Restriction of ectopic recombination by interhomolog interactions during *Saccharomyces cerevisiae* meiosis

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In *Saccharomyces cerevisiae* meiosis, recombination occurs frequently between sequences at the same location on homologs (allelic recombination) and can take place between dispersed homologous sequences (ectopic recombination). Ectopic recombination occurs less often than does allelic, especially when homologous sequences are on heterologous chromosomes. To account for this, it has been suggested that homolog pairing (homolog colocalization and alignment) either promotes allelic recombination or restricts ectopic recombination. The latter suggestion was tested by examining ectopic recombination in two cases where normal interhomolog relationships are disrupted. In the first case, one member of a homolog pair was replaced by a homoeologous (related but not identical) chromosome that has diverged sufficiently to prevent allelic recombination. In the second case, *ndj1* mutants were used to delay homolog pairing and synapsis. Both circumstances resulted in a substantial increase in the frequency of ectopic recombination between *arg4*-containing plasmid inserts located on heterologous chromosomes. These findings suggest that, during normal yeast meiosis, progressive homolog colocalization, alignment, synapsis, and allelic recombination restrict the ability of ectopically located sequences to find each other and recombine. In the absence of such restrictions, the meiotic homology search may encompass the entire genome.

homolog pairing | sequence divergence | *NDJ1*

During prophase of meiosis I, chromosomes move from a state of relative dispersal to a highly organized paired conformation. Sequences on one parental homolog are brought close to corresponding sequences on the other, resulting in end-to-end homolog alignment. We will refer to this progressive process as homolog pairing. Pairing culminates at pachytene, when coaligned homologs are tightly synapsed by the synaptonemal complex (reviewed in ref. 1). Homolog juxtaposition can also occur in nonmeiotic cells and ranges in extent from the close homolog alignment seen in *Drosophila* (2) to the intermittent colocalization of short chromosome segments seen in mitotic and premeiotic *Saccharomyces cerevisiae* cells (3, 4). In *S. cerevisiae*, segmental colocalization disappears during meiotic S phase. Restoration of colocalization is nonuniform and gradual, with homolog segments colocalized or even synapsed when the bulk of chromosomes appear to be dispersed (4, 5). In other organisms, end-to-end homolog alignment can occur at times when most chromosomal sequences are still separated by a significant distance (6–9). Alignment at a distance may also occur in *Saccharomyces*, but the nonuniform homolog pairing seen in this organism precludes its detection.

Meiosis is also characterized by elevated frequencies of genetic recombination, including both nonreciprocal (gene conversion) and reciprocal (crossing-over) events. Meiotic recombination in *S. cerevisiae* (and most likely in other organisms) is initiated via the formation and subsequent repair of double-strand DNA breaks (DSBs) induced early in meiosis I prophase (1, 10–13). Recombination usually occurs at levels sufficient to ensure at least one crossover per homolog pair and is needed to ensure correct homolog disjunction during the first meiotic division (14). The contribution of recombination to meiotic homolog colocalization and alignment remains an issue of some controversy. Recombination is clearly dispensable for pairing in *Drosophila* and in *Caenorhabditis elegans*, inasmuch as recombination-null mutants display apparently normal synapsis at pachytene (12, 13). In contrast, *S. cerevisiae* Rec$^-$ mutants display defects in homolog pairing and synapsis (1, 4, 15, 16). It remains to be determined whether these defects reflect an obligate role for recombination in homolog pairing, a checkpoint-mediated block to meiotic progression in the absence of recombination, or a structural role for recombination proteins or intermediates in synaptonemal complex assembly (17–19).

Aspects of the relationship between meiotic recombination and homolog pairing are revealed by studies of ectopic recombination (20–24). Ectopic recombination occurs during *S. cerevisiae* meiosis but is limited by chromosomal context. For example, we have shown that recombination between *arg4* inserts located on heterologous chromosomes is 10- to 20-fold reduced relative to interhomolog recombination between inserts at allelic loci (22). Inserts located on homologs at sites about 20 kb apart recombine as efficiently as do allelic inserts, and the likelihood of ectopic recombination declines with increasing distance between insert loci. These results were interpreted as suggesting that most meiotic recombination occurs at a time when homologs are colocalized and are in end-to-end alignment.

Two possible explanations could account for the reduced efficiency of ectopic relative to allelic recombination seen in *S. cerevisiae* meiosis. The first explanation suggests that multiple interhomolog interactions are needed for efficient recombination between homologous sequences; the lack of such interactions between heterologous chromosomes would reduce the likelihood of interheterolog ectopic recombination. The second explanation suggests that homologous sequences, unless otherwise constrained, would find each other and efficiently recombine irrespective of their location. In this view, homolog pairing and synapsis would reduce the ability of dispersed sequences to find each other during meiosis, while preserving the ability of allelic sequences to recombine.

We examined meiotic recombination between dispersed copies of the *ARG4* gene in two situations where normal interhomolog interactions might be disrupted. In the first, ectopic recombination was measured in hybrid *S. cerevisiae* strains containing one or two homoeologous chromosomes from *Saccharomyces carlsbergensis*. These homoeologous chromosomes

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Abbreviations: DSB, double-strand DNA break; *IIIcere*, chromosome *III* of *Saccharomyces cerevisiae*; *IIIcarl*, chromosome *III* of *Saccharomyces carlsbergensis*; *Vcere*, chromosome *V* of *Saccharomyces cerevisiae*; *Vcarl*, chromosome *V* of *Saccharomyces carlsbergensis*.

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are 15% diverged in coding sequences, with even greater differences seen in intergenic regions (ref. 25; T. Nilsson-Tillgren, personal communication). This sequence divergence severely reduces (by at least 100-fold) rates of mitotic and meiotic recombination between diverged *S. cerevisiae* and *S. carlsbergensis* sequences (26–28). Furthermore, studies using LacI-green fluorescent protein fusions bound to the arms of chromosomes *V* from *S. cerevisiae* and *S. carlsbergensis* indicate that these two diverged chromosomes do not undergo end-to-end alignment in meiosis I prophase (R. Boumil, B. Kemp, and D. Dawson, personal communication). Consistent with this lack of alignment and recombination, homoeologous *S. cerevisiae–S. carlsbergensis* chromosome pairs display a level of nondisjunction typical of that produced by the distributive disjunction system that segregates single pairs of nonhomologous chromosomes (refs. 29 and 30 and B. Kemp and D. Dawson, personal communication; unpublished data).

Ectopic recombination was also examined in strains homozygous for a loss-of-function *ndj1* mutation. Ndj1p is produced early in meiosis and is located primarily at chromosome ends. *ndj1* mutants display defects in telomere clustering (bouquet stage), which normally occurs early in meiosis I prophase (ref. 31; H. Scherthan, personal communication). Perhaps as a result, homolog colocalization is reduced early in meiosis I prophase (H. Scherthan, personal communication), and complete synapsis is delayed relative to wild type (32, 33). Genetic studies of haploid meiosis in *ndj1* strains are also consistent with a defect in homolog pairing (34). Nevertheless, allelic gene conversion and crossing over occur at near-normal frequencies in *ndj1* mutants (32, 33).

We report here that both of these disruptions in normal homolog interactions result in a significant increase in ectopic recombination between sequences on affected chromosomes. This finding is consistent with the view that meiotic homolog pairing and synapsis, accompanied by allelic recombination, serve to limit an otherwise promiscuous homology search, thus actively discouraging ectopic interactions between homologous sequences on heterologous chromosomes.

Materials and Methods

Strains. All yeast strains (the complete list will be supplied upon request) are congenic to SK1 (35). The *S. carlsbergensis* chromosome *V* (abbreviated *Vcarl*), marked with *ilv1*, was introduced into SK1 by S. Priebe and M. Resnick (National Institute on Environmental Health Sciences, Triangle Park, NC), who performed five backcrosses; we performed five more backcrosses. All hybrid diploids containing chromosome *Vcarl* were homozygous for an unlinked single-locus modifier, not present in SK1, that is necessary for germination of *ilv1* spores. The *S. carlsbergensis* chromosome *IIIcarl* was provided in an SK1 background by C. Newlon (University of Medicine and Dentistry, New Jersey Medical School, Newark, NJ) and was introduced by using standard crosses. *URA3-arg4* inserts on *S. cerevisiae* chromosomes (at *LEU2, MAT, CHA1, URA3, PHO11, PHO12*, and *PUT2*) have been described (22, 36) and were introduced during backcrossing. A *MAT*::*URA3-arg4-bgl* insert on chromosome *IIIcarl* was created by transformation as described (37), except that an *Eco*NI/*Bgl*II digest was used to direct integration. *ppx1*::*URA3-arg4* inserts were made by using plasmids pMJ443 and pMJ444, which contain chromosome *VIII* sequences from bp 501400 to bp 503284 at the *Eco*RI site of PMJ113 and PMJ115, respectively (36), and were inserted at *PPX1* as described (22). Diploids are homozygous for *lys2 ho*::*LYS2* and for *arg4-nsp,bgl* at the normal *ARG4* locus. All except those with *IIIcarl* are *leu2-K/leu2-R*. Strains containing the *rad50S* (*rad50-K81I*) allele (38) were constructed as described (36). Haploid *ndj1*::*KanMX6* strains were obtained by transformation with a digest of plasmid MCB253, a gift of M. Conrad and M. Dresser (Oklahoma Medical Research Foundation, Oklahoma City, OK).

Genetic Methods. *ARG4* and *LEU2* recombination frequencies and efficiencies were determined as described (22). At least two independent determinations were made. Crossover-associated *ARG4* recombinants were detected in allelic crosses with inserts at *MAT* by scoring for nonmating haploids (39) and in *LEU2* × *URA3* or *PPX1* × *URA3* ectopic crosses by pulsed-field gel analysis (22). In diploids used to determine crossover association, the two inserts had the same orientation relative to their centromeres, thus allowing the recovery of viable translocations containing crossover-associated *ARG4* recombinants.

Species-Specific PCR. Primers used to detect unique sequences at the left end of chromosome *V* of *S. cerevisiae* (*Vcere*) were TGTTGCTCAGTTAACTGCCGAGGC (bp 16373–16396) and GTTGGTTCTCCAGAGGGAAAGGC (bp 17262–17243); primers for the right end were CGATCAAATCGTGGCAGCGG (bp 566235–566254) and CTCCAGTGTTCTGAGCACCG (bp 566641–566622). Coordinates are from the chromosome *V* sequence [J. M. Cherry, C. Ball, K. Dolinski, S. Dwight, M. Harris, J. C. Matese, G. Sherlock, G. Binkley, H. Jin, S. Weng, and D. Botstein (2000) *Saccharomyces* Genome Database, http://genome-www.stanford.edu/Saccharomyces/].

DSB Quantitation. DSBs were detected in DNA from meiotic *rad50S/rad50S* diploids by Southern blotting, as described (36). A Fuji BAS2000 phosphorimager and MACBAS 2.5 software were used to quantify radioactivity on blots.

Results

The experiments reported here examine the relationship between meiotic homolog pairing and meiotic recombination, specifically the impact of interhomolog interactions on ectopic recombination. We determined the effect of either homoeology or an *ndj1* mutation on the frequency of *ARG4* spores produced by ectopic recombination between *arg4* mutant alleles (*arg4-nsp* and *arg4-bgl*) present on dispersed pairs of an 8-kb *URA3-arg4* insert (Fig. 1*a*). DSBs occur at three places in the insert at all loci, and comparisons of DSB and *ARG4* recombinant frequencies indicate that most if not all meiotic *ARG4* recombinants are induced by breaks formed in the insert (22, 36, 40). Under normal circumstances, a DSB break formed during meiosis can be repaired by interhomolog allelic recombination, by sister chromatid recombination, or by ectopic recombination with homologous sequences if they are present elsewhere in the genome. The current study used inserts that were hemizygous (present on only one member of a homolog or homoeolog pair) and were located on heterologous chromosomes (Fig. 1*b*). These inserts could not directly participate in allelic pairing or recombination. Thus the experiments described below specifically address the issue of how interhomolog interactions, occurring outside the 8-kb insert, affect the ability of sequences within that insert to encounter and recombine with an insert located elsewhere in the genome.

Homoeologous Chromosomes Increase the Frequency of Ectopic Recombination. To examine the effect of homoeology on ectopic meiotic recombination, we measured the frequency of *ARG4* spores produced by ectopic recombination between *URA3-arg4* inserts located on chromosome *III* at *MAT*, on chromosome *V* at *URA3*, or on chromosome *VIII* at *PUT2*. Ectopic recombination was measured in three types of diploids: nonhybrid control strains with two copies each of chromosome *Vcere* and chromosome *IIIcere*; hybrid strains containing a single homoeologous chromosome *Vcere/Vcarl* pair; and hybrid strains containing both homoeologous chromosome pairs *Vcere/Vcarl* and *II-*

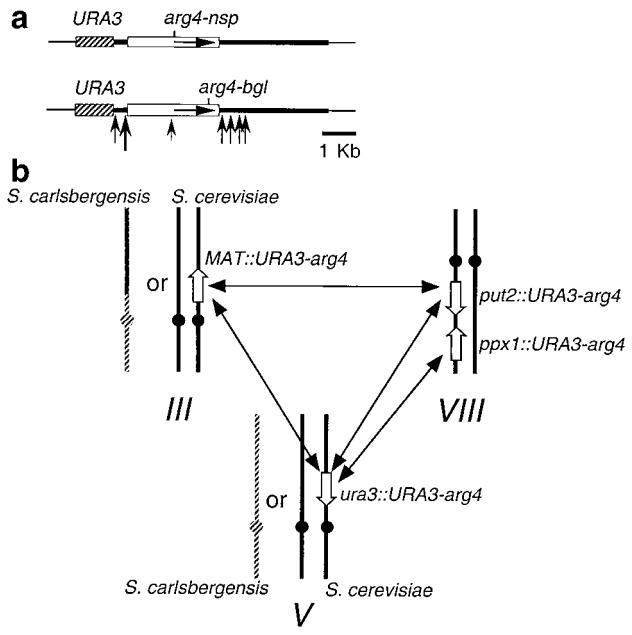

Fig. 1. Experimental design. (*a*) Structure of the *URA3-arg4* recombination substrates. Thick line, pBR322 sequences; hatched box, 1.2-kb *Hin*dIII *URA3* fragment; gray box, 3.3-kb *Pst*I *arg4* fragment containing either *arg4-nsp* or *arg4-bgl*; thin lines, flanking genomic sequences; horizontal arrows, direction of *ARG4* transcription; vertical arrows, meiosis-induced DSBs seen in all inserts (36). (*b*) Ectopic recombination was measured between pairs of *arg4* inserts (open arrows, indicating insert orientation) on chromosomes *III*, *V,* and *VIII*. Ectopic recombination was measured in three different diploid types: (*i*) nonhybrid diploids with only *S. cerevisiae* chromosomes (solid lines), (*ii*) diploids with a single homoeologous *S. carlsbergensis* chromosome *V*, and (*iii*) diploids with homoeologous copies of *S. carlsbergensis* chromosomes *III* and *V*. Hatched lines on *S. carlsbergensis* chromosomes indicate regions of divergence from *S. cerevisiae*.

Table 1. Effect of homoeologous chromosomes on ectopic recombination

Location (chromosome–locus)			
arg4-nsp	*arg4-bgl*	f(*ARG4*) × 10^3*	Relative to nonhybrid
Nonhybrid strains			
III–MAT	*V–ura3*	0.25 ± 0.04	
V–ura3	*III–MAT*	0.40 ± 0.10	
VIII–put2	*V–ura3*	0.15 ± 0.02	
V–ura3	*VIII–put2*	0.15 ± 0.02	
VIII–ppx1	*V–ura3*	0.36 ± 0.04	
V–ura3	*VIII–ppx1*	0.32 ± 0.01	
III–MAT	*VIII–put2*	0.31 ± 0.08	
VIII–put2	*III–MAT*	0.65 ± 0.1	
Homoeologous chromosome *V*			
III–MAT	*V–ura3*	0.82 ± 0.06	3.3
V–ura3	*III–MAT*	1.1 ± 0.17	2.8
VIII–put2	*V–ura3*	0.98 ± 0.1	6.5
V–ura3	*VIII–put2*	0.72 ± 0.17	4.8
VIII–ppx1	*V–ura3*	1.9 ± 0.36	5.3
V–ura3	*VIII–ppx1*	1.7 ± 0.19	5.3
III–MAT	*VIII–put2*	0.34 ± 0.06	1.1
VIII–put2	*III–MAT*	0.67 ± 0.06	1.0
Homoeologous chromosomes *V* and *III*			
III–MAT	*V–ura3*	1.70 ± 0.05	6.8
V–ura3	*VIII–put2*	0.81 ± 0.11	5.4
III–MAT	*VIII–put2*	0.69 ± 0.09	2.2

arg4 mutant alleles were present on hemizygous copies of a *URA3-arg4* insert (see Fig. 1) at *MAT* (chromosome *III*), at *URA3* (chromosome *V*), at *PUT2* (chromosome *VIII*), or at *PPX1* (chromosome *VIII*). Strains with homoeologous chromosomes *V* or *III* contain one chromosome each from *S. cerevisiae* and *S. carlsbergensis.* The *arg4* insert was always present on the *S. cerevisiae* chromosome.

**ARG4* spores/total viable spores, mean ± standard deviation.

Icere/IIIcarl (Fig. 1*b*). Chromosomes *Vcarl* and *Vcere* are diverged along their entire length and do not exhibit end-to-end alignment or recombine with each other during meiosis I prophase (refs. 27 and 28; R. Boumil, B. Kemp, and D. Dawson, personal communication). The absence of meiotic recombination between *Vcarl* and *Vcere* was confirmed in our genetic background, using as markers an *ilv1* mutation on *Vcarl* and the unique sequences closest to each end of *Vcere*. Only parental marker configurations were observed among 72 *ILV1* and 59 *ilv1* spore colonies, indicating that crossing over between *Vcere* and *Vcarl* occurs in less than 3% of meioses. Chromosomes *IIIcarl* and *IIIcere* diverge in the region from the left telomere to within 1 kb of the *MAT* locus but are homologous at *MAT* and in the remainder of the chromosome (ref. 28; M.L., unpublished data). Diploids hybrid for chromosomes *IIIcere* and *IIIcarl* do not undergo meiotic recombination in the homoeologous region to the left of *MAT*, whereas recombination is normal in the homologous region to the right of *MAT*. For example, crossovers between *MAT* and *THR4* occur at nearly identical frequencies in hybrid *IIIcere/IIIcarl* and in nonhybrid *IIIcere/IIIcere* strains (28).

In the presence of a homoeologous chromosome *Vcarl*, the frequency of recombination between inserts on chromosomes *V* and *III* increased by about 3-fold relative to nonhybrid controls (Table 1). Ectopic recombination between inserts on chromosomes *V* and *VIII* also increased in *Vcere/Vcarl* hybrids, by about 5-fold. Ectopic recombination between inserts on chromosomes *III* and *VIII* was not affected by the presence of a homoeologous chromosome *Vcarl*. Thus the presence of diverged chromosome *V* homoeolog caused a specific increase in the ability of inserts on chromosome *Vcere* to participate in ectopic recombination, without affecting the global level of ectopic recombination between inserts on unrelated chromosomes.

A further test of the effect of homoeology on ectopic recombination used double-hybrid strains with both *Vcere/Vcarl* and *IIIcere/IIIcarl* homoeologous chromosome pairs (Fig. 1*b*). Ectopic recombination between inserts on chromosomes *V* and *III* was further increased, by about 7-fold relative to nonhybrid control strains and by about 2-fold relative to strains containing a single *Vcere/Vcarl* pair (Table 1). Ectopic recombination between inserts on chromosomes *III* and *VIII* also increased by about 2-fold relative to nonhybrid control strains or *Vcere/Vcarl* strains. In contrast, ectopic recombination between inserts on chromosomes *V* and *VIII* showed no further increase beyond the 5-fold increase conferred by the homoeologous chromosome pair *Vcere/Vcarl*. This confirms the conclusion that a homoeologous chromosome affects only events involving sequences located on its partner.

A further control for global effects measured recombination between the *leu2-K* and *leu2-R* mutant alleles, present in all strains with two copies of chromosome *IIIcere*. *LEU2* recombinant frequencies in nonhybrid control strains ($3.2 \times 10^{-3} \pm 0.6 \times 10^{-3}$) did not differ significantly from those in *Vcere/Vcarl* hybrids ($2.9 \times 10^{-3} \pm 0.6 \times 10^{-3}$).

DSBs in the *URA3-arg4* Insert Are Not Affected by a Homoeologous Chromosome. An alternative explanation for the above results is that recombination initiates more frequently within *arg4* inserts when they are opposite a homoeologous partner. To test this possibility, we measured DSBs within a hemizygous

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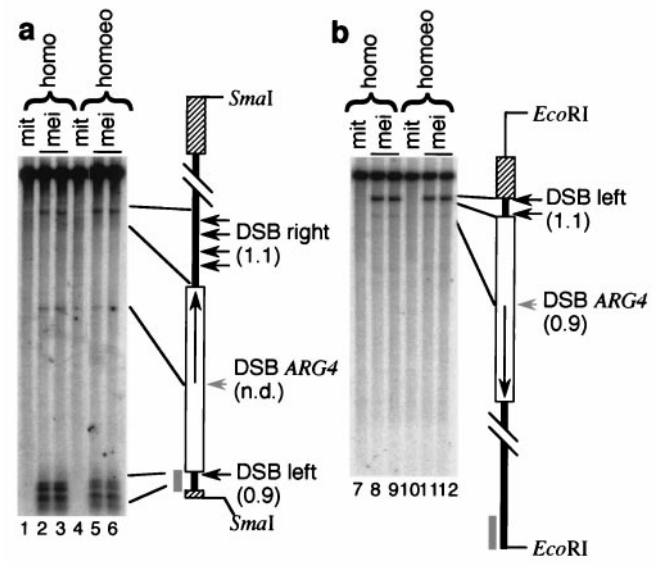

Fig. 2. DSBs at *ura3*::*URA3-arg4* in normal and hybrid diploids. Southern blots contain DNA from diploids with a hemizygous *ura3*::*URA3-arg4* insert and homozygous for the *rad50-K81I* mutation, containing either two *S. cerevisiae* chromosomes *V* (homo) or an *S. cerevisiae* and an *S. carlsbergensis* chromosome *V* (homoeo). DNA samples from premeiotic cells (mit, lanes 1, 4, 7, and 10) and cells 5 and 6 h after induction of meiosis (mei, 5 h, lanes 2, 5, 8, and 11; mei, 6 h, lanes 3, 6, 9, and 12) were restriction enzyme-digested, displayed on 0.5% agarose gels, transferred to membranes, and hybridized with radioactive probe (36). (*a*) Restriction enzyme, *Sma*I; probe, pBR322 *Hin*dIII–*Bam*HI fragment. (*b*) Restriction enzyme, *Eco*RI; probe, pBR322 *Pst*I–*Eco*RI fragment. Horizontal arrows, meiosis-induced DSBs at *ARG4* (DSB *ARG4*) and in pBR322 sequences on either side of *ARG4* sequences (DSB left and DSB right). Numbers in parentheses are ratios of DSB frequencies for each site (6-h samples, DSB-homoeo/DSB-homo; n.d., not determined). In the insert diagrams, thick lines, pBR322; hatched boxes, *URA3*; open boxes, *ARG4*; thin lines, Ty sequences; thick gray lines, probe. A Ty element inserted in *URA3* provides one of the *Eco*RI sites in *B* (T.-C. Wu and M.L., unpublished data).

ura3::*URA3-arg4* insert in *rad50S* derivatives of hybrid *Vcarl/Vcere* and nonhybrid *Vcere/Vcere* strains. Neither the pattern nor the relative frequency of DSBs in the insert was altered by the presence of a homoeologous chromosome *Vcarl* (Fig. 2). Similar results were obtained when the same DNA samples were used to examine DSBs at a control locus, the *YCR47c-ARE1* region on chromosome *III* (data not shown).

Recombination Between *URA-arg4* Inserts in Hybrid Strains Is Associated with Crossing Over. Meiotic recombination between *URA3-arg4* inserts is frequently accompanied by crossing over, in the case of ectopic recombination producing reciprocal translocations (22). We examined the effect of homoeology on the fraction of crossover-associated *ARG4* recombinants in two circumstances: allelic recombination between *MAT*::*URA3-arg4* inserts in *IIIcere/IIIcere* nonhybrids and in *IIIcere/IIIcarl* hybrids, and ectopic recombination between inserts on chromosomes *Vcere* (at *URA3*)and *VIIIcere* (at *PPX1*) in a hybrid diploid containing a *Vcere/Vcarl* homoeologous pair. Crossover-associated *MAT*::*URA3-ARG4* allelic recombinants were recovered at similar frequencies from *IIIcere/IIIcere* and *IIIcere* and *IIIcarl* strains (63/105 and 137/198, respectively). *V–VIII* translocations were recovered in 23% (42/175) of *ARG4* ectopic recombinants between inserts at *URA3* and *PPX1* in a *Vcere/Vcarl* hybrid. Correcting for spore inviability due to unbalanced translocation segregation (22), this result corresponds to 56% of ectopic recombinants being crossover associated, in good agreement with values obtained for ectopic recombination in nonhybrid diploids [42–66% (22)].

Table 2. Effect of *ndj1* on allelic and ectopic meiotic recombination

Insert location	*ndj1*	*NDJ1*†	*ndj1/NDJ1*
Allelic recombination, f(*ARG4*) × 10³*			
LEU2	19	19	1.0
URA3	8.1	5.7	1.4
CHA1	5.0	4.7	1.1
PHO11	3.0	4.8	0.6
PHO12	1.3	1.6	0.8
MAT	7.4	9.4	0.8
Mean			0.9 ± 0.3
Ectopic recombination, E_{ab}‡			
On heterologous chromosomes			
MAT × *URA3*	0.29	0.12	2.4
LEU2 × *URA3*	0.29	0.13	2.2
PHO11 × *PHO12*	0.51	0.24	2.1
PHO11 × *CHA1*	0.59	0.27	2.2
PHO12 × *CHA1*	0.57	0.28	2.0
Mean			2.2 ± 0.1
On homologs			
LEU2 × *CHA1*	0.6	0.56	1.1
LEU2 × *MAT*	0.5	0.48	1.1
MAT × *CHA1*	0.62	0.4	1.6
Mean			1.3 ± 0.3

*Arg^+ spores/total spores from strains with *URA3-arg4* inserts at allelic locations.

†Data from ref. 22.

‡E_{ab}, efficiency of ectopic recombination (22). E_{ab} for a pair of insert loci is the sum of the ectopic recombination frequencies for both marker orientations divided by the sum of the two relevant allelic recombination frequencies, corrected for loss of recombinants on lethal crossover-associated rearrangements. For allelic inserts, $E_{ab} = 1$; $E_{ab} < 1$ indicates that two inserts recombine less frequently than would be expected if they were at allelic positions.

The *ndj1* Mutation Increases the Efficiency of Ectopic Recombination Between Sequences on Heterologous Chromosomes. *ndj1* mutants display a delay in early meiotic homolog colocalization and in homolog synapsis, although allelic meiotic recombination occurs at approximately wild-type frequencies (refs. 31–33; H. Scherthan, personal communication). To examine the impact of these defects on ectopic recombination, we determined frequencies of allelic and ectopic recombination between *arg4* inserts in wild-type and in *ndj1* strains (Table 2). Ectopic recombination was expressed in terms of the efficiency of ectopic recombination, a measurement that corrects for marker- and locus-specific effects by combining ectopic and allelic recombination frequencies into a single term (22).

The efficiency of ectopic recombination between *arg4* inserts on heterologous chromosomes was consistently 2.0- to 2.4-fold greater in *ndj1* mutants compared with wild-type cells. Ectopic recombination between dispersed inserts on homologs was less affected, with no significant increase in recombination between inserts at *LEU2* and *CHA1* (separated by about 70 kb) or *LEU2* and *MAT* (separated by about 105 kb), and a 1.6-fold increase in the efficiency of ectopic recombination between *MAT*::*URA3-arg4* and *cha1*::*URA3-arg4* (separated by about 175 kb). Allelic recombination frequencies were similar in *ndj1* and wild-type strains, with modest locus-specific deviations from equality in both directions.

Calculations of ectopic recombination efficiency include a correction for *ARG4* recombinants lost because of the segregation of crossover-associated reciprocal translocations (22). It was

therefore important to show that the *ndj1* mutation does not alter the fraction of *ARG4* recombinants accompanied by crossing over. This was done for allelic recombination between *MAT*::*URA3-arg4* inserts and for ectopic recombination between inserts at *LEU2* and at *URA3*. About 60% of *MAT*::*URA3-ARG4* allelic recombinants were associated with crossovers (63/105 in *NDJ1*, 66/106 in *ndj1*); 25–30% of viable *ARG4* ectopic recombinants between *leu2*::*URA3-arg4* and *ura3*::*URA3-arg4* were associated with *III–V* reciprocal translocation (26/99 in *NDJ1*, 28/98 in *ndj1*). These data further support the suggestion that the increase in ectopic recombination seen in *ndj1* mutants reflects increased interactions between dispersed homologous sequences, rather than a general effect on recombination initiation or on intermediate resolution.

Discussion

Previous studies have shown that, in *S. cerevisiae*, ectopic recombination between homologous sequences dispersed on heterologous chromosomes occurs less frequently than recombination between sequences at allelic locations on homologs (20–22). In the present study, we measured ectopic recombination between *URA3-arg4* inserts under two circumstances where normal interhomolog interactions are disrupted but where most meiotic products are recovered as viable spores. In the first case, chromosome arm pairing and allelic recombination on chromosome *V* were abolished by replacing a *S. cerevisiae* chromosome with a diverged *S. carlsbergensis* chromosome. This resulted in an increase (from 3- to 6-fold) in ectopic recombination frequencies, but only when at least one of the recombining inserts was on the *S. cerevisiae* partner of the homoeologous chromosome pair. In the second case, a delay in homolog pairing and synapsis caused by loss of *NDJ1* function was accompanied by a 2- to 2.4-fold increase in the efficiency of interheterolog ectopic recombination relative to allelic recombination. Similar increases in ectopic recombination have been reported for *rad17*, *rad24*, *mec1*, and *hop2* mutants, all of which confer defects in homolog synapsis but which also result in substantial meiotic lethality (41–43). Taken together, these results are most consistent with the suggestion that, in normal *S. cerevisiae* meiosis, interhomolog interactions prevent recombination between dispersed homologous sequences and that disruption of these interactions relieves the normal restriction on ectopic recombination.

Why Does the Frequency of Ectopic Recombination Increase in *S. cerevisiae*/*S. carlsbergensis* Hybrids and in *ndj1* Diploids? We believe that our data can be most economically explained by suggesting that meiotic recombination and chromosome pairing proceed along pathways that normally may be temporally linked by the assembly of common structures or by checkpoint mechanisms (1, 15) but have the potential to proceed independently. In normal yeast meiosis, homolog colocalization, alignment, recombination, and synapsis have the effect of reducing chromosome mobility as meiosis progresses. The pairing–synapsis process, which may be facilitated by early steps in allelic recombination, reduces the ability of dispersed sequences to encounter each other, thus creating a barrier to ectopic recombination that is not imposed on allelic events. According to this view, most ectopic recombination should occur during the early stages of homolog pairing, when interhomolog associations are either loose or transient. Delays or defects in pairing and/or synapsis would create a longer time period for dispersed sequences to encounter one another, thus resulting in increased frequencies of ectopic recombination. Sequence divergence between *S. carlsbergensis* and *S. cerevisiae* homoeologs may cause such a pairing defect or delay, either by preventing allelic recombination itself or by interfering with other homology-dependent interactions. Similarly, in *ndj1* mutants, a general delay in onset of homolog pairing would result in a corresponding increase in opportunities for interaction between sequences dispersed on heterologs.

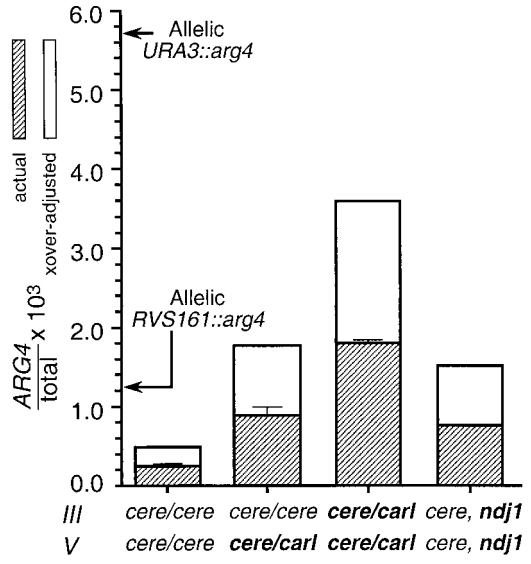

Fig. 3. Summary of homolog/homoeolog effects on *MAT*::*URA3-arg4-nsp* × *ura3*:*URA3-arg4-bgl* recombination. Hatched bars, uncorrected frequencies of *ARG4* spores; open bars, frequencies corrected for loss of recombinants associated with a crossover, assuming that half of all *ARG4* recombinants are crossover associated (22). The *MAT*::*URA3-arg4* and *ura3*::*URA3-arg4* inserts face in opposite directions relative to their respective centromeres; crossover-associated recombinants will reside on lethal rearrangements. For purposes of comparison, frequencies of allelic recombination at two insert loci are presented (40, 53).

While favoring the above interpretation, we recognize that the pairing defects conferred by homoeology or loss of *NDJ1* function are not completely understood, and the relationship of these defects to meiotic recombination has not been fully established. Although the extent of homoeology between *S. cerevisiae* and *S. carlsbergensis* chromosomes is sufficient to block meiotic recombination, the effect of divergence on other interchromosomal transactions remains to be determined. In other organisms, most notably cereal plants, diverged chromosomes can pair early in meiosis I and may even synapse if fully homologous partners are absent (44). A similar situation may hold for *Saccharomyces*, inasmuch as fully synapsed late pachytene nuclei can be detected in *cerevisiae*/*carlsbergensis* and *cerevisiae*/*paradoxus* single chromosome-hybrid strains (A.S.H.G. and R. M. Speed, unpublished data; R. H. Borts, personal communication). It remains possible that abnormal chromosome structures formed in both *cerevisiae*/*carlsbergensis* hybrids and *ndj1* mutants permit more flexibility in recombination partner choice, thus allowing increased ectopic recombination throughout or even late in meiosis I prophase. Temporal analysis of chromosome pairing and synapsis relative to the molecular events of meiotic recombination in hybrid strains and in *ndj1* mutants should distinguish between this and the above-mentioned possibility.

A Genome-Wide Homology Search? The most striking increase in ectopic recombination observed was between *MAT*::*URA3-arg4* and *ura3*::*URA3-arg4* inserts in double-hybrid *IIIcere*/*IIIcarl* and *Vcere*/*Vcarl* diploids. Once adjusted for crossover-associated recombinant lethality, these ectopic events occurred at a frequency (3.4×10^{-3}) roughly two-thirds that seen for allelic events at *URA3*, and more than one-third that seen for allelic events at *MAT*, and well within the range of allelic frequencies seen at other *arg4* inserts (1.2×10^{-3} to 1.9×10^{-2}) (Fig. 3). Chromosome *IIIcarl* is a composite chromosome, with *MAT*

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defining the most centromere-proximal point of homology with *IIIcere*. Interactions in the homologous parts of *IIIcere* and *IIIcarl* may partially restrict the ability of the *MAT*::*URA3-arg4* insert to engage in ectopic recombination and thus may account for the modest increase in ectopic recombination seen for *MAT*::*URA3-arg4* inserts opposite *IIIcarl*, versus the greater increase seen for *URA3*::*URA3-arg4* inserts opposite the fully homoeologous *Vcarl*.

The high level of *MAT*::*URA3-arg4* × *URA3*::*URA3-arg4* ectopic recombination seen in double-hybrid *IIIcere/IIIcarl* and *Vcere/Vcarl* diploids suggests that, if unimpeded, the meiotic recombination homology search can encompass the entire genome, as has been shown for DSB repair during vegetative yeast growth (45). However, it is also possible that centromere-mediated chromosome orientation (46, 47) or region-specific nuclear addressing (48) fortuitously place the insert loci (*MAT*, *URA3*, *PUT2*, and *PPX1*) in the same nuclear subcompartment. Were this true, a homology search encompassing only part of the nucleus could generate our results. We believe this to be unlikely, because inserts at other locations in nonhybrid diploids display interheterolog ectopic recombination efficiencies similar to those seen in the current study (ref. 22; A.S.H.G., unpublished observations). In fact, nuclear addressing and a genome-wide homology search are not mutually exclusive; extensive meiotic nuclear motions seen in many organisms (reviewed in ref. 1) might rearrange the nucleus sufficiently to allow a genome-wide homology search.

Meiotic Pairing and Recombination: Different Strategies in Different Species. It has been suggested that multiple, dispersed, homology-dependent DNA–DNA interactions initiate and advance homolog colocalization and alignment in *S. cerevisiae* (49). This presents the possibility of frequent contact between heterologous chromosomes and may explain the relatively modest (10- to 20-fold) differences between ectopic and allelic recombination frequencies seen in this organism (22). In contrast, *Drosophila*, *C. elegans*, and *Schizosaccharomyces pombe* all appear to use recombination-independent mechanisms to align homologs (12, 13, 50), and ectopic recombination is several orders of magnitude less frequent than allelic recombination (24, 51, 52). To account for these differences, we suggest that the amount of ectopic recombination seen in an organism reflects the timing of recombination initiation relative to homolog colocalization and alignment. In *Saccharomyces*, ectopic interactions can occur unless they are locked out by the multiple allelic interactions involved in homolog pairing. In the other organisms cited above, recombination-independent initiation of homolog association uses specific sites on each chromosome, and recombination is most likely initiated in the context of already-paired homologs.

In summary, our data support the suggestion that ectopic recombination is restricted by the homolog colocalization and coalignment events that occur during *Saccharomyces cerevisiae* meiosis. A variety of homolog pairing strategies may be used in diverse species, possibly in response to differences in genome structure and complexity. However, we expect that homolog colocalization, alignment, and synapsis will perform a similar function in all organisms, namely, to prevent recombination between repeated sequences, thus preventing deleterious rearrangements, and to ensure the faithful transmission of a complete genome to gametes.

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