Karyotype Variability in Yeast Caused by Nonallelic Recombination in Haploid Meiosis

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ABSTRACT

Chromosomes of altered size were found in the meiotic products of a haploid *Saccharomyces cerevisiae* strain by pulsed field gel electrophoretic separation of whole chromosomes. About 7% of haploid meioses produced chromosomes that differed by ≥10 kb from their wild-type counterparts. Chromosomes most often became enlarged or shortened due to recombination events between sister chromatids at nonallelic sequences. By this mechanism chromosome III acquired tandem arrays of up to eight extra copies of the ~100 kb *MAT-HMR* segment during repeated rounds of haploid meioses. Enlarged chromosomes III were unstable and changed their size during meiosis more often than remaining unchanged. Altered chromosomes appeared also as the products of intrachromatid recombination and of reciprocal translocations caused by ectopic recombination between nonhomologous chromosomes. In diploid meiosis, chromosomes of altered size occurred at least 10 times less frequently, whereas in mitotic cultures cells with altered karyotypes were virtually absent. The results show that various forms of ectopic recombination are promoted by the absence of allelic homologies.

DURING meiotic prophase homologous chromosomes pair to form a synaptonemal complex and recombine to form stable bivalents that ensure proper disjunction (VON WETTSTEIN *et al.* 1984; HAWLEY and ARBEL 1993). In cases where no homologous partner is available (*e.g.*, in haploids) nonhomologous chromosomes pair during meiotic prophase in plants (see, *e.g.*, LOIDL 1994) and yeast (LOIDL *et al.* 1994). In yeast, an extensive synaptonemal complex was found during haploid meiosis (LOIDL *et al.* 1991), and the question arises whether the synaptonemal complex is initiated at dispersed homologous stretches or whether its formation is totally random. The genomes of higher eukaryotes, but also the relatively compact yeast genome contain repeated sequences (*e.g.*, transposons, subterminal repeats, multiple gene families, ancient duplications) that could act as secondary sources of homology. Whereas ectopic recombination (mostly nonreciprocal) between artificially induced duplications is frequent, there seem to exist mechanisms that suppress ectopic recombination (and hence chromosome rearrangements) between naturally occurring repeats (PARKET *et al.* 1995). It has been suggested, however, that the nonavailability of allelic homology in haploid meiosis promotes ectopic recombination (WAGSTAFF *et al.* 1985).

It was found that meiotic double strand breaks are efficiently repaired in haploid yeast and it is assumed that they are repaired by using the sister chromatid or homologous sequences elsewhere in the genome as template (DE MASSY *et al.* 1994; GILBERTSON and STAHL 1994). In yeast, there exists ample genetic evidence for both nonreciprocal and reciprocal recombination within chromatids, between sister chromatids, and between nonhomologous chromosomes (PETES and HILL 1988). Ectopic (unequal) recombination between sister chromatids may result in a triplication or loss of a repeat, and recombination between nonhomologous chromosomes leads to a translocation that may cause size changes of the chromosomes involved (PETES and HILL 1988; KLEIN 1995).

Here we studied whether the extensive pairing of nonhomologous chromosomes in haploid *Saccharomyces cerevisiae* is matched by an increased level of ectopic recombination all over the genome. To this aim, we searched for chromosomes of altered sizes as the physical products of ectopic recombination by pulsed field gel electrophoresis (PFGE) of intact DNA molecules. The advantage of PFGE over genetic assays for nonallelic recombination is that the detection of events is not limited to genetic marker regions but rather the whole chromosome complement can be scored for alterations.

MATERIALS AND METHODS

Strains: We used two haploid and a diploid derivative of the fast sporulating yeast strain SK1 (KANE and ROTH 1974). The diploid strain is *MAT***a**/*MATα HO/HO lys2/lys2 leu2::hisG/leu2::hisG his4/his4 ura3/ura3 trp1::hisG/trp1::hisG ade2::hisG/ade2::hisG spo13::hisG/spo13::hisG.* It performs a single division in meiosis producing two diploid spores. One haploid is *MAT***a** *ho::LYS2 lys2 leu2::hisG his4X ura3 trp1::hisG*

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spo13::hisG sir3::LEU2. The *sir3* disruption derepresses the silent mating type cassette *HMLα* and allows this strain to enter meiosis. Due to the disruption of the *SPO13* gene, it performs a single meiotic division, which produces two viable haploid spores (WAGSTAFF *et al.* 1982). The other haploid is *MAT***a** *ho::LYS2 lys2 leu2::hisG his4X ura3 trp1::hisG spo13::hisG* + p *MATα*. It carries the *α* mating type allele on the YCp50 *CEN* plasmid which enables it to undergo meiosis.

Growth and sporulation regimens: For checking the frequency of mitotic chromosome alterations, a whole single mitotic colony was transferred from a plate into 1 liter of liquid medium and grown to stationary phase. The number of cells in the culture was determined and the number of mitotic cycles calculated [no. of cycles = ^{2}log(no. of cells)]. A sample of cells was taken and grown to single colonies on YEPD plates (ROSE *et al.* 1990). The occurrence of an altered chromosome in the cells of a colony would then indicate that a chromosomal rearrangement event has occurred during one of the mitotic cycles between the ancestor and the isolated cell. (This procedure is permissible for estimating the frequency of rearrangements if they are rare enough to make multiple recombination events in a cell line and the clonal origin of a particular rearrangement unlikely.) Colonies were used to inoculate liquid cultures from which cells were harvested for DNA preparation.

To examine meiotic karyotype changes, cells were sporulated in liquid 2% KAc at 30° and the dyads were dissected and grown on YEPD plates. Single colonies were used to inoculate liquid YEPD cultures for DNA preparation.

Electrophoresis: Chromosome-sized DNA was prepared in agarose plugs (GERRING *et al.* 1991). Loading of the gels was done with a glass Pasteur pipette the tip of which had been molded into a rectangular shape under heat, with the opening slightly smaller than the gel slots. Pieces of agarose were cut out from the plugs with the pipette and released into the slots. Agarose gels (1%) with 30 slots were used for fast prescreening and with 15 slots for closer inspection of the bands of selected clones. Pulsed field gels were run on a Contour-Clamped Homogenous Electric Field (CHEF) apparatus in 0.5× TBE buffer (*e.g.*, see GERRING *et al.* 1991) at 200 V, 10° for 14 hr with 60-sec pulses, 10 hr with 90-sec pulses and 3 hr with 120-sec pulses. The yeast strain YPH149, for which chromosome sizes in kilobase have been given (GERRING *et al.* 1991), was used as a marker for determining sizes and size-changes of chromosomes in our strains.

Southern blotting and hybridization: Gels were blotted onto Nylon-membranes (Hybond-N, Amersham) by alkaline transfer. Probes were either generated by PCR or prepared from cosmids or plasmids by restriction digest. Appropriate fragments were purified from gels by glassmilk-elution (Elu-Quick, Schleicher and Schuell) according to the manufacturer's instructions and radioactively labeled by random priming using ^{32}P-dATP (NEG). Blots were stripped by incubation in 2× SSC, 60% formamide at 70°. Southern hybridization was performed according to CHURCH and GILBERT (1984). Quantification was performed on a PhosphorImager (Molecular Dynamics).

For chromosome III probes, appropriate primers were chosen from the total chromosome III sequence (OLIVER *et al.* 1992) and ca. 500-bp-long sequences were produced by PCR (Figure 1). Primer sequences are available upon request. In addition, *THR4* was probed with an 0.88-kb *Hin*dIII fragment from pMJ338 (provided by MICHAEL LICHTEN), and *HIS4* with a mixture of 0.7- and 0.9-kb *Eco*RI-*Pst*I fragments from pNKY155 (provided by NANCY KLECKNER). For confirming the breakpoint of the III/IV translocation on chromosome III, the *LEU2* locus was probed with a 0.5-kb *Cla*I-*Eco*RI fragment and a 0.93-kb *Eco*RI-*Sal*I fragment (Probes A and B in Figure 7). As a probe for the left arm of chromosome *IV*, we used a 1.7-kb fragment from *Eco*RI-*Hin*dIII digested cosmid ATCC71003, and for the right arm, a 1.9-kb fragment from cosmid ATCC71013 digested with the same enzymes. For confirming the breakpoint of the III/IV translocation on chromosome IV, the *TRP1* locus was probed with a 0.39-kb *Eco*RI-*Eco*RV fragment and a 0.47-kb *Eco*RV-*Bgl*II fragment.

RESULTS

Nonallelic recombination is more frequent in haploid than in diploid meiosis: To examine and quantify karyotype changes we used two isogenic strains, a *spo13* diploid, which performs a single division in meiosis (producing two diploid spores), and a *sir3 spo13* haploid, which can perform haploid meiosis with a single division, producing two viable haploid spores (WAGSTAFF *et al.* 1982). In the diploid, sporulation frequency was ~35% and spore viability was 27% (712 of 2600); in the haploid sporulation frequency was ~39% and spore viability was 19% (443 of 2362). In both, spore viability thus was lower than reported for *spo13* mutants in other strain backgrounds (WAGSTAFF *et al.* 1982; HUGERAT and SIMCHEN 1993). The reduced spore viability in our haploid compared with our diploid may be mainly due to the frequent failure of individual chromosomes to segregate equationally during the single meiotic division, which produces asci with a disomic and a nullisomic spore in haploid meiosis (HUGERAT and SIMCHEN 1993). Disomies could be readily detected on CHEF gels due to higher relative intensity of the corresponding band (arrows in Figure 2 and Figure 3a) and could be verified by the presence of two chromosome-specific signals after *in situ* hybridization (not shown). In addition, some inviable spores must be attributed to the loss of chromosome segments due to unequal recombination (see below).

From liquid cell cultures that had been grown for ~37 mitotic generations, 240 clones were isolated and tested by PFGE. None showed an aberrant chromosomal pattern except for chromosome IX, meaning that the karyotype remained essentially unchanged over a total of ~8880 mitotic divisions (Table 1). Chromosome IX was found to be smaller by ca. 10 kb in 3–4% of both mitotically and meiotically generated colonies. Of 487 clones that were recovered from 415 diploid *spo13* meioses, two had altered chromosomes (VII and XII) other than IX (Table 1).

In sharp contrast, of the offspring produced by 428 haploid meioses, 32 showed chromosomes (other than IX) with altered sizes (Figure 3), *i.e.*, in one out of ~13 haploid meioses a visible (≥10 kb) change in chromosome size takes place (Table 1).

To exclude the possibility that the increased frequency of ectopic recombination events in the haploid was due to an effect of the *sir3* mutation, we included as a control a *spo13* haploid in which *MATα* was expressed from a plasmid. In this strain, five chromosome

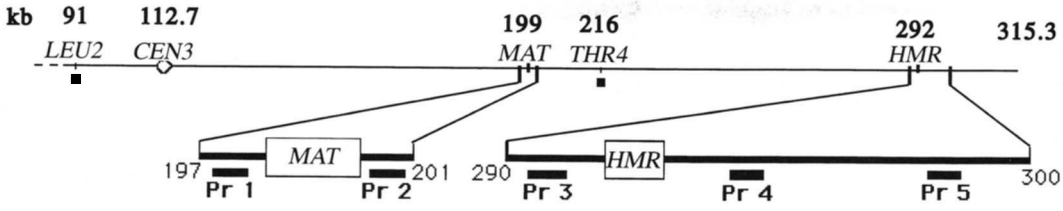

FIGURE 1.—Map of yeast chromosome III with the locations of hybridization probes *LEU2*, *THR4* and Pr1–Pr5 (thick bars) to delimit the repeated segment on chromosome III.

alterations occurred in 110 haploid meioses. This is comparable to the frequency in the *sir3* haploid.

Unequal sister chromatid recombination: In 26 of the colonies with altered karyotype, a single chromosome was enlarged (Table 2). In all but one case no viable sister spores were recovered (whereas 11 would be expected since 40% of all colonies grew from dyads with two viable spores). This suggests that the enlarged chromosomes resulted from unequal sister chromatid recombination events, leading to the gain of a segment on one chromatid and the corresponding loss on the other (KLEIN 1995), rendering inviable the spore carrying the deletion. There was one case, however, where a chromosome X was enlarged by ~20 kb, and a corresponding shortening of chromosome X was found in the sister colony. Chromosome X shortening, which was observed four times, suggests that this chromosome has a 20-kb region, possibly a duplication, in the wild-type SK1 strain whose loss does not noticeably impair growth on rich medium. In cases where one of two viable sister spores showed a shortened chromosome IX (see above), the other had a wild-type chromosome IX. This suggests that the deletions in chromosome IX are not due to unequal sister chromatid recombination but rather to intrachromatid recombination by which a segment of the chromosome is excised (see below). The

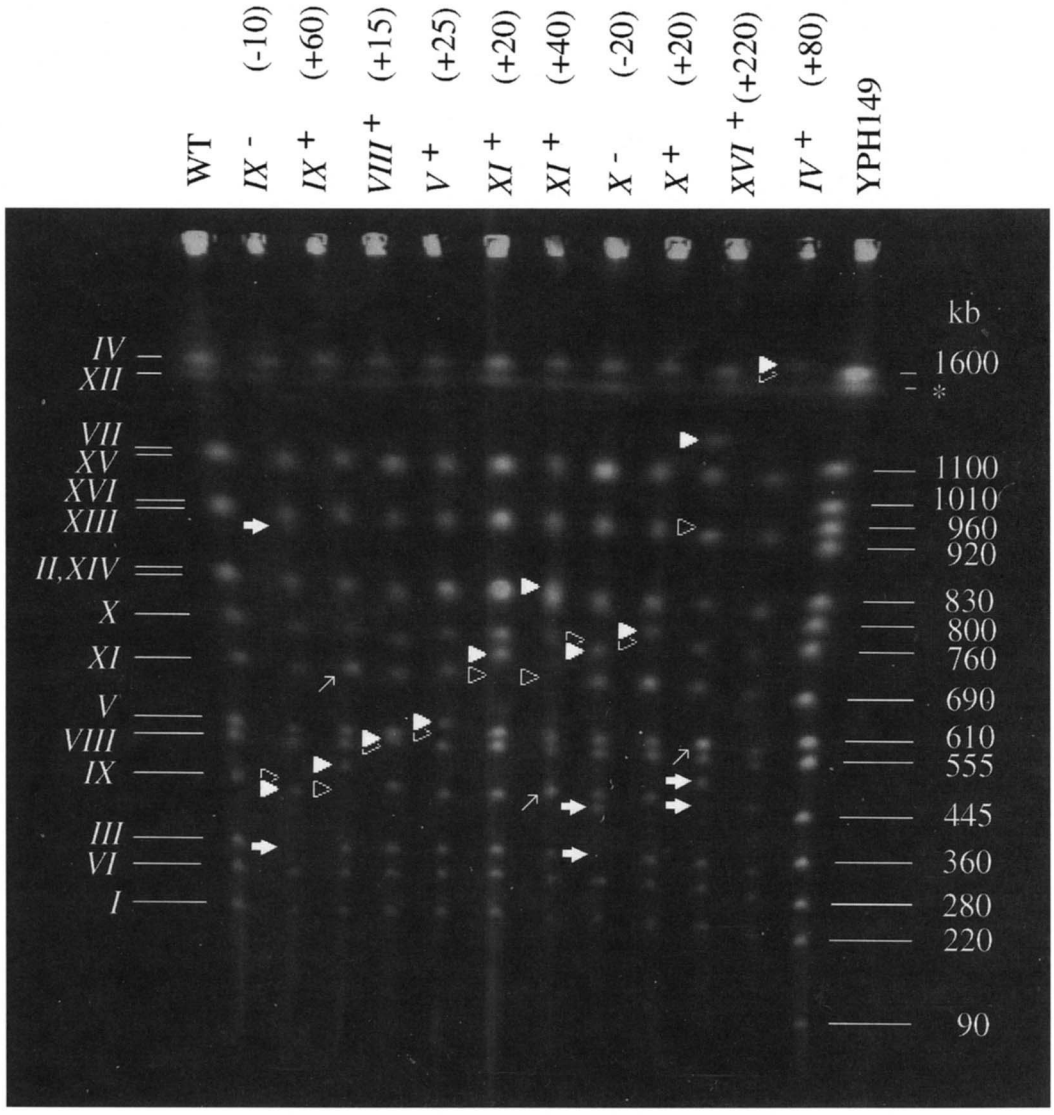

FIGURE 2.—CHEF gel with examples of haploid clones with enlarged (+) or shortened (−) chromosomes. The increase or decrease in kilobase (kb) as compared with the wild type (WT) is given in brackets. Solid arrowheads designate the altered chromosomes, open arrowheads indicate the positions where the corresponding wild-type chromosomes would run. In several clones, also other deviant chromosomes (thick arrows) can be seen, as those had been acquired during previous rounds of haploid meioses (the positions of these chromosomes in the WT are also indicated by thick arrows). The yeast strain YPH 149 (GERRING *et al.* 1991) was used as size marker. *Chromosome XII does not run proportionally to its molecular size. Notice disomic chromosomes as indicated by strong bands (thin arrows).

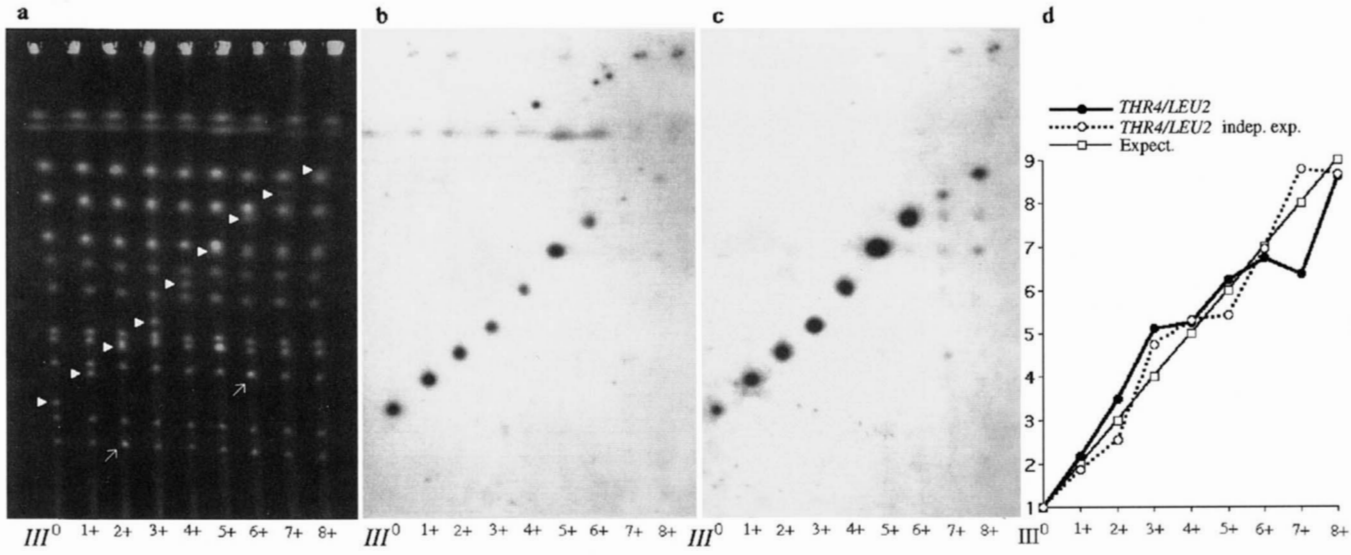

FIGURE 3.—(a) CHEF gel showing clones with wild-type (III^0) and enlarged (III^{1+}–III^{8+}) chromosomes III (arrowheads). Southern hybridization to chromosomes III with probes *LEU2* from outside (b) and *THR4* from inside (c) the *MAT-HMR* segment (see Figure 1). Whereas intensities of the *LEU2* signals are proportional to the amount of DNA run for the different clones, intensities of *THR4* signals increase with the size of chromosomes. (d) The relative intensities of *THR4/LEU2* were measured by phosphor imaging and normalized to a value of 1 in III^0 (●); ○, the *THR4/LEU2* ratios from an independent experiment on a different CHEF gel. Both fit the expectation (□) of the increase in relative intensities of *THR4 vs. LEU2* signals from 1:1 to 9:1 for enlarged chromosomes III. Comparable results were obtained with the other pairs of probes from inside *vs.* outside the *MAT-HMR* segment (see Figure 1). This provides evidence that the multiplication of the 100-kb segment between *MAT* and *HMR* causes the gain of size of chromosome III. Chromosomes III^{7+} and III^{8+} tend to lose copies of the amplified segment during mitotic growth (presumably by intrachromatid excision: Figure 4b) and shortened chromosomes III appear as additional bands on Southern blots (b and c). Notice disomies for chromosomes I and IX as indicated by the intense bands (arrows) in a.

cause of the high frequency of this event in both the mitotic and meiotic cycles is unknown. The position of chromosome XII was frequently found changed (Table 2). The frequency and extent of size changes could not be quantified, however, since chromosome XII does not run proportionally to its size on CHEF gels, and band positions vary between experiments.

Of the 16 yeast chromosomes, 11 were found subject to size changes, which were most likely caused by unequal sister chromatid recombination. (Theoretically, karyotypes with a single enlarged chromosome could also arise from reciprocal translocations with one breakpoint in a distal region whose loss would not confer lethality to an unbalanced segregation product. Such translocations must be considered rare, however.) Four chromosomes showed the same modification more often than once (Table 2), which suggests that unequal recombination events have preferences for certain chromosome regions (which share ectopic homologous sequences) rather than being illegitimate.

The *MAT* and *HMR* loci are substrates for reciprocal ectopic recombination: When we subjected a clone that

TABLE 1

Frequencies of ectopic recombination events

	Unequal sister chromatid recombination observed	Translocations		Total ectopic recombination (%)
		Observed	Calculated	
Mitotic cycle (haploid)	0/8880	0/8880	—	<0.01
Diploid meiosis	2/415	0/415	—	~0.5
Haploid meiosis (*sir3* strain)	26/428 (~6%)	6/428	24/428 (~6%)	>12[a]

Unequal sister chromatid recombination events were observed as single chromosomes of altered lengths on CHEF gels. Altered chromosomes IX, which possibly occur by a different mechanism (see RESULTS), are not included. Translocations were observed as complementary size changes of two different chromosomes and verified by Southern hybridization with chromosome-specific probes. The calculated frequency takes into account that 75% of translocation events will result in genetic imbalance and cause spore lethality (see DISCUSSION).

[a] Ectopic recombination is likely to occur in more than the calculated 12% of meioses because some recombination events may produce size changes <10 kb that are not resolved by PFGE.

TABLE 2

Enlarged and shortened chromosomes among the offspring of 428 haploid meioses due to unequal sister chromatid recombination

Chromosome	I	II	III	IV	V	VIII	IX	X	XI	XII	XVI
±kb	+30 (1)	+10 (1)	+100 (2)[a]	+80 (1)	+25 (1)	+15 (1)	−10[b] +60 (5)	−20 (4) +20 (5) +50 (1)	+20 (1) +40 (1)	variable ± (14)[c]	+220 (2)

Enlargement (+) and shortening (−) of chromosomes given in kilobase (kb). Cases observed are in parentheses.

[a] In two cases, a wild-type chromosome III was enlarged. Further size increases (see RESULTS) are not included in the table.

[b] Shortened chromosomes IX occur in 3–4% of both mitotic and meiotic colonies (see RESULTS).

[c] In addition to four clones where chromosome XII has altered its size considerably (probably by >100 kb), smaller size changes in up to up to 10% of chromosomes XII could be detected in some of the CHEF gels whereas in others all chromosomes XII ran uniformly. This suggests that minor size changes of chromosome XII, although they may be frequent, are poorly detectable because of the erratic mobility of this chromosome (see RESULTS).

had acquired an enlarged chromosome III (III 1+) by unequal sister chromatid recombination, to another round of meiosis, we found among the progeny some colonies with an even further enlarged chromosome III (III^{2+}). In subsequent rounds of haploid meioses, we could obtain a set of chromosomes III up to III^{8+}, each enlarged by the same amount of ~100 kb (Figure 3). Also this regular increase shows that recombination is ectopic rather than illegitimate (see above). To determine the amplified region, we prepared a set of hybridization probes along chromosome III (Figure 1). Southern hybridization to chromosomes III^{0} (wild-type III) to III^{8+} on CHEF gels showed that the intensities of bands probed with Pr2, *THR4* and Pr3 increased with chromosome size relative to the intensities of *LEU2*, Pr1, Pr4 and Pr5 signals (Figure 3). This suggests that probes Pr2, *THR4* and Pr3 are from within the amplified region and that the substrates for the initial ectopic sister chromatid recombination that has led to chromosome III^{1+} are located in or close to the *MAT* and *HMR* loci, which share ca. 650 bases of continuous homology (HERSKOWITZ and OSHIMA 1981; OLIVER *et al.* 1992).

Unequal recombination between tandem repeats: The more copies of the repeat a chromosome III had acquired, the more frequent were further size-changes during subsequent rounds of haploid meiosis. Whereas in the original haploid only two changed chromosomes III were observed among the products of 428 meioses (Table 2), 76% (166 of 217) of the meiotic products of clones with enlarged chromosomes III showed a further growth or a shortening of this chromosome. In 26% (17 of 65) of meioses of a III^{3+}-clone, chromosome III was enlarged further by one or several copies of the repeat, thus, by inference, unequal sister chromatid recombination occurred in >50%. This high frequency can be explained by the whole 100-kb interval between *MAT* and *HMR* being present as a multiple tandem repeat and serving as substrate for unequal sister chromatid

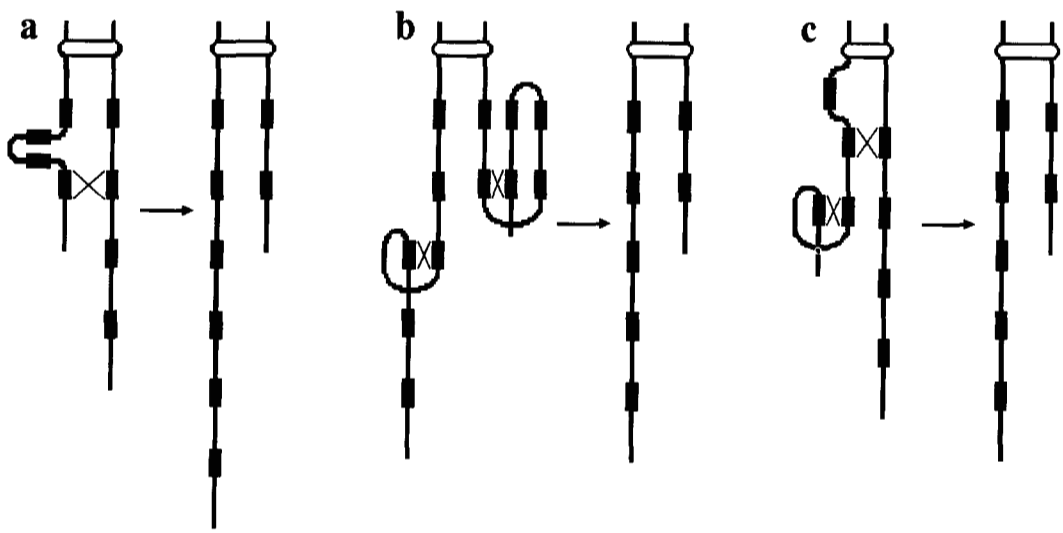

FIGURE 4.—Types of ectopic recombination events that could cause the observed size-changes of single chromosomes in the haploid. With unequal sister chromatid recombination (a), the total number of copies of the amplified region in the two products remains constant; with intrachromatid recombination (shown here as the excision of a loop) that can involve one or both chromatids (b) there is a loss. If both unequal sister chromatid and intrachromatid recombination occur simultaneously (c), there can be a gain of copies in one product accompanied by a reduction in the total number of copies.

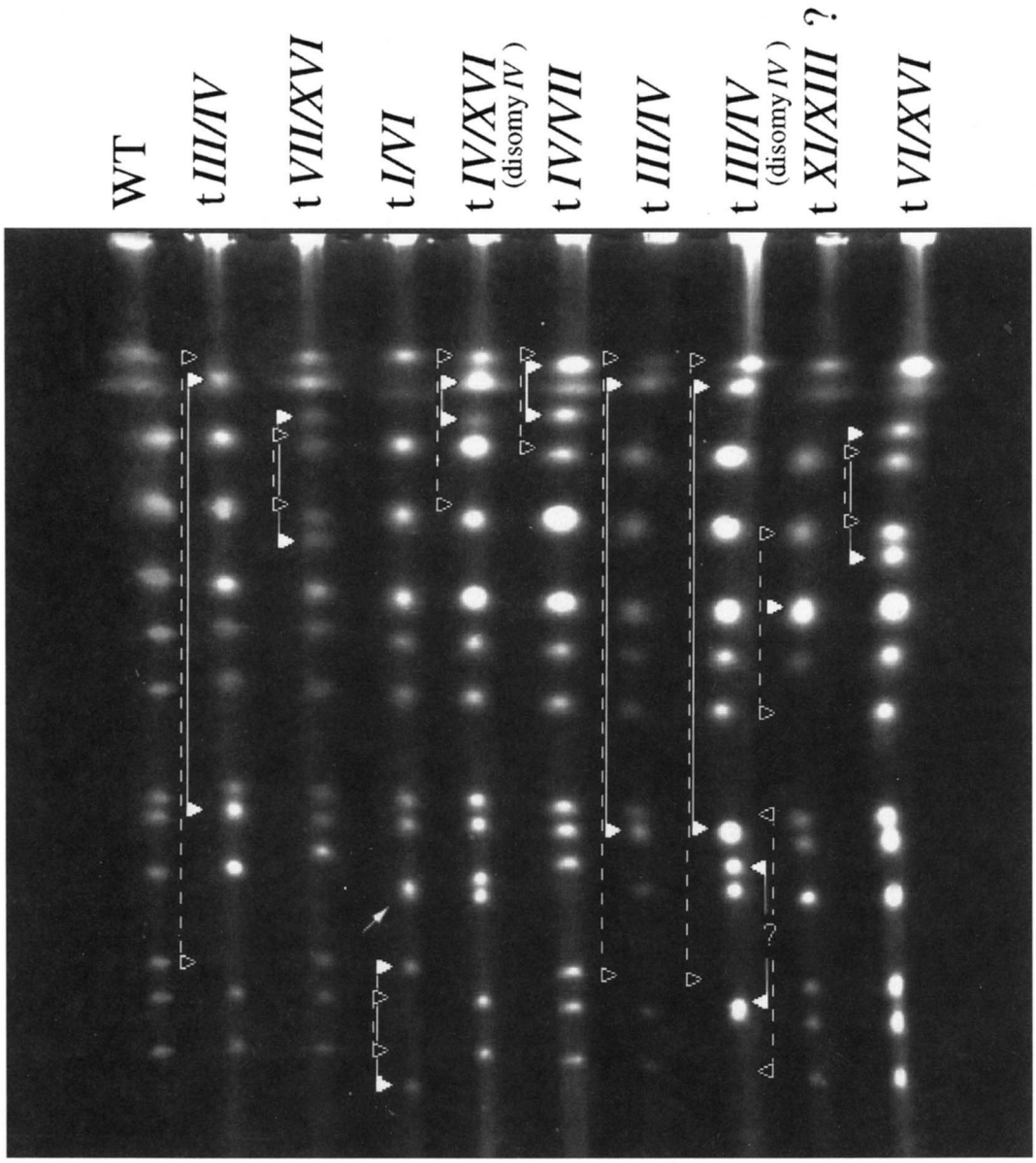

FIGURE 5.—Translocations resulting from ectopic recombination during haploid meiosis. (Examples from both haploid strains are included.) Translocation products can be identified in CHEF gels as pairs of chromosomes (solid arrowheads) that are smaller and larger than the corresponding wild-type chromosomes (open arrowheads), but add up to the same total length. In three independent translocation events where chromosome IV is involved, there is a band at the wild-type position of chromosome IV because of a disomy. Chromosome alterations other than translocations have occurred independently in some of the clones. In the fourth lane, one product of a I/VI translocation runs at the position of chromosome III in the wild type (compare Figure 2), whereas chromosome III is present as an enlarged III^{1+} (arrow; compare Figure 3).

recombination. Likewise, the frequent occurrence of altered chromosomes XII (Table 2) is probably due to unequal sister chromatid recombination between copies of the rDNA tandem repeat (see also DISCUSSION).

Intrachromatid recombination: When a spore from a clone with an enlarged chromosome III showed further growth of this chromosome after haploid meiosis, then its sister had a shortened copy. This is as expected from unequal sister chromatid recombination where the total number of repeats in the offsprings remains constant (Figure 4). In 73% (27 of 37) of pairs of sister spores, however, there was a reduction in the combined lengths of chromosomes III. (Pieces of as large as 600 kb, *i.e.*, six copies of the repeat, could be lost in a single step.) The reduction of chromosome size therefore must have been caused also by intrachromatid recombination either due to the excision of loops of the repeat (PETES and HILL 1988; KLEIN 1995) (Figure 4) or due to single-strand annealing events (OZENBERG and ROEDER 1991).

Vigor of clones with enlarged chromosomes up to III^{6+} was not notably affected although they carry six extra copies of the 100-kb segment between *MAT* and *HMR* and thus are highly imbalanced for the genes in this segment. They were mitotically fairly stable. Of 90 colonies derived from a III^{3+}-clone that had been grown for ~37 vegetative generations and thus had gone through ~3330 mitotic cycles, only one showed a shortening of III^{3+} to III^{1+}. Clones with III^{7+} and III^{8+} are increasingly unstable, however, as can be seen from the appearance of several shortened copies of chromosome III in CHEF gels of colonies grown from III^{7+} and III^{8+} spores (Figure 3c). Obviously, cells with shortened chromosomes III, which rarely originate during vegetative growth (not shown), outgrow cells with larger chromosomes III rapidly.

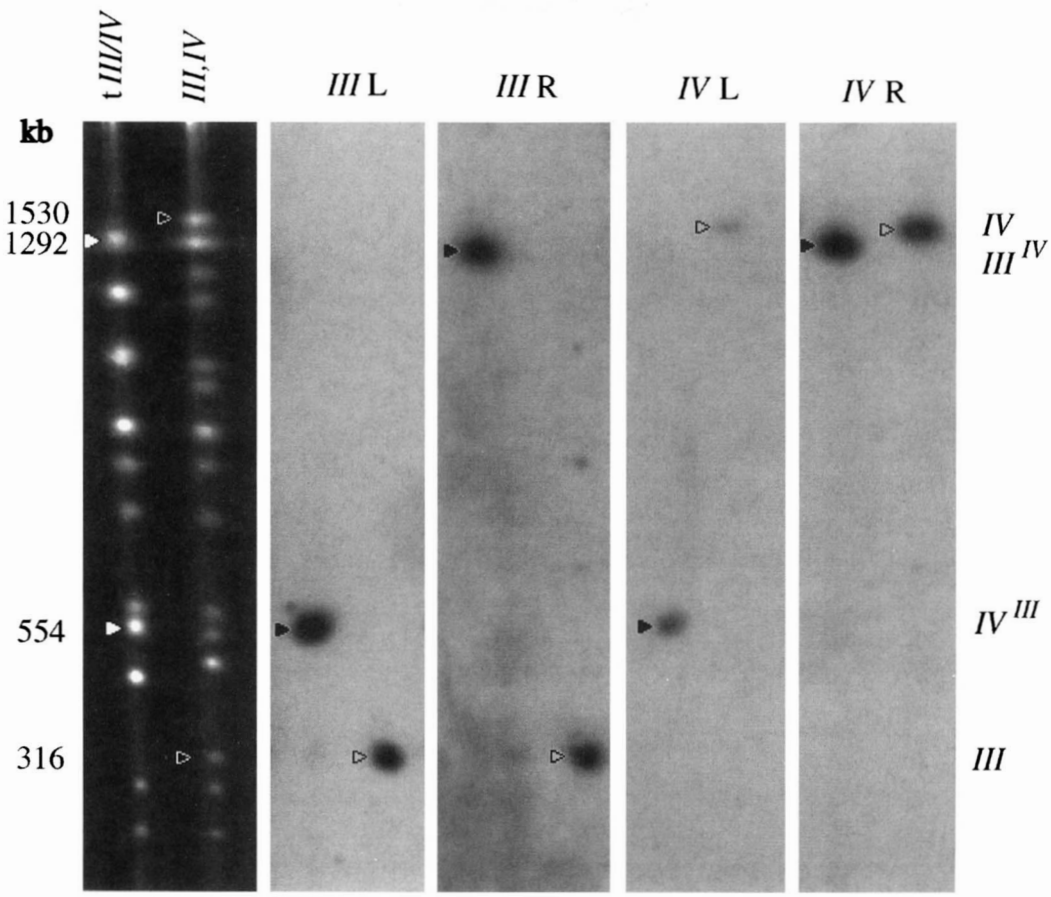

FIGURE 6.—Example of the confirmation of a translocation by Southern hybridization with chromosome-specific probes. A CHEF gel with a putative III/IV translocation clone (t III/IV) and a clone with wild-type chromosomes III and IV (III, IV) was probed with sequences from the left (IIIL, *HIS4*) and right (IIIR, *THR4*) arms of chromosome III and from the left (IVL) and right (IVR) arms of chromosome IV. Solid arrowheads indicate the translocation products, open arrowheads the wild-type chromosomes III (lower) and IV (upper). Probes IIIL and IVL colocalize in the small, and probes IIIR and IVR in the large translocation product. The other translocations (Figure 5) were confirmed in similar ways. Sizes of chromosomes III and IV and of the translocation products are shown in kilobase (kb).

Recombination between nonhomologous chromosomes: Six spores of the haploid *sir3* strain showed both an enlarged and a shortened chromosome whose combined sizes were the same as the combined sizes of the corresponding wild-type chromosomes (Figure 5). These presumed reciprocal translocations were confirmed by Southern hybridization with probes from the corresponding wild-type chromosomes. Figure 6 shows a typical experiment. For a case of a translocation between chromosomes III and IV, we located the breakpoints in the 1.1-kb bacterial *hisG* sequence (Figure 7), which is inserted in our strains for disruption of the *LEU2* and *TRP1* genes (ALANI *et al.* 1987). Two other independent III/IV translocations may also involve *hisG* sequences whereas the other translocations seem to rely on endogenous repeats.

The identification of some translocations was complicated by the fact that a wild-type chromosome was not missing because of a disomy and/or that a translocation product colocalized with another chromosome and therefore did not appear as an additional band on ethidiumbromide-stained gels (see Figure 5). Since only a fraction of translocation products are viable and some of these may not be detected by changed chromosome sizes, the actual frequency of translocations is expected to be much higher than the observed (see DISCUSSION and Table 1).

DISCUSSION

From the observation of high frequencies of sister chromatid recombination in the meiosis of a haploid yeast, WAGSTAFF *et al.* (1985) had concluded that the absence of allelic homologies in haploid meiosis promotes recombination between sister chromatids. However, recombination was not compared to an isogenic diploid control, and it was objected that the *spo13* mutation present in the haploid might influence recombination (PETES and PUKKILA 1995). We have used pulsed-field gel electrophoresis of intact yeast chromosomes to detect altered karyotypes, and we frequently found chromosomes of altered size in the meiotic products of

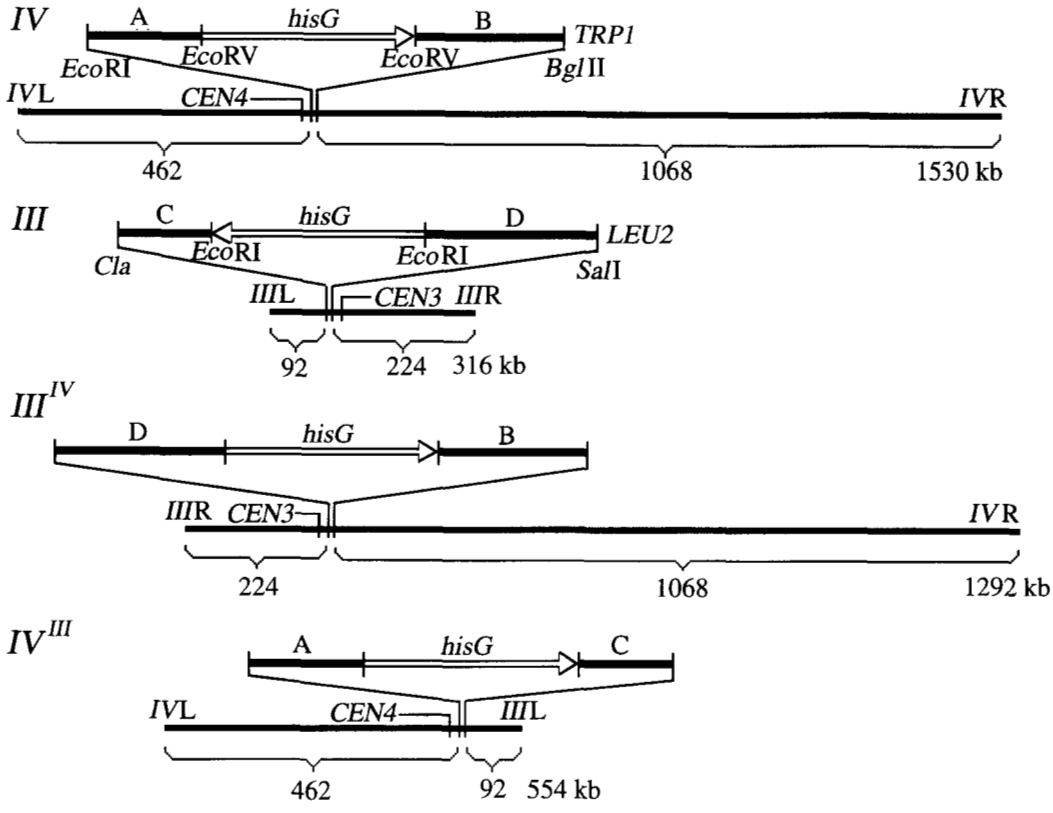

FIGURE 7.—Determination of the translocation breakpoints in the III/IV translocation (see Figure 6). The lengths of translocation products III^{IV} and IV^{III} as seen in the CHEF gel (Figure 5) gave a first clue to the approximate location of the breakpoints. With several pairs of hybridization probes the left and right borders of the putative breakpoints were narrowed down to the regions near the *TRP1* and *LEU2* loci. Finally, Southern hybridizations were performed with probes A and B on both sides of the *Eco*RV site, where the *hisG* is inserted at the *TRP1* locus on chromosome IV, and probes C and D at the *Eco*RI integration site of *hisG* in the *LEU2* locus on chromosome III. hisG sequences are inserted on opposite strands (W and C). The redistribution of probes B and D to the III^{IV}, and of probes A and C to the IV^{III} translocation product proves that the translocation breakpoints are within the 1.1-kb *hisG* sequences. Physical lengths and positions in the chart are approximate since in SK1 they may differ from the published data from the sequencing project.

a haploid *S. cerevisiae* strain caused by ectopic recombination within and between chromosomes. In the isogenic diploid, altered chromosomes occurred rarely (Table 1). Most length change likely is due to unequal sister chromatid recombinations between sequences up to 100 kb apart. We could not monitor equal sister chromatid recombination events, but it is safe to assume that increased unequal sister chromatid recombination in haploids indicates a corresponding increase also in equal exchanges (GAME *et al.* 1989). Our findings support the conclusion by WAGSTAFF *et al.* (1985) that sister chromatid and nonsister homologous recombination events compete for limiting quantities of components of the genetic exchange machinery. The same rule might apply to recombination within the multiple tandem repeats (~150 copies) of rDNA where intrachromosomal recombination is increased at the cost of homologous recombination. In meiosis, no SC is formed between the nucleolus organizing regions on chromosome XII (BYERS and GOETSCH 1975) and recombination between the homologues is very low at these regions (PETES and BOTSTEIN 1977). On the other hand, sister chromatid recombination is high (PETES 1980; SZOSTAK and WU 1980). Although in this case corresponding regions are present on the homologue, they are probably not available as substrates for recombination.

In haploid plants, meiotic recombination between nonhomologous chromosomes is common, as was shown by the occurrence of chiasmata (RIEGER 1957; SADASIVAIAH and KASHA 1971; CRANE *et al.* 1982; NEIJZING 1982; DE JONG *et al.* 1991; SANTOS *et al.* 1994). The presence of heterozygous heterochromatin bands as cytological markers for segregation allowed the demonstration that these chiasmata were associated with genuine crossovers (NEIJZING 1982; DE JONG *et al.* 1991; SANTOS *et al.* 1994). We scored for crossovers in haploid yeast by the presence of reciprocal translocations, which are the result of recombination between homologous sequences on nonhomologous chromosomes. Chromosomal rearrangements (duplications, translocations) occur in vegetative cells and more frequently in meiosis (SCHERER and DAVIS 1980; JACKSON and FINK 1985; JINKS-ROBERTSON and PETES 1985, 1986; LICHTEN *et al.*

1987; MALONEY and FOGEL 1987; KUPIEC and PETES 1988; PETES and HILL 1988). Normally, however, there seem to exist mechanisms that suppress ectopic recombination (PARKET *et al.* 1995), and meiotic recombination between allelic regions on homologous chromosomes is the rule.

We found six translocations among the offspring of 428 haploid meioses, however, they may actually be over four times as frequent. Fifty percent of translocation events between heterologous chromosomes are expected to occur between sequences that are oriented in opposite directions with respect to the centromeres. They will produce dicentric chromosomes plus acentric fragments that are inviable. Of the remaining 50% (between sequences of the same orientation), half will segregate to produce dyads with two nonviable, genetically imbalanced spores and half will produce spores with one unchanged and one genetically balanced translocation. Thus only 25% of translocations between heterologous chromosomes will be present in viable offspring. Some of these may go unnoticed if translocation products are of roughly the same size as the corresponding wild-type chromosomes. Furthermore, crossovers between nonallelic sites within single chromosomes (intrachromosomal translocations) would also go undetected by our screen since they lead to inversions without size-change or to nonviable dicentric/deletion karyotypes (NEIJZING 1982). Taking into account these concealed events, one can estimate that somewhat >0.06 ectopic crossovers occur per meiosis (Table 1). On the other hand, there were found 0.38 (SANTOS *et al.* 1994) and 0.47 (DE JONG *et al.* 1991) chiasmata per meiocyte in haploid rye plants and 0.8 chiasmata per meiosis in haploid Antirrhinum (RIEGER 1957). A possible reason for the lower incidence of ectopic recombination in yeast may be the relative rareness of repeated sequences compared with higher plants.

The emergence of altered chromosomes as seen by PFGE shows that reciprocal unequal sister chromatid recombination, intrachromatid recombination, and recombination between sequences on nonhomologous chromosomes are more frequent in haploid than in diploid meiosis. Thus it seems that in haploids the recombination machinery is directed toward sister chromatids and dispersed sequences on nonhomologous chromosomes, whereas the presence, or more precisely, the availability of homologous chromosomes suppresses ectopic and sister chromatid recombination (Table 1). This conclusion concurs with the observation in Drosophila that ectopic recombination between interspersed repeats of the *roo* transposon is higher when an allelic copy is absent (MONTGOMERY *et al.* 1991).

So far, ectopic crossovers in yeast have not been confirmed cytologically. Whereas *zip1* diploids show multiple axial associations between homologous chromosomes, which indicate interactions between homologous sequences, axial associations are missing in the mutant haploid (ROCKMILL *et al.* 1995). Associations that accompany translocation events may go unnoticed in cytological preparations because they are rare (about six in 100 meioses) or because single axial associations are more easily disrupted than an array of multiple associations between homologous chromosomes.

It is an open question whether synapsis in haploids is completely random or whether synaptonemal complex is initiated at sites where short homologous or partially homologous sequence tracts are interacting. This would parallel the *XY* pairing in some mammals where the synaptonemal complex is initiated in the pseudoautosomal region but may extend far into the heterologous portions of the sex chromosomes (*e.g.*, ASHLEY 1987). In view of the occurrence of heterologous crossovers (and probably a much higher incidence of conversion-type recombination and nonrecombinogenic homologous interactions), it is possible that the initiation of synapsis in haploids is based on ectopic homologies.

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