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A role for centromere pairing in meiotic chromosome segregation

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In meiosis I, exchanges provide a connection between homologous chromosome pairs that facilitates their proper attachment to the meiotic spindle. In many eukaryotes, homologous chromosomes that fail to become linked by exchanges exhibit elevated levels of meiotic errors, but they do not segregate randomly, demonstrating that mechanisms beyond exchange can promote proper meiosis I segregation. The experiments described here demonstrate the existence of a meiotic centromere pairing mechanism in budding yeast. This centromere pairing mediates the meiosis I bipolar spindle attachment of nonexchange chromosome pairs and likely plays the same role for all homologous chromosome pairs.

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Exchanges (crossovers), which occur between homologous chromosomes in meiosis I, are critical for the production of viable gametes because they link the homologs together (Bascom-Slack et al. 1997). This linkage allows the homologs to remain joined during the process of microtubule attachment and enables the homologous pair to achieve a bipolar spindle attachment (the centromeres of the homologs attached to microtubules from opposite spindle poles), analogous to sister chromatids in mitotic cells. The importance of recombination in ensuring high-fidelity chromosome segregation might suggest that a pair of nonexchange chromosomes, in an otherwise normal meiosis I, would segregate randomly. However, this is not always the case (Wolf 1994). In several organisms, it has been shown that a single nonexchange pair of chromosomes will be segregated properly in most meioses. Most notably, female *Drosophila melanogaster* use the pairing of centric heterochromatin to mediate the segregation of the nonexchange fourth chromosome pair (Dernburg et al. 1996; Karpen et al. 1996). In the budding yeast *Saccharomyces cerevisiae*, a single chromosome pair without an exchange segregates properly in ~90% of meioses (Dawson et al. 1986; Mann and Davis 1986; Guacci and Kaback 1991; Ross et al. 1996). These examples of nonexchange segregation demonstrate that there are mechanisms, other than exchange, that contribute to the ability of a chromosome pair to assume a bipolar spindle attachment in meiosis I. The nature of these mechanisms remains largely mysterious. In yeast, nonexchange segregation is not based on DNA sequence homology; numerous studies have shown that nonexchange chromosome pairs that are nonhomologous, even at their centromeres, segregate with the same fidelity (90%) as nonexchange pairs that are perfectly homologous (Ross et al. 1996).

The experiments described here explore the mechanisms, beyond exchange, that contribute to meiotic segregation fidelity in *S. cerevisiae*. For these experiments we have constructed a novel yeast strain that has one obligate nonexchange chromosome pair. This strain was created by replacing one copy of *S. cerevisiae* Chromosome *V* with Chromosome *V* from *Saccharomyces carlsbergensis*, which provides full function in haploid *S. cerevisiae*. These "homeologous" chromosomes are ~30% divergent at the DNA level and virtually never experience meiotic recombination, yet like other yeast nonexchange meiotic chromosome pairs, they segregate from each other in ~90% of meioses (Boumil et al. 2003).

Results and Discussion

Synaptonemal complex does not mediate nonexchange segregation

During meiotic prophase, homologous chromosomes are typically linked by both exchanges and synaptonemal complexes (SC). In most eukaryotes, the SC disassembles at diplotene, leaving the chromosomes joined by chiasmata (the cytological manifestation of exchanges). In yeast, diplotene is brief (Padmore et al. 1991), allowing for the possibility that an association of the nonexchange chromosomes, mediated by SC, might persist through diplotene, and orient the centromeres toward opposite poles of the meiotic spindle. This model would be consistent with the demonstration that SC can form between nonhomologous chromosomes (Loidl et al. 1991) and the observation of unusual SC-like structures in cells carrying nonexchange partners (Loidl et al. 1994). We tested this notion by deleting *ZIP1*, the gene that encodes the central element of the SC (Sym et al. 1993), then scoring the meiotic segregation of the homeologous chromosomes by tetrad analysis. The nondisjunction (both chromosomes moving to the same pole at meiosis I) frequency of the homeologous pair in the *zip1* mutant was not distinguishably different from that exhibited by the wild-type control (5% vs. 7% nondisjunction; $n = 68$). The segregation of the nonexchange chromosomes was not randomized by the absence of SC. Therefore, SC is not critical for dictating nonexchange segregation in budding yeast.

Nonexchange chromosomes experience meiotic centromere pairing

Models to explain the segregation of nonexchange chromosomes can be divided into those that invoke pairing of the chromosomes versus those that do not (such as counting models in which the cell directs equal numbers of chromosomes to each pole at anaphase). To distinguish between these possibilities, we localized GFP to the homeologs (Straight et al. 1996) and used

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fluorescence microscopy to monitor their interactions in meiotic prophase. The meiotic behavior of the homeologous chromosomes was compared with the interactions of GFP-tagged homologous Chromosome *V*s, and to GFP-tagged heterologous chromosomes (*V* and *III*).

Meiotic cells were harvested at a time corresponding to pachytene, the stage of maximal homolog pairing, and pairing of the GFP-tagged loci was assayed on chromosome spreads using indirect immunofluorescence. To monitor pairing of the homeologous chromosome arms, GFP was localized to the *ILV1* locus, on the right arm of Chromosome *V*, 180 kb from the centromere (Fig. 1a). Tagged loci were scored as "paired" if the centers of the dots were within 0.7 µm (Fig. 1b) and unpaired if the dots were farther apart (Fig. 1c).

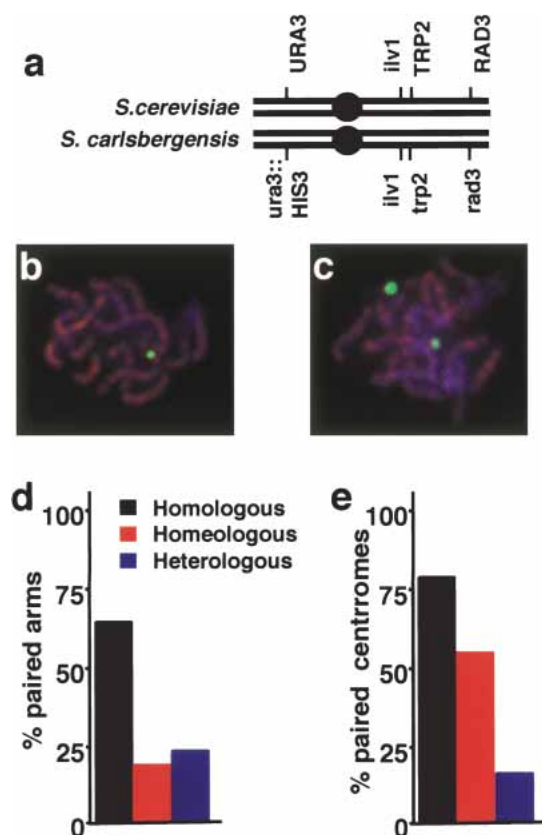

Figure 1. Analysis of pairing of meiotic nonexchange chromosomes in yeast. (*a*) Maps of the homeologous Chromosome *V* pair. (*b*,*c*) Chromosome spreads from synchronous meiotic culture. (*b*) An example of a spread with paired GFP dots. (*c*) An example of unpaired dots. (*d*) Analysis of chromosome arm pairing. Three diploids were evaluated. Homologous arm pairing was evaluated with a strain bearing a homologous *S. cerevisiae* Chromosome *V* pair tagged at the *ILV1* locus (black bars; DBK215). Heterologous pairing was evaluated with a strain in which one copy of Chromosome *V* (*ILV1*) and one copy of Chromosome *III* (*LEU2*) was tagged (blue bars; DBK217). Homeologous arm pairing was evaluated in a strain with homeologous *V*s tagged at *ILV1* (red bars; DBK216). (*e*) Analysis of chromosome centromere pairing. Homologous centromere pairing was evaluated with a strain bearing a homologous *S. cerevisiae* Chromosome *V* pair tagged 12 kb from *CEN5* (black bars; DBK205). Heterologous pairing was evaluated with a strain in which one copy of Chromosome *V* (12 kb from *CEN5*) and one copy of Chromosome *IV* (12 kb from *CEN4*) was tagged (blue bars; DBK201). Homeologous centromere pairing was evaluated in a strain with both homeologous *V*s tagged 12 kb from *CEN5* (red bars; DBK203).

Homologous Chromosome *V* arms were scored as paired in >63% of the meiotic chromosome spreads examined (Fig. 1d, black bars). Homologous arms synapse in virtually 100% of meioses; thus, this assay underestimates the true level of pairing. The failure to observe 100% pairing could be due to disruption of pairing by the spreading technique or may be attributable to the fact that synapsis is not synchronous and some of the spreads we examined might not have yet synapsed the Chromosome *V* pair. In contrast, both the heterologous arms and the homeologous arms were separated in >80% of nuclei (Fig. 1d). These data suggest that whereas the arms of homologous chromosomes are aligned in pachytene, the arms of the homeologous chromosomes are no more likely to be paired than the arms of two heterologous chromosomes (each with its own homologous partner). In both the heterologous and homeologous strains we observed a fraction (~20%) of nuclei with only one visible dot. It is likely this does not represent true pairing but instead may be caused by juxtaposition of dots by random chance, failure to detect one of the dots for technical reasons, or low levels of association of the GFP-lacI clusters (Aragon-Alcaide and Strunnikov 2000).

Very different results were obtained when GFP was localized to a position 12 kb from the centromere of Chromosome *V*. Both homologous and homeologous centromeres were paired in pachytene spreads (77% and 54%, respectively; Fig. 1e). Therefore, unlike the arms, the centromere regions of the homeologous chromosomes are paired in pachytene. In contrast, the centromeres of heterologous chromosomes were nearly always separated (>80%; Fig. 1e).

To test whether the pachytene centromere pairing of the nonexchange chromosomes is the basis of their disjunction, we took advantage of a previously described aspect of nonexchange segregation. Genetic experiments have shown that the segregation fidelity of a nonexchange pair is disrupted when a third nonexchange chromosome or centromere plasmid is introduced into the strain (Dawson et al. 1986; Guacci and Kaback 1991; Ross et al. 1996). The three nonexchange chromosomes typically act as nearly partners. That is, the segregation behavior of three nonexchange chromosomes is consistent with the model that, in each meiosis, any two of the three chromosomes can be chosen as partners and moved to opposite poles, while the third chromosome segregates randomly. This behavior predicts three kinds of meiosis I segregation patterns that should occur with nearly equal frequency if the chromosomes are all equally likely to pair, and this is what is observed (Fig. 2a). The homeologous Chromosome *V*s have a slight preference for each other as partners, probably because of their extensive sequence homology (Boumil et al. 2003), and typically exhibit ~25% nondisjunction (Fig. 2a, Type C) in the presence of a third nonexchange chromosome or centromere plasmid. If centromere pairing is the basis of the nonexchange segregation, then introduction of a third nonexchange chromosome should lower pachytene centromere pairing of the homeologous chromosomes, presumably by pairing with one of the two homeologs, and consequently raising nondisjunction of the homeologs. This is what we observed when a centromere plasmid (*CEN6*) was added to a strain bearing the homeologous pair. The plasmid acted as a competitor and increased nondisjunction of the homeologs from 7% to 25%, consistent with previous studies (Boumil et al.

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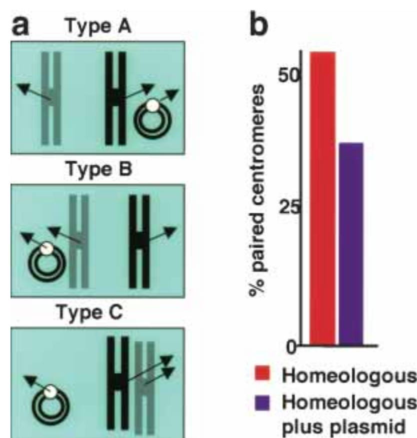

Figure 2. Disruption of centromere pairing with a competing nonhomologous centromere. (*a*) The three observed meiosis I segregation outcomes in cells with three nonexchange chromosomes; in this case two homeologous Chromosome *V*s and a *CEN6* plasmid (pRK21). In each outcome, the single chromosome going to one pole has presumably paired with one of the two chromosomes that have gone to the opposite pole, while the third has segregated randomly. (*b*) Pairing of GFP-tagged homeologous centromeres in strain DBK203 with or without the competitor plasmid. The evaluation of centromere pairing was as described in Figure 1. (Red bar) Without competitor; (purple bar) with competitor.

2003). Cytological analysis of the pairing of the GFP-tagged homeologous centromeres in this strain showed that the competing centromere plasmid decreased homeologous centromere pairing 17% (from 54% to 37%; Fig. 2b), which mirrors the 18% increase in nondisjunction caused by the competitor plasmid. This is the result that would be predicted if the centromere plasmid is able to participate as a pairing partner with the homeologous chromosomes and nonexchange segregation is driven by this centromere pairing. An alternate explanation (addressed below) is that the extra centromere plasmid indirectly disrupts meiotic behavior of the homeologous chromosome pair.

Meiotic centromere pairing is DNA-sequence-independent

We tested whether a centromere plasmid disrupts the segregation of the homeologous chromosomes directly, by pairing with them, by assaying pairing interactions between the homeologous chromosomes and a competing yeast artificial chromosome (YAC) bearing *CEN3*. The methods used were similar to those used for studying pairing of the homeologous *V*s except that the YAC was visualized using a myc-epitope tag, and one of the homeologous *V* pair (the *S. cerevisiae V*) was tagged with GFP. The 65-kb YAC (YLp61; Ross et al. 1992) is composed mostly of bacteriophage λ DNA and carries a 5-kb interval of Chromosome *III* that includes *CEN3*. The YAC was myc-tagged by introducing a *tet* operator array 3 kb from the *CEN3*. We then introduced into these cells a construct that would express a TetR-13xmyc hybrid protein that would localize to the tetracycline operator arrays and allow detection of the YAC with antibodies against the myc-epitope. The *S. cerevisiae* Chromosome *V* was GFP-tagged at *URA3* (~35 kb from *CEN5*). Cells bearing the homeologous Chromosome *V* pair and the YAC were induced to undergo meiosis, chromosome spreads were prepared, and the positions of the YAC and Chromosome *V* "dots" were determined using indirect immunofluorescence microscopy. The relative positions of the myc (YAC) and GFP (Chromosome *V*) dots were measured in chromosome spreads of cells that were determined to be in late prophase by DAPI staining (the condensed chromosomes give a wormy appearance; Supplementary Fig. 1). Tetrad analysis revealed that the YAC elevated the nondisjunction of the homeologs to 24%. If all of these nondisjunctions occur when the centromere of one of the homeologous Chromosome *V*s pairs with the centromere of the YAC, then this predicts the YAC pairs with each homeolog in ~24% of meioses (Fig. 3a). In these cases of pairing between the YAC and one of the Chromosome *V*s, there is a 50% chance that the unpaired homeolog will segregate to the same pole as its homeolog, yielding a nondisjunction. When the pairing of the YAC with the GFP-tagged Chromosome *V* was monitored in meiotic chromosome spreads, we observed that they were paired in ~19% of the spreads (Fig. 3b). This value is slightly below the predicted 24%, but note that with homologous centromeres that are synapsed in virtually 100% of meioses (Fig. 1e), we only detect 77% of the pairing with this method. As a control, the pairing of the YAC with the GFP-tagged Chromosome *V* when it had a homologous (exchange) partner was assayed using the same methods. In this experiment we observed pairing of the GFP and myc signals in only 6% of meioses (Fig. 3b), a value significantly less than the 19% pairing observed when the Chromosome *V* did not have an exchange partner ($p < 0.001$). These results demonstrate first, that nonhomologous centromeres can be paired in meiosis I, and second, that as in the experiments with homologous and homeologous tagged centromeres, there is a correlation between the level of centromere pairing of the centromere plasmid and Chromosome *V* and their level of disjunction, the result expected if centromere pairing leads to disjunction.

If centromeres are able to pair in a DNA-sequence-independent fashion, then what prevents random pairing

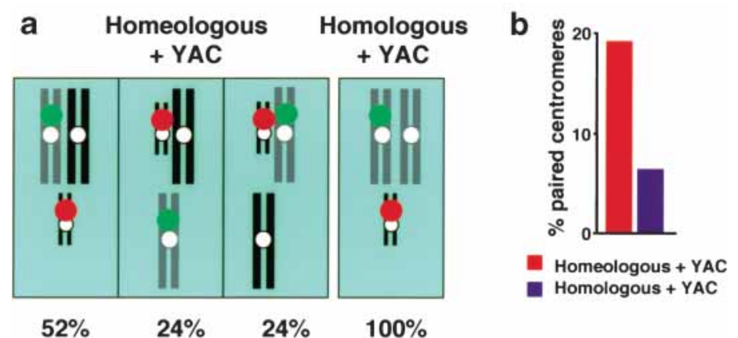

Figure 3. Analysis of pairing of a YAC with an *S. cerevisiae* Chromosome *V*. Experiments were performed with isogenic strains carrying a GFP-tagged Chromosome *V* and a myc-tagged YAC. (*a*) The predicted meiosis I pairing outcomes in cells with three nonexchange chromosomes, either two homeologous Chromosome *V*s and a YAC (*left* panel), or two homologous Chromosome *V*s and the YAC (*right* panel). (*b*) The level of pairing that was observed in meiotic chromosome spreads between a myc-tagged YAC (3 kb from *CEN3*) and a *S. cerevisiae* Chromosome *V*, GFP-tagged at the *URA3* locus 35 kb from *CEN5*, in the presence of a homeologous Chromosome *V* (red bar; DMS173), or a homologous Chromosome *V* (purple bar; DMS174; $n = 101$ for each strain).

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of the centromeres? The ability of an exchange partner to block the pairing of the YAC and the GFP-tagged Chromosome *V* suggests that it is exchange, and the synapsis that follows exchange, that juxtaposes homologous centromeres and blocks random centromere pairing.

Paired centromeres separate precociously at metaphase I

Chromosome pairs that have achieved a bipolar spindle attachment remain linked by crossovers until the signal to proceed into anaphase, at which time dissolution of sister-chromatid cohesion, distal to the crossover, permits the chromosomes to separate (for review, see Cohen-Fix 2000). To test whether the centromere association of the nonexchange chromosomes is sufficient to maintain the linkage of homeologs until anaphase, we examined the positioning of the homeologous chromosomes on the metaphase spindle. The similar morphologies of metaphase and early anaphase spindles in meiosis I of *S. cerevisiae* make them difficult to distinguish. To circumvent this problem, cells were delayed in metaphase I by expressing a nondegradable form of Pds1p (*PDS1-Δdb*) under the control of a meiotic promoter (P_{IME2}; Shonn et al. 2000). In these cells, we monitored positioning of the centromere-associated GFP-tags on both the homologous and homeologous Chromosome *V* pairs.

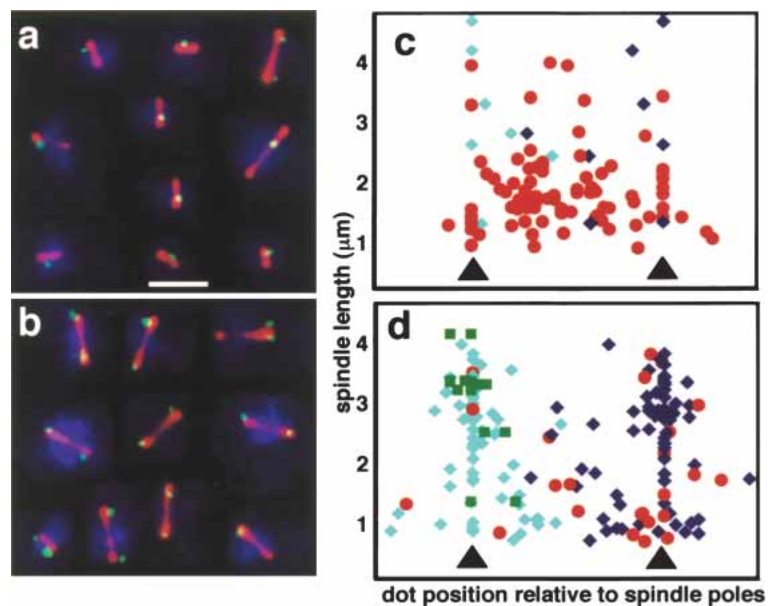

Figure 4. Metaphase behavior of nonexchange chromosomes. Indirect immunofluorescence was used to visualize spindles and GFP-tagged centromere regions in fixed, metaphase-arrested cells bearing either homologous (*a*; DBK270), or homeologous (*b*; DBK271) Chromosome *V*s. A collage of representative cells is shown for each strain (white bar, 5 µm). (*c*,*d*) Scatterplots showing the positions of the tagged centromeres relative to the spindle poles for cells with homologous (*c*) or homeologous (*d*) Chromosome *V*s (n = 100 for each strain) as a function of spindle length (*Y*-axis). The *X*-axis represents the metaphase spindle, with the black triangles indicating the positions of the poles. The spindle positions of paired centromeres (one GFP dot) are represented by orange dots (one dot for each cell measured). The positions of separated centromeres are represented by pairs of blue diamonds (one light and one dark). The homeologous centromeres were sometimes observed to be separated but at the same end of the spindle (within 10% of the total spindle length from one pole). These centromeres are represented by pairs of green squares in *d*.

On the meiosis I metaphase spindles, 91% of the homologous centromere *V*s appeared as a single (paired) dot (Fig. 4a). The homologous centromeres are likely under tension and may even be pulled toward the poles as is seen in mitotic cells (Goshima and Yanagida 2000; He et al. 2000), but because the GFP tag is ~12 kb from the centromere, this stretching might not be detected by our assay. Conversely, only 24% of the homeologous GFP-tagged centromeres were paired. Instead, most (76%) had separated to give two dots (Fig. 4b). The positions of the centromeres on the metaphase spindles were measured and plotted as a function of spindle length (Fig. 4c,d). The paired homologous centromeres were usually positioned between the spindle poles (Fig. 4c; each orange circle represents the position of the paired centromeres in one cell). Instances in which the GFP dots were separated were limited to long spindles (Fig. 4c; each separated pair of GFP dots is represented by a pair of light- and dark-blue diamonds). In these cases, the separated dots were usually located on opposite ends of the spindle. Because the cells eventually escape the Pds1-Δdb-induced metaphase delay after several hours (data not shown), the spindles with separated homologous centromeres (Fig. 4c, blue diamonds) may represent those that have proceeded into anaphase I.

In contrast, the homeologous centromeres were separated and positioned very close to the spindle poles in most metaphase cells (Fig. 4d, blue diamonds), demonstrating that the chromosomes had not simply drifted apart, but instead were actively disjoined by spindle-mediated forces. The fact that the nonexchange chromosomes are separated on even the shortest spindles suggests that they are separated, in most cases to opposite poles, immediately upon attachment to microtubules. In 6% (6 of 100) of the cells, both homeologous centromeres were at one spindle pole (Fig. 4d, pairs of black squares; see upper right spindle in Fig. 4b as an example). This was never seen for the homologous centromeres and likely corresponds to the 7% meiosis I nondisjunction exhibited by these nonexchange chromosomes.

Centromere pairing and nonexchange segregation

The above experiments suggest the basis of nonexchange segregation in *S. cerevisiae*. We propose that centromere regions in yeast are able to undergo meiotic pairing that is sequence-independent, and that this pairing orients the kinetochores of the nonexchange partners such that they are likely to encounter microtubules that radiate from opposite spindle poles. The fact that the pairing is able to orient the kinetochores, coupled with the fact that even core centromere regions of 125 bp support nonexchange segregation, suggests that the centromere pairing occurs at, or very close to, the actual kinetochores. These results suggest a model for nonexchange segregation (Fig. 5). Cells enter meiosis with decondensed chromatin and their centromeres clustered (Hayashi et al. 1998). In prophase, condensation begins and homologous recombination triggers the synapsis of homologous partners, which pulls their centromeres away from the centromere cluster (Fig. 5, center; Hayashi et al.

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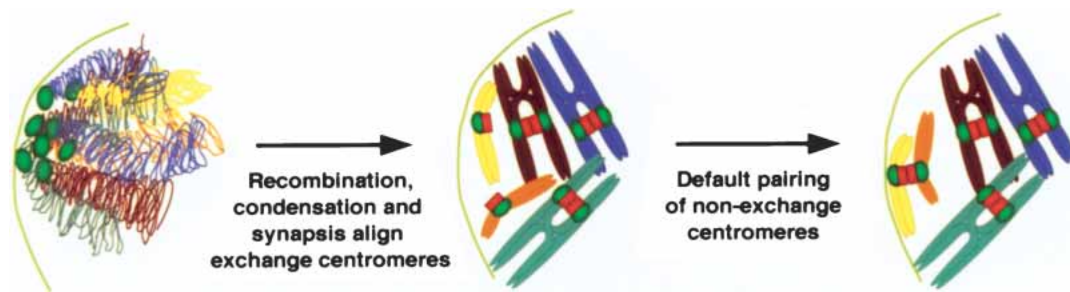

Figure 5. Model for centromere pairing in meiosis I. Cells enter meiosis I with decondensed chromosomes and clustered centromeres (green ovals; Hayashi et al. 1998). Recombination between homologs, followed by condensation and synapsis, pulls homologous centromeres into juxtaposition, mediating their pairing. Nonexchange centromeres pair by default, as all other potential pairing partners have been removed by synapsis. In wild-type cells, it is unlikely that more than one chromosome pair will fail to recombine. Thus, in these cells the default centromere pairing will mediate the segregation of homologous chromosomes even though the process is sequence-independent. As a consequence of centromere pairing, the microtubule attachment sites for each bivalent face in opposite directions.

1998; R. Shanks and D.S. Dawson, unpubl.), leaving the centromeres of the nonexchange chromosomes (Fig. 5, yellow and orange chromosomes) as default partners, rather like attractive students at a school dance pairing off, leaving the two least popular students as partners.

The yeast nonexchange segregation mechanism bears both similarity to and differences from the nonexchange process described in *Drosophila* females (Dernburg et al. 1996; Karpen et al. 1996). In *Drosophila*, pairing of centric heterochromatin appears to mediate the segregation of homologous nonexchange chromosomes. Unlike the yeast system, this pairing is homology-dependent. It is yet unclear how the pairing of nonexchange chromosomes ensures their disjunction in *Drosophila*; however, the pairing is not of kinetochores, per se, but of adjacent chromosomal regions. It has been proposed that the pairing may play a role similar to chiasmata, by providing a link that opposes the pull of microtubules (Dernburg et al. 1996).

The meiotic role of centromere pairing

A mechanism for partitioning nonexchange chromosomes would not be needed in most budding yeast meioses, as nonexchange chromosomes are probably rare (Kaback et al. 1992). Why, then, does the mechanism exist? Although homologous centromeres are clearly juxtaposed when homologs experience end-to-end meiotic alignment in pachytene, there has been little evidence in yeast to suggest that the centromeres undergo active pairing, rather than simply being positioned next to one another by the process of synapsis. We suggest that the centromere pairing seen here with the homeologous pair also occurs between the centromere regions of homologous chromosomes (Fig. 5, red rectangles).

What might be the role of meiotic centromere pairing? Early studies of meiotic chromosome dynamics led Östergren (1951) to suggest that pairing of the centromeres of homologous chromosomes might outwardly orient the kinetochores, optimizing the chances for bipolar spindle attachment (as shown in Fig. 5). Our results suggest that, in yeast, the proper segregation of nonexchange chromosomes occurs by this type of process. This mechanism would increase the probability of an initial bipolar spindle attachment of all chromosome pairs, not just nonexchange chromosomes. Genetic evidence for such a model has been provided by the observation that the segregation fidelity of exchange chromosome fragments is improved if their centromeres are in register upon homologous alignment (Guerra and Kaback 1999). Chromosome pairs that have experienced exchanges can experience tension at their kinetochores when they attach to microtubules from opposite poles. Pairs with inappropriate attachments fail to experience this tension, which leads to a checkpoint-mediated signal that leads to a delay (Li and Nicklas 1995), during which chromosomes can reorient on the spindle (Nicklas and Koch 1969). In budding yeast, exchange chromosomes can benefit from this process (Shonn et al. 2000), but nonexchange chromosomes probably cannot. Thus, meiotic centromere pairing can be thought of as an alternative to the tension-mediated mechanism for attaining a bipolar spindle attachment. This mechanism is clearly critical for the segregation of nonexchange chromosomes, but may contribute to the segregation of exchange chromosomes as well.

In humans, such a mechanism could have special significance, as many birth defects are the result of errant segregation of nonexchange chromosomes (Hassold and Hunt 2001). In humans, as in yeast, these nonexchange chromosomes presumably cannot benefit from the tension-based mechanisms that ensure proper spindle attachments for exchange chromosomes. Instead, the segregation of these error-prone chromosomes must either be left to chance, or dependent on alternative processes like the centromere pairing described here in yeast.

Materials and methods

Genetic manipulation of yeast

Alteration of the genome by transformation with DNA was performed using published methods (Burke et al. 2000). Tetrad analysis was used to monitor the effect of a competitor plasmid on segregation of the homeologous chromosome pair using previously described methods (Boumil et al. 2003).

Tetrad analysis was performed using published methods (Burke et al. 2000). All strains were derived from S288C progenitors. The genotypes are in Supplementary Table 1.

Meiotic chromosome pairing assays

Clusters of the *lac* operon operator sequence or the *tet* operon operator sequence were targeted to specific chromosomal loci using established methods (Straight et al. 1996; Michaelis et al. 1997). Diploids contained a P_{CYC1}-*GFP-lacI* fusion gene, and where described in the text, a P_{URA3}-*tetR-13xmyc* gene for detecting *lac* operator and *tet* operator arrays, respectively. Meiotic chromosome spreads were prepared using previously

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described methods (Kamieniecki et al. 2000). Chromosome pairing was evaluated in spreads with multiple worm-like structures visualized with either, antibodies directed against the Zip1 synaptonemal complex protein, or by DAPI staining. GFP-tagged or myc-tagged chromosomes were visualized using indirect fluorescence microscopy. GFP was detected with either rabbit or chicken anti-GFP (Chemicon) antibody, and the myc-epitope was detected using mouse 9E10 monoclonal antibody. Secondary antibodies were FITC-conjugated donkey anti-rabbit (Jackson), Alexa 488 goat anti-chicken, and Alexa goat anti-mouse (Molecular Probes) antibodies. Dots with centers that were within 0.7 µm were scored as paired. Images were collected and processed using a Hamamatsu CCD and OpenLabs 3.0 software.

Analysis of centromere position in metaphase

A *PDS1* open reading frame with a deletion of the coding region for the destruction box (Shonn et al. 2000) was placed under the control of the meiotic *IME2* promoter. This construct was integrated into the genome, leaving both native copies of *PDS1* intact. Cells bearing the P_{IME2}-*PDS1-Δdb* construct showed no vegetative growth defects. Cells were harvested from synchronous meiotic cultures 24 h after induction of meiosis. Cells were fixed with formaldehyde, and indirect immunofluorescence was used to visualize tubulin and GFP using previously described methods (Kamieniecki et al. 2000).

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