Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 331–334, January 1994
Genetics

Physical association between nonhomologous chromosomes precedes distributive disjunction in yeast

(meiosis/chromosome pairing/segregation/*Saccharomyces cerevisiae*)

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Communicated by Allan C. Spradling, October 6, 1993 (received for review May 24, 1993)

ABSTRACT **During meiosis homologous chromosomes normally pair, undergo reciprocal recombination, and then segregate from each other. Distributive disjunction is the meiotic segregation that is observed in the absence of homologous recombination and can occur for both nonrecombinant homologous chromosomes and completely nonhomologous chromosomes. While the mechanism of distributive disjunction is not known, several models have been presented that either involve or are completely independent of interactions between the segregating chromosomes. In this report, we demonstrate that distributive disjunction in *Saccharomyces cerevisiae* is preceded by an interaction between nonhomologous chromosomes.**

Meiosis is the specialized form of nuclear division that reduces the chromosome number from the diploid to the haploid state. During meiosis I, homologous chromosomes normally associate, form synaptonemal complexes (SCs), undergo reciprocal recombination, and disjoin from each other. In *Drosophila melanogaster* females, *Saccharomyces cerevisiae*, and several other eukaryotes, two homologous chromosomes that have not recombined or two univalent nonhomologous chromosomes also segregate from each other at the first meiotic division (1–9). This behavior is believed to ensure the proper meiotic segregation of both the smallest *Drosophila* chromosome and the occasional nonrecombinant chromosome in other eukaryotes (2, 3). Based on the assumption that the nonrecombinant chromosomes had to contact each other in some manner, this phenomenon was first called distributive pairing (1). However, no evidence for the precise type of pairing that had been proposed has ever been presented. Accordingly, this behavior has been more properly described as distributive disjunction, distributive segregation, or achiasmate segregation (10–14). The mechanism of distributive or achiasmate segregation is not understood and it is unclear whether the nonrecombinant segregational partners physically interact with each other prior to disjunction. Several models have been suggested that do not require segregational partners to physically interact (9, 10). In this report, we demonstrate that distributive disjunction in the yeast *S. cerevisiae* is associated with a physical interaction between nonhomologous chromosomes. Strains monosomic for both chromosomes I and III and diploid for all other chromosomes undergo distributive disjunction of the two univalent chromosomes ≈90% of the time (6). Silver staining and fluorescence *in situ* hybridization (FISH) with chromosome-specific probes (15, 16) were used to show that the two nonhomologous chromosomes indeed paired with each other during meiosis I and formed an unusual structure.

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MATERIALS AND METHODS

Preparation of Yeast Spheroplasts and Silver-Stained Nuclei. VG72-DM was derived from and is isogenic to VG72 (6). Genotypes were confirmed by tetrad dissection and electrophoretic karyotyping as described (6). Diploids SK1 (17) and VG72 and the chromosome I–III double monosome VG72-DM were grown and sporulated as described (15, 18). Cell suspensions can be stored overnight at 0°C prior to shifting to sporulation medium. This storage allows greater convenience and caused no appreciable loss of synchrony or delay of sporulation. SK1 cells were harvested from sporulation medium at 4.5 hr, while VG72 and VG72-DM were harvested at 7.5–9 hr depending on the experiment. Spheroplasts prepared using Zymolyase 20T (0.5%, wt/vol), were spread, stained with $AgNO_3$, and transferred to electron microscope grids as described (15, 19). Only those nuclei showing mature well-spread SCs were examined. In all nuclei, chromosome XII appears divided because it passes through the nucleolus (16).

FISH. Nuclei from sporulating cells were prepared as described above but were left unstained on glass slides and were not transferred to electron microscope grids. Probes were labeled as described (16). The chromosome III probe was labeled with biotin-14-dATP (BRL) and was made up of an approximately equimolar mixture of plasmid YIp5 subclones that included the following *Bam*HI fragments: E5F, J10A, G2F, M5G, C1G, C2G, D12B, J11D, K3B, and I2B (20). These fragments contained ≈130 kb from this 315-kb chromosome and included the centromere and sequences from both arms. The chromosome I probe was labeled with digoxigenin-11-dUTP (Boehringer) and was composed of an approximately equimolar mixture of bacteriophage λ clones including λD39c, λK3c, λG4a, and λF58f (21). These clones contained ≈60 kb from this 230-kb chromosome and included the centromere and sequences from both arms. The chromosome V probe was labeled with a mixture of both digoxigenin-11-dUTP and biotin-14-dATP and contained ≈180 kb from this 580-kb chromosome. This probe contained sequences from both arms and its composition has been described (16). FISH was carried out as described and the hybrids were decorated with avidin–fluorescein isothiocyanate (FITC) (Sigma) and tetramethylrhodamine B isothiocyanate (TRITC)-labeled secondary and tertiary antibodies (Sigma) to a mouse anti-digoxigenin monoclonal antibody, respectively. Preparations were embedded in antifade solution (Vector Laboratories) containing 4′,6-diamidino-2-phenylindole (DAPI) (0.2 μg/ml) as a counterstain. Preparations were examined in a Zeiss Axioplan microscope equipped with filters for excitation of DAPI, FITC, and TRITC fluorescence. Enhanced

Abbreviations: FISH, fluorescence *in situ* hybridization; SC, synaptonemal complex; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine B isothiocyanate; DAPI, 4′,6-diamidino-2-phenylindole.

high-contrast images were obtained from a cooled CCD camera (Photometrics, Tucson, AZ) attached to the microscope and image analysis software (NRU200, NIH-image, Gene Join) run on a Macintosh Quadra computer.

RESULTS AND DISCUSSION

To show that nonrecombinant chromosomes that are known to disjoin distributively physically interact during meiosis I, a strain of *S. cerevisiae* that was doubly monosomic for chromosomes I and III (VG72-DM; ref. 6) and two diploid control strains (VG72 and SK1; refs. 6 and 17) were sporulated to induce meiosis. At appropriate times, cells were harvested and treated to display the meiotic pachytene nuclei by silver staining and electron microscopy (15). Nuclei from both control diploids produced pachytene arrays of up to 16 perfectly paired homologous chromosomes present in SCs corresponding to the 16 pairs of homologous yeast chromosomes (Fig. 1*A*). In contrast, nuclei from the double monosome contained no more than 14 SCs. In addition, these nuclei contained either an unusual structure that appeared to be due to the association of two small chromosomes (Fig. 1*B*) or two small unpaired chromosomes (Fig. 1*C*), I and III being the smallest and third smallest yeast chromosomes, respectively (22). The structures appeared to be composed of silver-stainable axial elements and few if any tripartite structures characteristic of genuine SCs. The structures were present in 67% of the nuclei while the two unpaired chromosomes were found in 33% of the nuclei. Occasional failure to observe 16 distinct SCs in the diploids and 14 distinct SCs in the double monosome was attributed to the fact that these structures often line up end to end, making resolution of an individual SC for each chromosome impossible (15). Irrespective of these difficulties, the presence of the unusual structure in only the double monosome suggests it is a complex composed of both chromosomes I and III.

To prove that chromosomes I and III were interacting with each other in the double monosome, we used FISH (16) with probes derived from chromosomes I, III, and V. Meiotic nuclei from the double monosome VG72-DM and the two diploid controls VG72 and SK1 were prepared as described above and incubated with a mixture of the differentially labeled probes and the resulting hybrids observed by differential fluorescence microscopy (Fig. 2). In this experiment, paired and unpaired homologous chromosomes can be discriminated based on the presence of single or double signals for each chromosome, respectively (16). Therefore, nuclei were first assigned to the pachytene stage by observing a single signal for the chromosome V-specific probe, indicative of paired bivalents. In the diploid controls, chromosomes I and III behaved identically to chromosome V, each giving single signals that were spatially distinct from each other and from the chromosome V signal (Fig. 2*A*). Such behavior is indicative of proper homologous pairing of all chromosomes (16). In contrast, the double monosome produced two classes of nuclei when chromosome V was paired. In the first class, representing 69% of the nuclei (Fig. 2*B*), the signals produced by chromosomes I and III were spatially coincident, indicative of a physical interaction. The remaining 31% had spatially distinct signals for chromosomes I and III, indicating these chromosomes were not paired (Fig. 2*C*). The combined results of electron microscopic and FISH studies demonstrate that chromosomes I and III undergo a physical interaction in the majority of the pachytene nuclei from the double monosomic strain where these chromosomes undergo distributive disjunction.

Distributive disjunction of chromosomes I and III was genetically observed in 89% of the cells in VG72-DM (6). Totaling the results in both cytological experiments, chro-

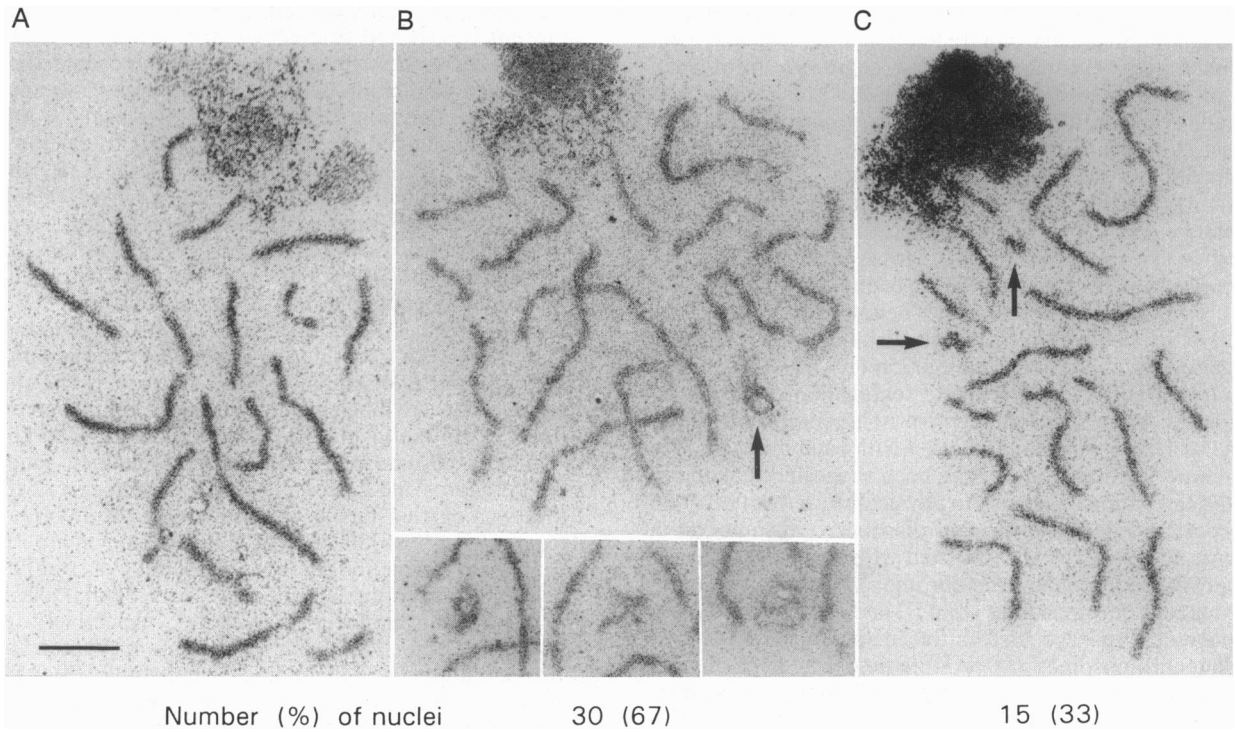

FIG. 1. Electron micrographs of silver-stained pachytene nuclei from diploid and chromosome I–III double monosomic yeast cells. (*A*) Diploid nucleus containing 16 paired chromosomes assembled in full-length SCs. (*B*) Double monosomic nucleus containing 14 paired chromosomes assembled in full-length SCs and an unusual paired structure due to association of monosomic chromosomes (arrow). (*Inset*) Several forms of the unusual structure that were observed. (*C*) Double monosomic nucleus containing 14 paired chromosomes assembled in full-length SCs and two unpaired chromosomes containing axial elements (arrows). Number and percentage of nuclei from the double monosomic strain that resembled *B* and *C* are shown below. (Bar = 2 μm.)

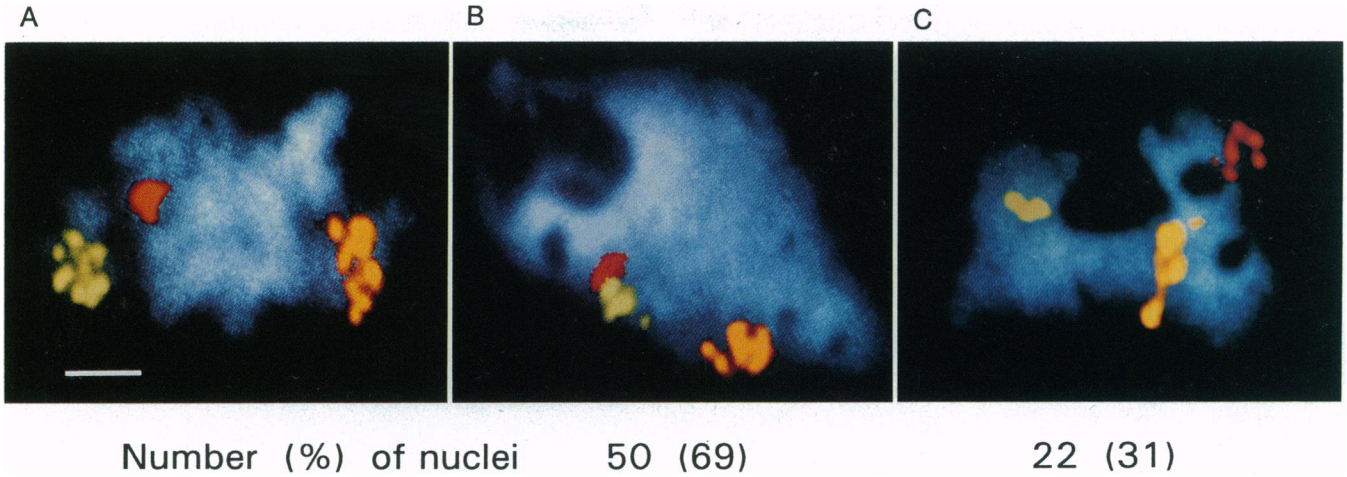

FIG. 2. FISH of meiotic nuclei from diploid and the chromosome I–III double monosomic strains. Chromosome I appears red due to TRITC fluorescence, chromosome III is green due to FITC fluorescence, and chromosome V is orange due to simultaneous green and red fluorescence from both labels. Blue background staining is by the DNA-specific dye DAPI. (*A*) Diploid nucleus showing single hybridization signals for chromosomes I, III, and V due to meiotic pairing of homologous chromosomes. (*B*) Double monosome showing adjacent green and red signals due to pairing of chromosomes I and III and single orange signal due to homologously paired copies of chromosome V. (*C*) Double monosome showing spatially distinct green and red signals due to unpaired copies of chromosomes I and III and single orange signal due to homologously paired copies of chromosome V. Note that in all nuclei from VG72-DM, the signals from chromosomes I and III were approximately half the size found in the diploids, confirming the double monosomy for these two chromosomes. Number and percentage of nuclei from the double monosomic strain that resembled *B* and *C* are shown below. (Bar = 2 μm.)

mosomes I and III were physically associated 68% of the time. This percentage is in complete agreement with the genetic results. If the paired chromosomes segregate from each other efficiently and the remaining unpaired chromosomes (32%) segregate randomly, half of the time (16%) the unpaired chromosomes will segregate from each other. Accordingly, 68% plus 16% would produce a total of 84% proper distributive disjunction of chromosomes I and III, very close to what was actually observed in the genetic experiment.

The physical interaction between nonhomologous chromosomes demonstrates that they are capable of interacting during meiotic prophase. Additional evidence for nonhomologous chromosomal interactions comes from the observation of ectopic recombination between homologous sequences located on different chromosomes (23–25) and the formation of inter- and intrachromosomal SCs in haploid meiosis (15). The mechanism of the interaction between the nonhomologous chromosomes is unknown. It may be dependent on small regions of homology such as centromeres, telomeres, transposons, or other repetitive sequences that are shared between segregating chromosomes (26). While several of the structures shown in Fig. 1 suggest an interaction between either centromeres (X-shaped structures) or telomeres (circular structures), it is too early to speculate that these chromosomal DNA elements are directly involved in the pairing process. Since circular minichromosomes undergo distributive disjunction, it is likely that this process is not dependent on functional telomeres (4–6). It is also possible that the observed chromosomal interactions are dependent on pairing sequences similar to those proposed by Simchen and coworkers (27) and Hawley (28). Alternatively, there may be absolutely no dependence on any specific DNA sequences.

We have observed an interaction that takes place during pachytene. For this interaction to be effective for distributive disjunction, we suggest that some physical linkage is maintained between the segregational partners through metaphase I. However, the nature of this linkage is not known.

Nonhomologous chromosomal interactions may be an essential component of the normal search mechanism whereby homologous chromosomes find each other, undergo recombination, and properly synapse (29). Alternatively, the observed interactions could be part of a different mechanism enabling nuclei containing the rare nonrecombinant pair of chromosomes to yield a balanced set of meiotic products (2, 3). In either case, this mechanism has the potential to be highly efficient since proper segregation of the smallest *Drosophila* chromosome may be dependent on it (2). Additional studies using probes from specific chromosomal regions, antibodies to specific chromosomal components (30, 31), and/or larger natural or stable synthetic chromosomes in yeast should help to further characterize the pairing mechanism associated with distributive disjunction.

We would like to thank Gudrun Holzinger for careful technical assistance, Carol Newlon and Maynard Olson for probes from chromosomes III and V, respectively, and Adelaide Carpenter and Hannah Klein for insightful comments on the manuscript. This research was supported by grants from the Austrian Fund for the Advancement of Scientific Research (S-5807) and the National Science Foundation.

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