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Patterns of meiotic double-strand breakage on native and artificial yeast chromosomes

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Abstract. The preferred positions for meiotic double-strand breakage were mapped on *Saccharomyces cerevisiae* chromosomes I and VI, and on a number of yeast artificial chromosomes carrying human DNA inserts. Each chromosome had strong and weak double-strand break (DSB) sites. On average one DSB-prone region was detected by pulsed-field gel electrophoresis per 25 kb of DNA, but each chromosome had a unique distribution of DSB sites. There were no preferred meiotic DSB sites near the telomeres. DSB-prone regions were associated with all of the known "hot spots" for meiotic recombination on chromosomes I, III and VI.

Introduction

The first meiotic division (meiosis I) is preceded by a high rate of homologous recombination, which is required for the accurate segregation of homologous chromosomes. In the yeast *Saccharomyces cerevisiae*, homologous recombination is induced about 1000-fold during meiosis.

A major pathway of meiotic recombination in *S. cerevisiae* is initiated via double-strand breakage in the DNA of one chromatid. Certain regions of the chromosome contain recombination "hot spots", where the frequency of meiotic recombination is particularly high. At several of these hot spots, specific meiotic double-strand breaks (DSBs) are induced early in meiosis (Sun et al. 1989; Goldway et al. 1993; Zenvirth et al. 1992; Collins and Newlon 1994). In wild-type strains the transient DSBs can be converted into recombinant molecules (Goyon and Lichten 1993; Schwacha and Kleckner 1994; Collins and Newlon 1994; Storlazzi et al. 1995). In *rad50S* mutant strains, however, the DSBs cannot be processed. Instead, the broken molecules accumulate for several hours, facilitating their analysis (Sun et al. 1991; Alani et al. 1990).

Using pulsed-field gel electrophoresis (PFGE), we previously showed that all *S. cerevisiae* chromosomes have preferred sites for meiotic double-strand breakage and mapped these positions on chromosome III (Zenvirth et al. 1992). Comparison of the DSB and the genetic maps of chromosome III revealed an association between meiotic hot spots for recombination and meiotic DSB sites: the three known hot spots each had an associated DSB-prone region. (It is not yet known whether the other nine meiotic DSB sites on chromosome III also coincide with recombination hot spots.)

Here we report the locations of the preferred meiotic DSB positions on two additional yeast chromosomes, I and VI. On the three smallest *S. cerevisiae* chromosomes, every known hot spot for meiotic recombination was associated with a DSB-prone region. Furthermore, yeast artificial chromosomes (YACs) comprising mainly human-derived DNA are also subject to meiotic double-strand breakage at specific sites (Klein et al. 1996). Here we show that the positioning of preferred meiotic DSB sites in the native and the artificial chromosomes is similar, inasmuch as the number of DSB-prone regions detected by PFGE was proportional to chromosome length (~1 DSB site/25 kb), and no preferred DSB-prone regions were detected near the telomeres.

Materials and methods

Strains of S. cerevisiae. Meiotic double-strand breakage of native chromosomes I and VI was examined in strain NKY1002 (*MAT***a**/*MATα*, *ura3*/*ura3*, *lys2*/*lys2*, *ho::LYS2*/*ho::LYS2*, *rad50S-k181::URA3*/*rad50S-k181::URA3*, Alani et al. 1990). YACs were transferred by Kar$^-$ transfer (Hugerat et al. 1994) into one or both of the following haploid strains: 2850 (*MAT***a**, *ho*, *ura3*, *lys2*, *trp1*, *rad50S*::*ura3*, *can*^{r}; Hugerat et al. 1994) and 2851 (*MATα*, *ho*, *ura3*, *lys2*, *trp1*, *rad50S*::*ura3*, *cyh*^{r}; Klein et al. 1996). Haploids of opposite mating type were mated to obtain diploids for examination of meiotic DSBs in the YACs. In some cases, YAC-carrying haploids were mated with NKY1179 (*MATα*, *ho::LYS2*, *ura3*,

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lys2, leu2, rad50S::ura3) or NKY1730 (*MATα, ho::LYS2, ura3, lys2, trp1::hisG, arg4, rad50S::URA3*). Specific double-strand breakage also occurred when only one of the haploid parents carried a YAC, suggesting that DSBs occur on monosomes. This result is consistent with studies showing that DSBs occur in haploid meiosis (de Massy et al. 1994; Gilbertson and Stahl 1994). All of the *rad50S* strains had an SK1 background.

Chromosome fragmentation was performed in strain JD1, a spontaneous *ura3* derivative of S288C-derived strain AB972 (Link and Olson 1991), and in the SK1-derived strains NKY560 and NKY278 (Alani et al. 1990). Strains with designation NKY were kindly provided by N. Kleckner.

Media and growth conditions. Yeast were grown on standard rich (YPD) and defined (SD) media (Sherman 1991). YAC-containing strains were grown on defined medium lacking the nutrients provided by the selectable markers on the YACs. Sporulation was in SPM sporulation medium, following pregrowth in YEPA medium, as previously described (Kassir and Simchen 1991).

Mapping meiotic DSBs. The scheme for mapping meiotic DSBs by hybridization to PFGE blots is outlined below and diagrammed in Fig. 1. All procedures were performed as described previously (Zenvirth et al. 1992).

Chromosome-length DNA was prepared from samples withdrawn before ("time 0") and 6 h after transfer to SPM sporulation medium and analyzed by contour-clamped homogeneous electric field gel electrophoresis in a CHEF-DR apparatus (Bio-Rad). Southern blots were hybridized to a series of chromosome-specific ^{32}P-labeled probes (Tables 1–3) and the autoradiograms compared.

In the time 0 sample, a given probe hybridized to one band, corresponding to the full-size chromosome. In the meiotic sample, the probe hybridized to the full-size, unbroken band and to an array of smaller bands, corresponding to the various fragments arising from double-strand breakage. The number of meiotic fragments observed with a probe from near the end of a chromosome (e.g. probe A in Fig. 1) corresponded to the number of potential meiotic DSB sites in that chromosome, and the locations of the DSBs were estimated from the lengths of the fragments. For the native chromosomes, the positions of the DSBs were confirmed by using internal probes from either side of the DSBs (e.g. probes B, C in Fig. 1), which hybridized to "complementary" fragments. A probe from the left of a given DSB hybridized to the chromosome fragment extending from that DSB to the left telomere; a probe from the right of the DSB hybridized to the complementary fragment extending from the DSB to the right telomere (See Fig. 1). Since a single molecule generally was not broken more than once, the sum of the lengths of the complementary fragments was equal to the total length of the chromosome. Often, a single meiotic band detected on PFGE blots was shown on restriction digests to result from several close DSB sites (e.g. Fig. 3). Furthermore, when DSBs were mapped precisely on sequencing gels, each "site" was found to extend over 50–100 nucleotides (e.g. de Massy et al. 1995; Liu et al. 1995). For the YACs, the positions of the DSB sites were estimated using probes from either end of the chromosome (Table 3); internal probes were not always available. The number of DSB sites on the YACs may have been slightly underestimated, as the use of internal probes provides added sensitivity for detecting weaker DSBs.

For each probe, several different exposures were analyzed; the weakest signals were detected only after overexposure of the blots. The proportion of chromosomes broken at any given site was estimated by scanning densitometry of the autoradiograms.

There were several differences between strain NKY1002 and the reference strains for the sequencing projects. The DSB maps in Figs. 2 and 4 were based on the published sequences of chromosome VI (Murakami et al. 1995) and chromosome I (Bussey et al. 1995), except where our data clearly showed a different map arrangement for strain NKY1002.

Chromosome fragmentation. Yeast chromosome fragmentation (Vollrath et al. 1988) employed fragmentation plasmids derived from vector YCFT4 (Goldway et al. 1993). The relevant DNA segment for targeting the fragmentation was inserted into the polylinker of vector YCFT4 in the appropriate orientation.

Fragmentation plasmid pSR117 carried a 2.5 kb *EcoRI* fragment from YRp7-CDC24 (Coleman et al. 1986), for construction of chromosome fragments extending from the left telomere to *CDC24*. Plasmid pSR117 gave a chromosome fragment of ~70 kb in strain JD1. Plasmid pSR119 carried a 2.1 kb *HindIII* fragment from pLF171 (Barton and Kaback 1994), for construction of chromosome fragments extending from the left telomere to *FUN31*. This plasmid gave chromosome fragments of ~125 kb in strains JD1 and NKY278.

Fragmentation plasmid pSR115 carried a 4 kb *EcoRI* fragment from pLF40, [a subclone of YCp50(ADE1-CDC15)7A, encompassing the *FUN2* gene; Crowley and Kaback 1989] for construction of chromosome fragments extending from *FUN2* to the right telomere. pSR115 gave a chromosome fragment of ~80 kb in strain JD1 and a chromosome fragment of ~75 kb in strain NKY278.

All plasmids were linearized and transformed into strains JD1, NKY560 and NKY278, with selection for Ura$^+$. The plasmid pSR117 yielded chromosome fragments in strain JD1 only; the other plasmids yielded chromosome fragments in all of the strains. The structures of the chromosome fragments were confirmed by PFGE and hybridization to suitable probes (data not shown).

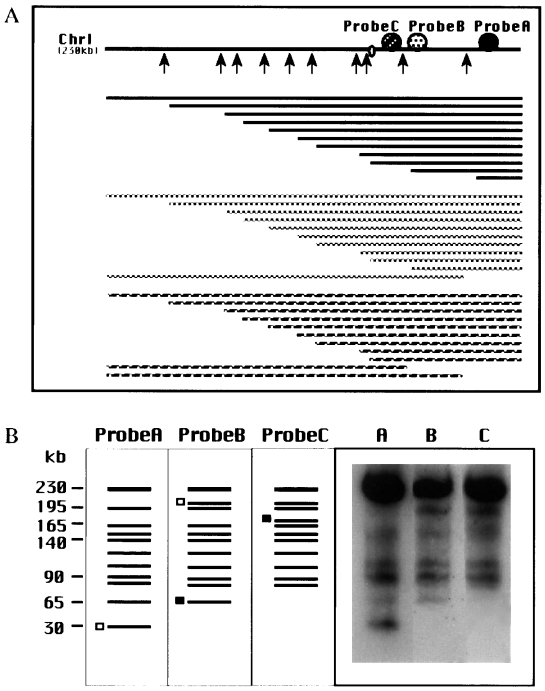


Fig. 1. Mapping of preferred meiotic double-strand breaks (DSBs). **A** The scheme shows the sizes of full-length chromosome I and the meiotic fragments obtained from double-strand breakage at each of the preferred DSB sites (*arrows*), as visualized by Southern hybridization with three different probes. **B** *Left* Pattern of Southern hybridization after pulsed-field gel electrophoresis (PFGE), using the same three probes. The *boxes* indicate the complementary fragments. *Right* Actual autoradiograms. Probe A = probe 32; probe B = probe 31; probe C = probe 30 (Table 3). [Contour-clamped homogeneous electric field (CHEF) electrophoresis conditions: 200 V, 5–35 s pulses, 17.3 h; 1% agarose in 0.5XTBE]

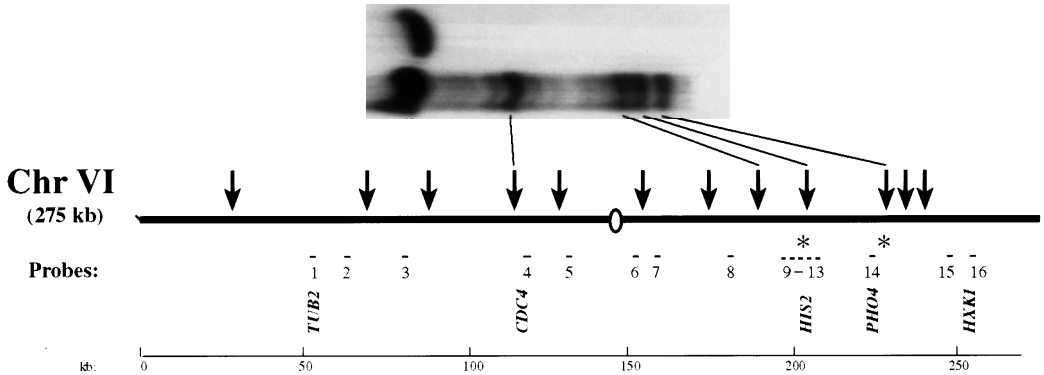


Fig. 2. Meiotic double-strand breakage of chromosome VI. Chromosome-sized DNA extracted from *Saccharomyces cerevisiae* NKY1002 after 0 (*top lane*) and 6 h (*bottom lane*) in sporulation medium (SPM) was separated by PFGE, blotted and hybridized to a series of probes from chromosome VI. The hybridization pattern obtained with probe 15 (YMR31), from the right end of the chromosome, is shown. The strong band on the left is the full-length chromosome. The *arrows* show the preferred meiotic DSB sites mapped on chromosome VI. The *guidelines* indicate the strongest break sites. The *asterisks* indicate known hot spots for meiotic recombination. (CHEF electrophoresis conditions as in Fig. 1)

Table 1. Chromosome VI Probes

Probe	Gene	Plasmid	Fragment	Source
1	*TUB2*	pRB129	1.6 kb EcoRI-EcoRI	Neff et al (1983)
2	*YFL035c*	p15-2	4.2 kb BamHI-KpnI	R. Perlman
3	*STE2*	yCpSTE2	4.3 kb BamHI-BamHI	Nakayama et al (1985)
4	*SMC1*	pCDC4-73	1.5 kb EcoRI-BamHI	Yochem and Byers (1987)
5	*YFL004w*	pLA423	2.0 kb BamHI-PstI	P. Philippsen
6	*NIC96*	pLA305	3.2 kb BamHI-BamHI	P. Philippsen
7	*YFR006w*	pLA276	0.8 kb EcoRI-EcoRI	P. Philippsen
8	*YFR107c-YFR108c*	pLA303	2.0 kb EcoRI-EcoRI	P. Philippsen
9	*PES4*	pH21	2.0 kb BglII-BglII	Malone et al (1994)
10	3′-*HIS2*	pSR109	1.4 kb EcoRI-BamHI	Subclone of pH21
11	*HIS2*	pSR109	0.75 kb XhoI-XhoI	Subclone of pH21
12	5′-*HIS2*	pSR109	0.3 kb EcoRI- HindIII	Subclone of pH21
13	*YFR027-YFR026*	pH21	1.4 kb BglII-KpnI	Malone et al (1994)
14	*PHO4*	pFL1-PHO4	1.9 kb SphI-SphI	Legrain et al (1986)
15	*YMR31*	pMI-XbaI-1	0.5 kb SspI-SspI	Matsushita et al (1989)
16	*HXK1*	pUC19-HXK1	0.7 kb XbaI-EcoRV	Kopetzki et al (1985)

Results

Meiotic DSBs on chromosome VI

Meiotic DSBs can be mapped on whole chromosomes using PFGE; each chromosome has a characteristic pattern of meiotic DSB fragments (Zenvirth et al. 1992; Game 1992; see Materials and methods and Fig. 1). We mapped 12 meiotic DSB-prone regions on chromosome VI (~275 kb in strain NKY1002), at the following positions (±5 kb, from the left end of chromosome VI): 30, 70, 90, 115, 130, 155, 175 190, 205, 230, 235, and 240 kb (Fig. 2, Table 1). There was a cluster of DSB sites at the right end of chromosome VI: probe 1 (from the left end of the chromosome) gave a diffuse signal at about 235 kb, but probes 15 and 16 (from the right end of the chromosome) gave three distinct bands: faint bands at ~35 and 40 kb, and a very strong band at ~45 kb (Fig. 2). The strongest signals were obtained for the bands at 115 kb (left of *CDC4*), 190 kb (right of *FAB1*), 205 kb (right of *HIS2*), and 230 kb (right of *PHO4*). "Strong" signals were obtained when 5%–10% of chromosomes were broken at a given site. The 130 kb band (left of *CEN6*) varied in intensity in different chromosomal preparations, sometimes giving a strong signal and sometimes a weak one. The relative intensities of all the other DSBs were consistent in different preparations. The weakest detectable signals (at 235, 240 kb) each represented breakage of ~1% of chromosomes.

Meiotic DSB sites were found at both known hot spots for recombination on chromosome VI: hybridization with probes 9–12 showed the DSB at 205 kb to be at *HIS2*, a hot spot for gene conversion (Malone et al. 1992, 1994); hybridization with probes 14, 15 showed that the DSB at 230 kb was to the right of *PHO4*, in a region where the genetic map is expanded relative to the physical map, indicating hyperrecombination (Murakami et al. 1995).

All previously mapped DSB sites were in noncoding DNA at the 5′ ends of genes (de Massy et al. 1995; Liu et al. 1995; Fan et al. 1995). The DSB near *HIS2* was mapped more precisely using restriction digests. Using probes 10–12, the 205 kb fragment detected on CHEF gels

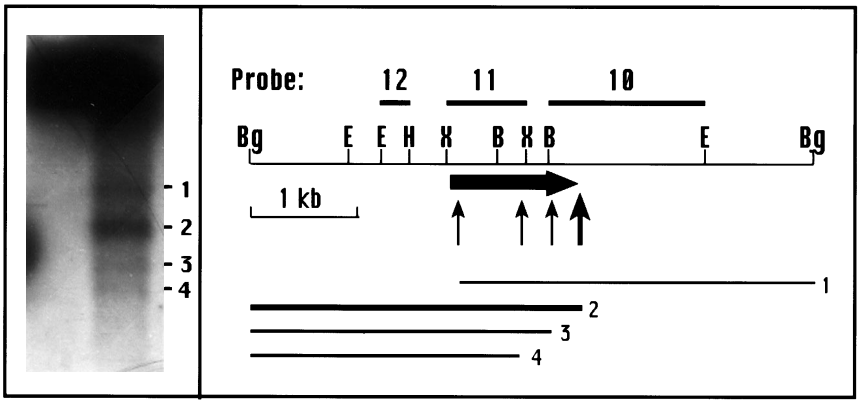


Fig. 3. Meiotic double-strand breakage at the *HIS2* locus. Southern hybridization of probe 11 to BglII digest of genomic DNA extracted from *S. cerevisiae* strain NKY1002 before transfer (*left lane*) and 6 h after transfer into SPM sporulation medium (*right lane*). Bands 1–4 are meiosis specific. The origins of probes 10–12 are shown on the restriction map of the *HIS2* region. The *thick horizontal arrow* indicates the direction of transcription of *HIS2*. The *vertical arrows* indicate the meiotic DSB sites, the strongest of which is at the 3′ end of transcription (band 2). The origins of meiotic bands 1–4 are indicated. Restriction sites: *B* BamHI, *Bg* BglII, *E* EcoRI, *H* HindIII, *X* XhoI

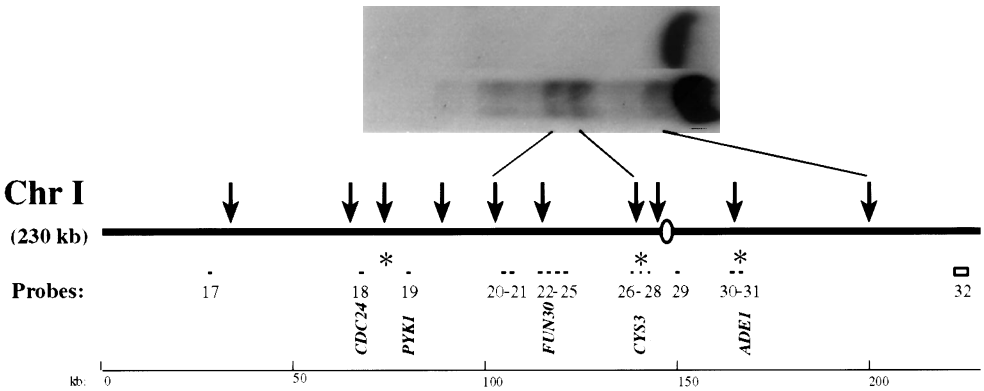


Fig. 4. Meiotic double-strand breakage of chromosome I. Chromosome-sized DNA extracted from *S. cerevisiae* NKY1002 after 0 and 6 h in sporulation medium (SPM) was separated by PFGE, blotted and hybridized to a series of probes from chromosome I. The hybridization pattern obtained with probe 17 (pYY54), from the left end of the chromosome, is shown. The strong band on the right is the full-length chromosome. The *arrows* show the preferred meiotic DSB sites mapped on chromosome I. The *guidelines* indicate the strongest break sites. The *asterisks* indicate known hot spots for meiotic recombination. The exact location of probe 32 (*hollow box*) is unknown, but it is at the right end of the chromosome in this strain (see text and Fig. 5). (CHEF electrophoresis conditions as in Fig. 1)

Table 2. Chromosome I Probes

Probe	Locus	Plasmid	Fragment	Source
17		pYY54	3.2 kb BamHI-SalI	Kaback et al (1989)
18	*CDC24*	YRp7-CDC24	2.3 kb BglII-HindIII	Coleman et al (1986)
19	*PYK1*	pBR322-PYK1	1.9 kb EcoRI-EcoRI	D. Frankel
20	*LTE1*	pLF164	1.2 kb EcoRI-HincII	Barton and Kaback (1994)
21	*PMT2*	pLF165	1.9 kb ClaI-BglII	Barton and Kaback (1994)
22	*ATS1-FUN30*	pLF169	2.7 kb EcoRI-PstI	Barton and Kaback (1994)
23	*FUN30*	pLF170	2.3 kb PstI-HindIII	Barton and Kaback (1994)
24	*FUN31*	pLF171	2.1 kb HindIII-HindIII	Barton and Kaback (1994)
25	*FUN31*	pLF172	1.2 kb HindIII-HindIII	Barton and Kaback (1994)
26	*FUN34*	pLF176	1.2 kb BglII-BglII	Barton and Kaback (1994)
27	*DEP1-CYS3*	pLF177	1.8 kb BglII-BglII	Barton and Kaback (1994)
28	*YAL011W*	pLF179	1.9 kb EcoRI-EcoRI	Barton and Kaback (1994)
29	*FUN17*	pLF72	1.0 kb HindIII-EcoRI	Steensma et al (1987)
30	*FUN2*	pLF42	2.0 kb SalI-SalI	Crowley and Kaback (1989)
31	*ADE1*	pLF29	1.2 kb EcoRI-EcoRI	Crowley and Kaback (1984)
32		pLF233	3.4 kb EcoRI-EcoRI	λ3029, Riles et al (1992)
33		pLF251	2.6 kb HindIII-HindIII	λ3029, Riles et al (1992)
34		pLF258	3.7 kb HindIII-HindIII	λ5010, Riles et al (1992)
35		pLF260	5.0 kb HindIII-HindIII	λ7467, Riles et al (1992)

was seen to be due to a cluster of DSBs in the *HIS2* region. A strong DSB site was at the 3′ end of *HIS2*, within 0.1 kb of the coding sequence, and three weaker DSB sites were within the coding sequence of the gene (Fig. 3). *HIS2* is unusual among genetic hot spots that have been characterized in detail, inasmuch as the gradient of polarity for gene conversion is highest at the the 3′ end and decreases toward the 5′ end of the gene (Malone et al. 1992, 1994). Hence, the strong meiotic DSB site at the 3′ end of the gene is likely to be an initiation site for gene conversion in *HIS2*. Kim and Malone also found a DSB site at the 3′ end of the gene (R.E. Malone, personal communication).

Meiotic DSBs on chromosome I

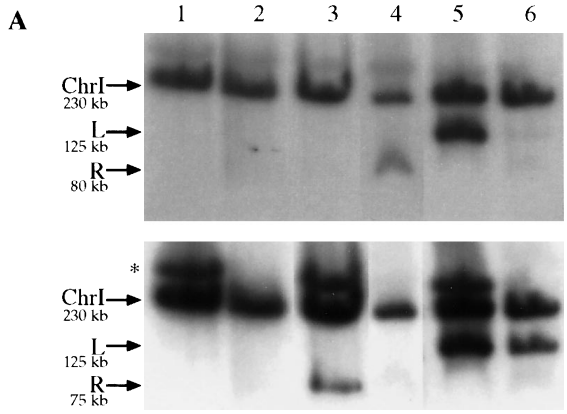
The positions of the preferred meiotic DSBs on chromosome I (~230 kb, in strain NKY1002) were also determined. We detected ten distinct meiotic DSB-prone regions at the following positions in strain NKY1002 (±5 kb, from the left end): 35, 65, 75, 90, 105, 115, 140, 145, 165, 200 (Fig. 4, Table 2). The strongest breaks were at 115, 140 (to the left of *CYS3*) and 200 kb; between 7% and 10% of chromosomes were broken at each of these positions. The weakest detectable breaks were at 35, 65 and 105 kb; ~1% of chromosomes were broken at each of these positions.

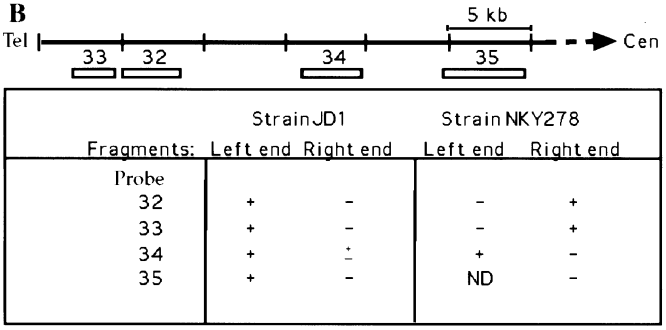
Meiotic DSBs were associated with the two known hot spots for meiotic recombination: at 75 kb between *PYK1* and *CDC 24*, in a hot spot of reciprocal recombination (Coleman et al. 1986; Kaback et al. 1989); and at 140 kb, at *CYS3*, a hot spot for gene conversion (Cherest and Surdin-Kerjan 1992). Another DSB site (165 kb) was adjacent to *ADE1*, which indeed also showed relatively high rates of meiotic gene conversion (D.B. Kaback, unpublished and Kaback et al. 1992).

In general, we were able to assign the DSB sites to known genetic locations, by hybridizing PFGE blots to probes from known genes from either side of the DSB site. The 115 kb site was an exception, as breakage at this site gave rise to complementary fragments of identical size. By hybridizing probes 22–24 to appropriate restriction digests, we identified at least two meiotic DSB sites in this region: upstream of *ATS1*, and upstream of *FUN30* (data not shown).

The availability of adjacent probes enabled us to pinpoint some DSB sites, using PFGE analysis. Probe 26 from *FUN34* hybridized to the ~140 kb DSB fragment, whereas probe 28 from *YAL011W* (*FUN36*) hybridized to the ~90 kb complementary DSB fragment, indicating that the DSB in question fell in the 2.3 kb region between the two probes. A 1.8 kb probe (probe 27) encompassing *FUN54* and *CYS3* hybridized to both the ~140 and ~90 kb DSB fragments, showing that the DSB fell within that 1.8 kb region. On restriction digests, the same 1.8 kb probe revealed two distinct DSB sites, one upstream of *DEP1* and another upstream of *CYS3*. The region between the two sites also appeared to be subject to enhanced meiotic double-strand breakage. The DSB site at *CYS3* was reported previously (Collins and Newlon 1994; de Massy et al. 1995).

Probes 30 and 31 (*FUN2* and *ADE1*) hybridize to complementary meiotic fragments (~165 and ~65 kb, respectively) (Fig. 1B). Since these probes are about 1 kb apart on the yeast genome, the DSB site was presumed to be within this 1 kb region. Hybridization to restriction digests placed the DSB site within 0.3 kb of the 5′ end of *ADE1*.





Fragments:	Strain JD1 Left end	Strain JD1 Right end	Strain NKY278 Left end	Strain NKY278 Right end
Probe				
32	+	–	–	+
33	+	–	–	+
34	+	±	+	–
35	+	–	ND	–

Fig. 5. Right and left end polymorphism on chromosome I from different strains of *S. cerevisiae*. **A** Hybridization of supernumerary chromosome fragments to probe 33 (*upper panel*) and probe 34 (*lower panel*). Lane 1, JD1; lane 2, NKY278; lane 3, JD1 carrying right-end fragment generated with pSR115; lane 4, NKY278 carrying right-end fragment generated with pSR115; lane 5, JD1 carrying left-end fragment generated with pSR119; lane 6, NKY278 carrying left-end fragment generated with pSR119. *L* left-end fragment, *R* right-end fragment, * cross-hybridization with chromosome VIII. (CHEF electrophoresis conditions: 100-V, 5–35 s pulses, 20 h; 1% agarose in 0.5XTBE.) **B** Positions of probes 33–35 on the left end of S288C-derived strains. The table shows the hybridization patterns of the four probes to supernumerary fragments in JD1 (S288C background) and NKY278 (SK1 background). *ND* not determined

Polymorphism at the ends of chromosome I

Probe 32 is located at ~10 kb from the left end of chromosome I in the *S. cerevisiae* strain AB972 used in the chromosome I sequencing project (Bussey et al. 1995). This probe hybridized to the right end of chromosome I in SK1-derived strain NKY1002: the pattern of meiotic chromosome fragments seen with this probe matched the pattern expected for a probe from the right end of the chromosome (Fig. 1B).

To prove that the sequence represented by probe 32 was at different positions in the two strains, we constructed supernumerary chromosome fragments from the left and right arms of chromosome I in strain JD1 (S288C de-

Table 3. Additional Probes

Probe	Description	Source
36	pGS301: 2.5 kb SphI-EcoRI fragment, hybridizes near centromere of YAC12	G. Simchen, unpub.
37	pGS304: 3 kb SphI-EcoRI fragment, hybridizes near non-centromeric end of YAC12	G. Simchen, unpub.
38	pGS346: 1.5 kb SphI-EcoRI fragment, hybridizes near centromere of YAC21.4	G. Simchen, unpub.
39	pGS342: 1.0 kb SphI-EcoRI fragment, hybridizes near non-centromeric end of YAC21.4	G. Simchen, unpub.
40	YCFT4: 1 kb BglII-PstI fragment containing Y′ subtelomeric sequences	Goldway et al. (1993)

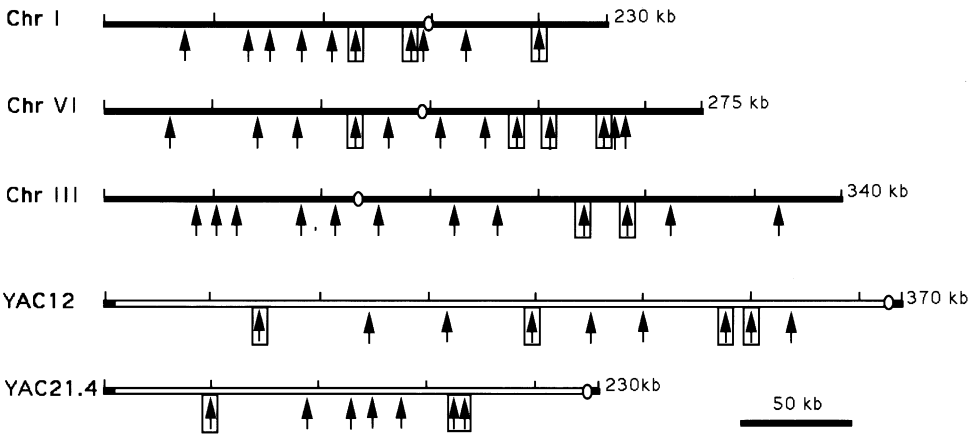


Fig. 6. Maps of the preferred meiotic DSB sites on native and artificial yeast chromosomes. The *arrows* show the meiotic DSB sites, the *boxed arrows* show the strong DSB sites, *solid lines* indicate yeast DNA, *hollow lines* indicate human-derived DNA. YAC12 contains DNA from human chromosome 2 and YAC21.4 contains DNA from human chromosome 21

rived) and strains NKY278 and NKY560 (SK1 derived), as described in Materials and methods. Left-end fragments extended from the left telomere inward, to *CDC24* (fragments of ~70 kb) or to *FUN31* (fragments of ~125 kb). Right-end fragments extended from the right telomere inward, to *ADE1* (fragments of 75–80 kb).

Following PFGE and Southern blotting, DNA from strains with the chromosome fragments was hybridized sequentially with probes 32–35, which had been mapped to the left end of chromosome I in strain AB972 (Fig. 5, Table 2). In strain JD1, all of these probes hybridized to left-end fragments, as expected. (Probe 34 also gave weak signals with the right-end fragments and chromosome VIII.) In strains NKY278 and NKY560, probe 34 hybridized to left-end fragments, but probes 32 and 33 hybridized to right-end fragments (Fig. 5). Hence, probes 32 and 33, which are on the left end of the chromosome in strain S288C, are on the right end in SK1-derived strains. One endpoint of the rearrangement between the two strains appears to be in the ~7 kb region between probes 32 and 34 in strain S288C. This region encompasses a *FLO1*-like pseudogene and the W′ subtelomeric repeat sequences (Bussey et al. 1995).

Meiotic DSBs on YACs

We also examined meiotic double-strand breakage in YACs carrying human DNA. To date we have analyzed over 20 YACs, with inserts ranging in size from 100 to 1250 kb, from several different locations on the human genome.

All of the YACs had specific, preferred meiotic DSB-prone regions, the locations of which depended on the human-derived DNA (Klein et al. 1996). The meiotic DSBs on YAC12 and YAC21.4 were mapped using unique human DNA from either end of each YAC as probes. YAC12 is 370 kb long and contains DNA from human chromosome 2 (Pavan et al. 1990; Sears et al. 1994). Nine meiotic DSB sites on YAC12 were identified using probes 36–37 (Table 3). YAC21.4 is 230 kb long and contains DNA from human chromosome 21 (McCormick et al. 1989; Sears et al. 1994). Seven meiotic DSB sites on YAC21.4 were identified using probes 38–39 (Table 3). The maps of the DSB sites on the YACs were similar to those of the native chromosomes (Fig. 6): all the YACs we examined had strong and weak DSBs; no DSBs were detected near the telomeres; and there was approximately one DSB-prone region per 30–40 kb of DNA.

No meiotic DSBs near the telomeres

No preferred meiotic DSB sites were found near the telomeres in any of the *S. cerevisiae* chromosomes, native and artificial, in which meiotic DSBs were mapped. To test the generality of this finding, meiotic chromosomal preparations were electrophoresed briefly, so as to retain the smallest meiotic fragments on the gel. We hybridized several chromosomal blots with the subtelomeric Y′ sequence (probe 40; Table 3). This probe hybridized to most NKY1002 chromosomes (data not shown). No hybridization was detected below 15 kb, even after extensive overexposure (Fig. 7). Above 15 kb, a smear was obtained. Within the smear, stronger signals were apparent with a periodicity of 25–30 kb (at 15–30 kb, 50–60 kb, and 80–90 kb; Fig. 7), suggesting that the average spacing of one preferred meiotic DSB site per 25 kb that we found on the three smallest yeast chromosomes may also be true of the larger chromosomes. The absence of any signal below 15 kb confirmed that there were no preferred meiotic DSB sites that generated fragments containing Y′ sequences within 15 kb of the *S. cerevisiae* telomeres.

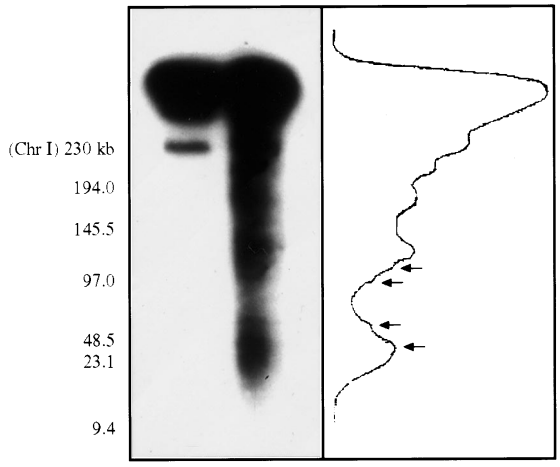


Fig. 7. Hybridization of Y′ subtelomeric repeats to meiotic DNA. *Left panel* Meiotic DNA was prepared from strain NKY1002, as in Fig. 2, and hybridized to probe 40. Mitotic DNA is shown in the left lane, and meiotic DNA in the right lane. No fragments of <15 kb were detected, even after prolonged overexposure. (CHEF electrophoresis conditions: 200 V, 2–26 s pulses, 13 h; 1% agarose in 0.5XTBE.) *Right panel* Scanning densitometry on the autoradiogram shown in left panel, showing periodicity in the pattern of meiotic breakage. The *arrows* indicate peaks that correspond to the smallest bands

Discussion

We present here the maps of the meiotic DSBs on *S. cerevisiae* chromosomes I and VI. The preferred meiotic DSBs on the three smallest *S. cerevisiae* chromosomes have now been mapped. On chromosomal blots, we identified 10 meiotic DSB-prone regions on chromosome I (~230 kb), 12 on chromosome VI (~275 kb), and 12 on chromosome III (~340 kb; Zenvirth et al. 1992). Thus, there is one preferred meiotic DSB-prone region per 25 kb of chromosomal DNA, on average, although each chromosome had a unique, nonrandom distribution of DSB sites. Similar frequencies of DSB-prone regions were found on YACs. On both the native and artificial chromosomes the DSBs range in characteristic intensity from weak (representing breakage of ~1% of chromosomes) to strong (≤10% breakage).

Every known hot spot for meiotic recombination on these three chromosomes was associated with a meiotic DSB site, accounting for a quarter of the DSB sites identified. Although it is not yet known how many of the other DSB sites are also hot spots for recombination, it is clear that there is an association between meiotic recombination at hot spots and meiotically induced double-strand breakage. Several years ago, it was shown that the initiation site for meiotic gene conversion at the *ARG4* hot spot, as defined by the polarity of gene conversion at that locus, coincided with a meiotically induced DSB site (Nicolas et al. 1989; Sun et al. 1989). Since then, a large body of evidence has accumulated showing that meiotic recombination at hot spots initiates at DSBs (reviewed in Klein et al. 1994). The DSBs are rapidly resected at their 5′ ends, to yield 3′ single-stranded tails (Alani et al. 1990). The DSBs can then apparently be converted into double Holliday junction recombination intermediates, and thence to mature recombinant molecules (Goyon and Lichten 1993; Schwacha and Kleckner 1994; Collins and Newlon 1994; Storlazzi et al. 1995).

What determines the positions of the preferred meiotic DSB sites? There is no obvious primary sequence motif common to the known DSB sites. Numerous factors appear to be involved in determining both the positions of the DSB sites and the extents of breakage at those sites. These may include *cis*-acting elements, local position effects and competition for limiting factors (de Massy and Nicolas 1993; Wu and Lichten 1995; V. Dror, MSc Thesis, Hebrew University of Jerusalem 1994). Several meiotic DSB sites are known to coincide with nuclease-sensitive regions in chromatin (Wu and Lichten 1994; Ohta et al. 1994; Fan and Petes 1996), suggesting that an "open" chromatin structure is required to allow access of the DSB machinery. At the *ARG4* DSB site, micrococcal nuclease sensitivity increased during meiosis (Ohta et al. 1994), and at the *HIS4* DSB site, DNase I (but not micrococcal nuclease) sensitivity increased during meiosis (Fan and Petes 1996). Hence, conformational changes in chromatin structure may be required for induction of meiotic double-strand breakage, at least at some sites. Transcription factors also have been shown to play a role in modulating the extent of DSB breakage at some sites (Fan et al. 1995; White et al. 1993). Most of the meiotic DSB sites that had been carefully analyzed to date were 5′ to transcribed sequences. The strong DSB at the 3′ end of *HIS2* (Fig. 3 and R.E. Malone, personal communication) is therefore an interesting exception. This site mapped to within 100 bp of the 3′ end of *HIS2* transcription, and the nearest transcriptional start site is ≥600 bases downstream of this DSB site (Malone et al. 1992; Murakami et al. 1995). It would be interesting to look at the nuclease sensitivity pattern around the *HIS2* DSB site. There is no apparent difference in transcription through *HIS2* during meiosis and mitosis (R.E. Malone, pers. commun.).

We found no meiotic preferred DSB sites within 15 kb of the telomeres (Figs. 6, 7 and S. Klein unpublished data), although our data do not rule out the existence of preferred DSB sites between the Y′ subtelomeric sequences and the chromosome ends. In most cases, no DSB sites were seen within 25 kb of the telomeres. The subtelomeric regions in *S. cerevisiae* appear to undergo much lower levels of homologous recombination than the rest of the chromosome (Steensma et al. 1989; Su, Barton and Kaback, unpublished results), consistent with the absence of preferred meiotic DSB sites, although meiotic rearrangements of the subtelomeric zones have been reported (Horowitz et al. 1984). We are investigating whether transcriptional silencing is involved in repressing recombination near the telomeres in *S. cerevisiae*. Recombination is likewise reduced near the chromosome ends in *Drosophila melanogaster* (Hawley et al. 1993), but is enhanced near the ends of many human chromosomes (Murray et al. 1994). Rare recombination events in the subtelomeric zone appear to provide a mechanism for telomeric repair in mitotic cells (reviewed in Zakian 1995; Louis 1995). Some form of recombination between the W′ subtelomeric repeats (Bussey et al. 1995) may well be responsible for the homology between the right ends of chromosomes I and

VIII (Steensma et al. 1989), as well as for the polymorphic differences we discovered on the left and right ends of the chromosome I from different strains.

We have demonstrated that YACs carrying human DNA are subject to double-strand breakage during yeast meiosis. The distribution of preferred DSB sites is similar to that on the native *S. cerevisiae* chromosomes. YACs carrying DNA from the plant *Arabidopsis thaliana* are also subject to double-strand breakage during yeast meiosis (M. Stammers, pers. commun.). Homologous YACs carrying human-derived DNA recombine at rates approaching those of native chromosomes during meiosis, and segregate with fidelity (Sears et al. 1992). The morphology of YACs with human-derived DNA during pachytene is like that of native yeast chromosomes (Loidl et al. 1995). These findings suggest that the mechanisms that promote accurate pairing and disjoining of native yeast chromosomes also operate on the artificial chromosomes. This is here shown to be true as regards meiotic double-strand breakage of the DNA. Nonetheless, we have shown that the cloned human DNA carries with it recognition signals that determine the specific locations of the DSB sites (Klein et al. 1996). The nature of these signals remains enigmatic.

The frequency of meiotic double-strand breakage varies not only from site to site, but also from chromosome to chromosome. Both the size and content of a chromosome appear to affect total levels of meiotic double-strand breakage. Larger chromosomes tend to be broken somewhat more frequently than smaller ones, reflecting the larger number of potential DSB sites that they carry. Certain native yeast chromosomes are subject to extraordinarily high levels of breakage, even compared with other chromosomes of similar size (Zenvirth et al. 1992 and unpublished data). We have also shown that YACs carrying DNA from human hot spots for recombination are broken more extensively during yeast meiosis than YACs from cold spots (Klein et al. 1996). Yet the overall distribution of the preferred meiotic DSB sites is remarkably similar for the different chromosomes that we have studied. Each chromosome has numerous potential preferred DSB sites, but a given chromosomal molecule is broken at most once or a few times, and may not be broken at all. The meiotic DSB-prone regions are scattered throughout the chromosome, with the exception of the zones adjacent to the telomeres. It is likely that meiotic DSBs span the entire chromosome; our assay detects those sites at which breakage is most frequent. For at least some of the preferred sites, the increased double-strand breakage is resolved as hyperrecombination. We have previously proposed that the preferred DSB sites may also be involved in disjunction of homologous chromosomes in meiosis I (Goldway et al. 1993; Zenvirth et al. 1992). In this regard, it is interesting that each chromosome has a small subset of strong DSB sites, which may be the best candidates for involvement in homolog disjunction.

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