simchen, 2000), and in mouse spermatocytes (Mahadevaiah *et al.*, 2001; Zenvirth *et al.*, 2003), we believe that they are common to all eukaryotes and meiotic recombination assumes similar features in different organisms. Moreover, YACs containing human DNA undergo DSBs during budding yeast meiosis (Klein *et al.*, 1996a, 1996b), as do YACs with DNA of the plant *Arabidopsis* (D. Zenvirth, unpublished results) and several mouse YACs (see above, Figure 2), supporting the universality of meiotic DSBs. YACs with DNA from recombination hot spots in the human genome show considerably more breakage during yeast meiosis than cold-spot YACs, implying similarities between the meiotic recombination machinery in humans and in yeast (Klein *et al.*, 1996b). These differences in DSBs between hot-spot and cold-spot YACs are also reflected by the frequencies of recombination on the YACs (Table 1B). YAC18ED5 has undergone considerably more recombination events than yWDX4932—in most meioses of the former two or more exchanges have occurred, and therefore the distribution of PD:TT:NPD is as expected if the two end-markers segregated freely from each other (1:4:1). YAC109 and yWDX4932 have undergone only one or two exchanges (or none at all), reflecting their shorter length, as well as their genomic origin: YAC109 can be expected to represent an average recombination potential in the mouse genome; yWDX4932 comes from pseudoautosomal region (PAR) on the human chromosome X, next to the hot spot covered by 18ED5 (Ried *et al.*, 1995)—this is a relatively cold region (compared with 18ED5), but even such “cold” regions in PAR may show at least 10-fold higher frequency than the genome average (Lien *et al.*, 2000).

In contrast to the behavior in meiosis of most YACs, the two containing DNA from the mouse MHC class III region did not undergo double-strand breakage during meiosis in *rad50S* yeast strains (Figure 1A) and showed very few recombination events (Table 1B). This peculiar behavior was unique to the YACs, because the native yeast chromosomes in the same cells behaved as expected during meiosis, showing meiotic DSBs and undergoing normal segregation. Absence of meiotic DSBs on YAC99 and YAC100 was observed in spite of the presence of CoHR DNA sequences (Figure 3), which are commonly associated with most preferred meiotic DSBs in *S. cerevisiae* (Blumental-Perry *et al.*, 2000). We presume that the MHC class III inserts on these YACs are responsible for their unusual behavior in meiosis, perhaps reflecting their origin and role in the mouse genome. These

two YACs also showed serious mal-segregation in meiosis: high rates of nondisjunction in meiosis I and II, and chromatid loss (together 12–14% of tetrads), and of premeiotic loss of one or both YACs, or duplication of one of the YACs (40–50% of tetrads). During vegetative growth on YEPD plates, and especially on SPO plates (before sporulation), these YACs also showed marked instability (Table 2), although to a lesser extent. We therefore speculate that these premeiotic changes could be induced by the poor nutritional conditions just at the onset of meiosis and sporulation. The meiotic NDJ events could of course be related to the absence of recombination, as was previously found for a pair of nonhomologous YACs in meiosis (Sears *et al.*, 1994). Moreover, a number of premeiotic gene-conversion events, with or without YAC loss, were also observed (Table 1B); these could also occur during meiotic DNA replication (also called premeiotic S). We conclude that the behavior of the MHC class III YACs might have also been abnormal in the mitotic divisions just preceding meiosis or during meiotic DNA replication. Some of these defects could reflect difficulties in DNA replication or centromere functioning on the YACs in these divisions, possibly resulting from a unique chromatin confirmation (see below).

We found that mutations in *SIR2* or *SIR4*, but not in *SIR3*, remove the inaccessibility of the MHC class III YACs to meiotic DSBs (Figure 4). This may suggest that the proteins Sir2 and Sir4 take part in a unique chromatin structure that makes the YACs refractory to the meiotic DNA breakage and recombination enzymes. This is a new role for Sir proteins, previously regarded mostly as transcriptional silencers (Gasser and Cockell, 2001). Meiotic DSB silencing may, for instance, be important near telomeres and centromeres. Meiotic DSBs are indeed silenced near the Telomeres of *S. cerevisiae* (Blumental-Perry *et al.*, 2000) and preliminary experiments suggest that such silencing may be removed by the *sir4* mutation (Raizman, Zenvirth and Simchen, unpublished results). A similar role was documented for Sir proteins in protecting the silent mating-type cassettes *HML α* and *HMR α* from DNA cleavage by the HO endonuclease, despite these loci having the HO recognition sequences (Klar *et al.*, 1981; Nasmyth, 1982). The unique chromatin structure of the MHC class III YACs could also account for their unusual segregation behavior in the premeiotic divisions, although at present we cannot offer a precise mechanism for the latter phenomenon.

What does meiotic DSB silencing of MHC class III YACs tell us about gametogenesis and meiosis in the mouse? We have previously suggested that the frequency of meiotic DSBs on human-DNA YACs may reflect their recombination potential in the human genome (Klein *et al.*, 1996b). Recombination in yeast meiosis of two YACs from the human pseudoautosomal region also reflects their recombination potential (Table 1C, above). By extrapolation to the MHC class III region in the mouse genome, we speculate that its refraction to DSBs and recombination in yeast meiosis may reflect similar characteristics during mouse spermatogenesis. Thus the recombination hot spot identified in this region (Snoek *et al.*, 1996, 1998) may not represent events that occur during meiosis in the mouse, but rather during premeiotic divisions. From the behavior of the MHC class III YACs in yeast, we speculate that the region may be protected from the recombination machinery during meiosis in the mouse, probably by a unique chromatin structure that is associated with unknown sequences in this region. These DNA sequences may be recognized by Sir2 or Sir4 in yeast or by proteins that are associated with the Sirs and by similar proteins in mouse. (No homologues were found in mamma-

lian genomes to Sir3 and Sir4, although homologues of the histone deacetylase Sir2 were found in humans, mice, and other eukaryotic organisms [Sherman *et al.*, 1999; Smith *et al.*, 2000; Yang *et al.*, 2000].) Support for the proposition that recombination in MHC class III occurs before meiosis comes from a PCR analysis of gene-conversion events in the MHC class II region at different stages of mouse spermatogenesis (Hogstrand and Bohme, 1997). On the other hand, recombination at the *Psmb9* (*Lmp2*) recombination hot spot in the mouse MHC class II region has been recently shown to occur during spermatogenesis in the pachytene stage of meiosis (Guillon and de Massy, 2002). Thus it seems that on chromosome 17 of the mouse, in the 4500-kb region containing the MHC genes, recombination in subregions occurs at different stages during spermatogenesis. We suggest that these differences in recombination timing may reflect temporary changes in accessibility of the subregions. Accessibility may at least partly be determined by elements of the chromatin, namely proteins that are homologous or similar to the yeast Sir proteins.

ACKNOWLEDGMENTS

We thank Ronald Stam and Huub van Vugt for their assistance in characterizing the mouse DNA YACs and Susan Gasser for reading the manuscript and for helpful discussions. This research was supported by grant 96-00086 from BSF (U.S.-Israel Binational Science Foundation).

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