Meiotic Chromosome Behavior in Spread Preparations of Yeast

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Abstract. Chromosome behavior in meiosis is well characterized from cytological and genetic descriptions but little is known of the underlying molecular mechanisms, largely because no one experimental system has been developed to support an integrated application of modern cytological, genetic, and molecular biological methods. To combine efficient analyses of meiotic chromosome structure and function in a single organism, we have extended to yeast methods for making spread preparations of nuclei. Features of yeast meiosis that parallel meiosis in large eukaryotes, such as bouquet formation and prophase chromosome condensation that occurs in concert with synaptonemal complex formation, are evident for the first time. The ability to analyze large numbers of nuclei at the light and electron microscopes in preparations amenable to a variety of cytological and immunocytological techniques will facilitate the description of meiosis at the molecular level in yeast.

CHROMOSOME pairing, synapsis, crossing over, and disjunction are cytologically defined events which correlate well with the genetically defined chromosome association, recombination, and reduction that characterize meiosis. The synaptonemal complex (SC),[1] a meiosis-specific nuclear organelle that forms along the axes of the paired chromosomes (25, 32), is a central feature of most models proposed to account for and to unite the cytological and genetic observations. However, the molecular mechanisms underlying SC assembly and meiotic chromosome behavior remain undetermined.

Detailed genetic descriptions of recombination events that occur during meiosis (8), genetic characterization of genes required for meiotic recombination (7), and manipulation of these genes in vitro followed by targeted replacement and analysis in vivo are well developed in the yeast *Saccharomyces cerevisiae* (30). Identification of gene products specifically required for meiotic recombination has begun with cloning of the yeast meiosis-specific gene SPO11 (9). However, cytological analysis of yeast has been encumbered by the small size of its chromosomes and its durable cell wall. Formation of SCs has been characterized, and condensed meiotic chromosomes have been identified (10, 18, 20, 35), but new and more efficient cytological methods will be required to correlate localization of gene products with analysis of morphogenesis of specific structures in the yeast meiotic nucleus.

Surface-spread preparations of meiotic nuclei from large eukaryotes allow efficient and detailed observation of large numbers of nuclei with both light microscopy (LM) and EM (6). Characteristic morphologies of the chromatin, synaptonemal complexes (SCs), and nucleoli provide internal markers for the progress of each nucleus through meiosis and indicate a sequence of events that is confirmed by independent means of staging, e.g., in mouse (4, 11, 26, 27). We have adapted to yeast these methods for making and analyzing spread preparations of meiotic nuclei. Nuclear structures are well preserved, allowing the staging and analysis of individual meiotic nuclei, and revealing previously unobserved features of yeast meiosis, such as bouquet formation and condensation of the chromatin during meiotic prophase.

1. *Abbreviations used in this paper*: LE, lateral element; LM, light microscopy; NDB, nuclear dense body; NLL, nucleolus; SC, synaptonemal complex; SPB, spindle pole body.

Materials and Methods

Sporulation Conditions

The homothallic diploid yeast strain, K65-3D (17), was grown in liquid culture in YPA presporulation medium (1% wt/vol potassium acetate, 2% wt/vol peptone, 1% wt/vol yeast extract) at 30°C to a density of 2×10^7 cells/ml. Cells were harvested by filtration and washed with an equivalent volume of water followed by an equivalent volume of SP2-CS sporulation medium (2% wt/vol potassium acetate, pH 7.0 containing an amino acid mix to supplement the auxotrophic requirements of the yeast strain). Cells were resuspended in SP2-CS at 4×10^7 cells/ml and shaken at 30°C to induce meiosis and sporulation.

Cytological Preparations

Spread meiotic nuclei were prepared from sporulating cells by spheroplasting, fixing, and drying the resulting preparations on slides, using modifications of established techniques (6, 10). Spheroplasting was carried out by removing 5-10 ml samples, washing the cells in 2% wt/vol potassium acetate/0.8 M sorbitol/pH 7.0, adding dithiothreitol to a final concentration of 10 mM for 10 min at 30°C, and then adding Zymolyase 100T (ICN Biomedicals, Inc., Irvine, CA) to 25 μg/ml final concentration. The percent of cells spheroplasted at a given time was determined by counting samples diluted into potassium acetate-sorbitol with versus without 2% wt/vol Sarkosyl;

only the spheroplasts lyse in 2% Sarkosyl. At 70–90% spheroplasting, the cells were washed once with ice-cold 0.1 M 2-(*N*-morpholino) ethane sulfonic acid/1 M sorbitol/1 mM EDTA/0.5 mM $MgCl_2$/pH 6.4 and kept on ice as a pellet with the supernatant removed. Spheroplasts in the pellet were mixed with 0.1 M 2-(*N*-morpholino) ethane sulfonic acid/1 mM EDTA/0.5 mM $MgCl_2$/pH 6.4 at a volume ratio of 1 part spheroplasts:25 parts buffer. Paraformaldehyde (4% wt/vol)/pH 7.0 (unbuffered) was added at a volume ratio of 1 part suspension:7 parts paraformaldehyde, and 0.4 ml of the mixture was placed on a glass microscope slide precoated with poly-L-lysine (for LM) or with plastic (for EM). After 10 min at room temperature, the slides were gently drained and 0.35 ml more of 4% wt/vol paraformaldehyde/pH 7.0 was added for another 5 min. The final fixative was gently drained off, the surface of the slide rinsed with 5 ml of 0.4% vol/vol PhotoFlo 200 (Eastman Kodak Co., Rochester, NY), and the preparations allowed to air dry.

Cytological Procedures

Preparations to be analyzed at the EM were stained with silver nitrate (15). Areas of the slides determined at the LM to be well populated with nuclei were transferred to 50-mesh copper grids which were then mapped for well-spread, well-preserved nuclei at the LM to facilitate EM analysis (6).

Preparations to be analyzed using fluorescence microscopy alone were stained in 0.1 mg/ml acridine orange in 70 mM phosphate buffer (22.4 mM Na_2PO_4/47.6 mM KH_2PO_4/pH 6.5) for 3 min at 4°C, then rinsed and mounted in 4°C buffer without acridine orange and photographed (at room temperature) on Kodak P400/800 slide film.

Results

Preservation of Nuclear Structures

In these preparations of silver-stained, whole nuclei, the SCs are evident at the EM as pairs of filaments (termed lateral elements, or LEs) surrounded by indistinct areas of chromatin (Figs. 1 and 2). Discontinuities in the LEs where synapsis is incomplete, and thinned segments along otherwise dense, continuous SCs, are common and are more likely to be of biological significance than to result from the spreading forces, as similar inhomogeneities have been reported from sectioned material of yeast (35). The SCs are also evident in brightfield at the LM after silver staining (not shown), which facilitates analysis by allowing mapping of the preparations on grids before observation at the EM.

The chromosomes are evident at the LM using acridine orange, a fluorescent stain selective for nucleic acids (Fig. 3) that does not extract chromatin (as does the silver-staining technique employed; unpublished observations). The intensity of chromatin fluorescence is higher in nuclei where the chromosomes are condensed and organized into linear structures than in nuclei with more diffuse chromatin (Fig. 3).

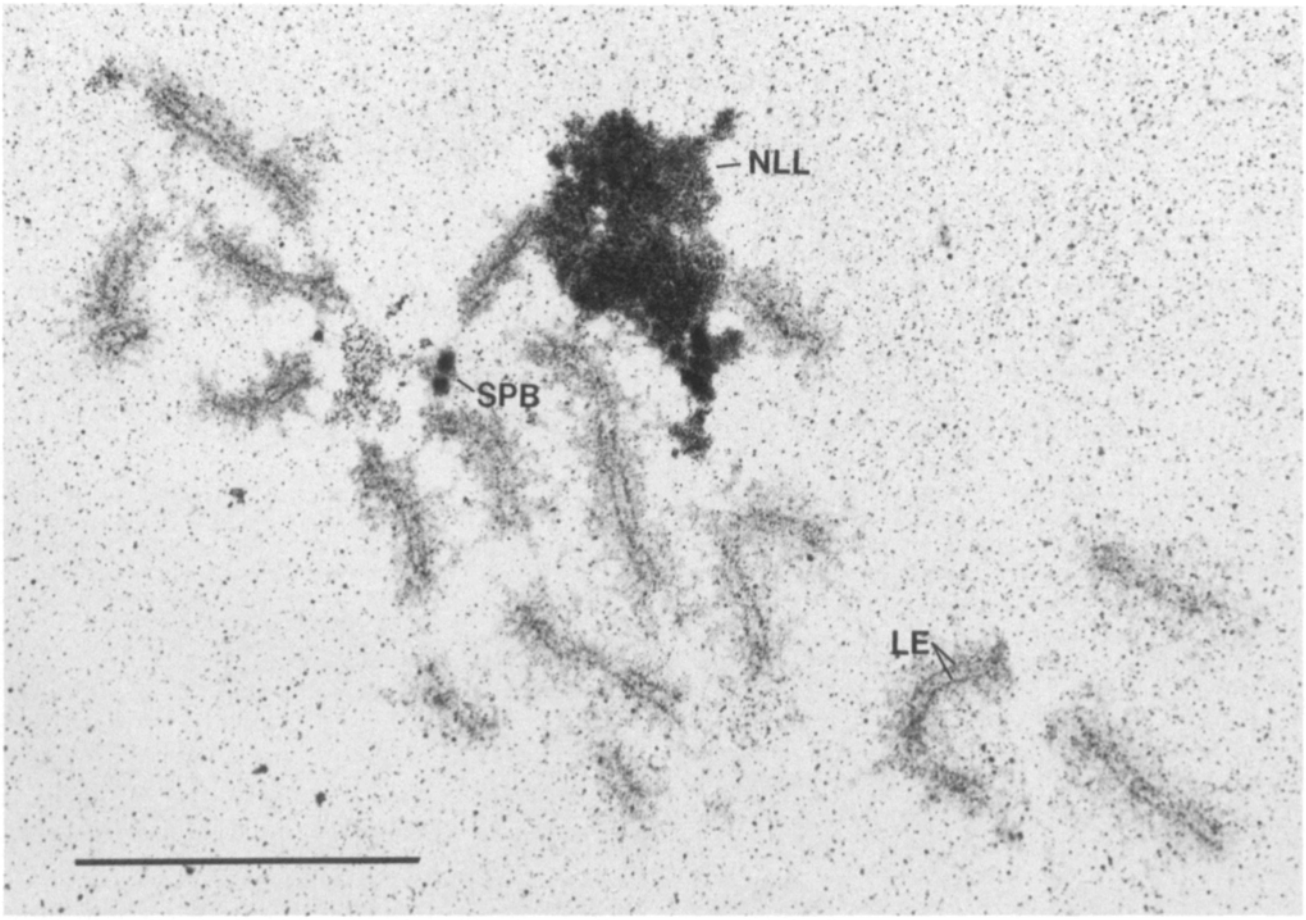

Figure 1. Electron micrograph of a silver-stained pachytene nucleus. The two lateral elements (*LE*) of each of the 16 SCs, the nucleolus (*NLL*), which divides bivalent XII into two parts, dense bodies in the nucleolus, and the duplicated spindle pole body (*SPB*) are well contrasted. Chromatin is partially extracted by this staining procedure and is visible as the less dense "fuzz" along each SC. In some regions the LEs are not continuous (see text). Bar, 3 μm.

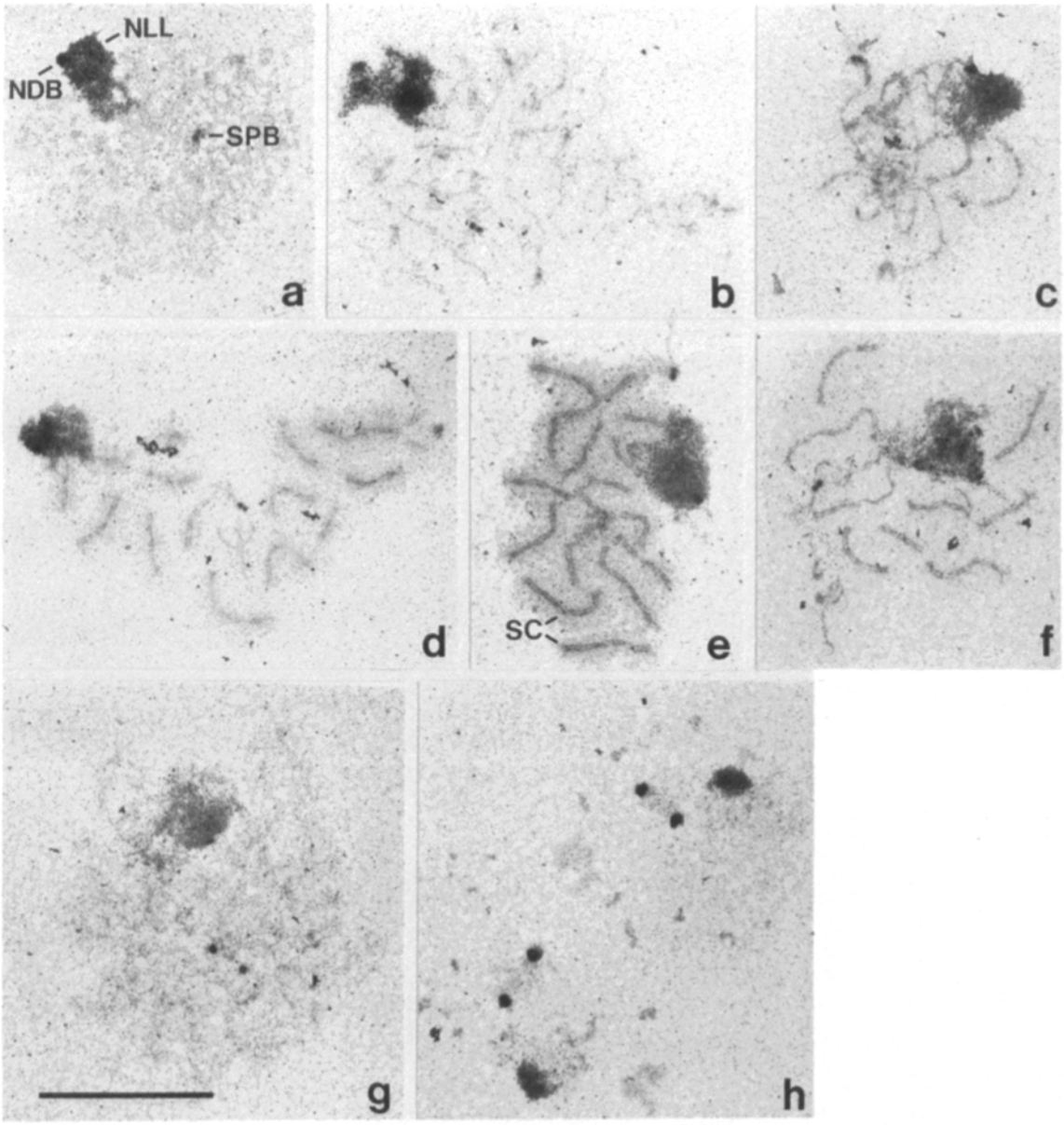

Figure 2. Electron micrographs of silver-stained nuclei illustrating the series of changes in nuclear morphology observed in cells undergoing sporulation, beginning with an early meiotic (or vegetative) cell and ending with a binucleate cell. (*a*) Early meiotic (or vegetative) nucleus with diffuse chromatin, a well-contrasted nucleolus (*NLL*) containing a nuclear dense body (*NDB*), and a spindle pole body (*SPB*) in the early stages of duplication. (*b*) Leptotene nucleus showing short, silver-stained segments of incompletely formed axial elements. (*c*) Zygotene nucleus showing nearly complete axial elements (which when synapsed are termed lateral elements) and some SC formation (synapsis). Many of the bivalent ends are collected together at one place in the nucleus, giving rise to the "bouquet" appearance often described in large eukaryotes. (*d*) Late zygotene nucleus showing incomplete synapsis at the nucleolus and of two entire bivalents (one medium and one short in length). In this nucleus there are no apparent end-to-end associations. (*e*) Pachytene nucleus showing complete synapsis. Some end-to-end associations are apparent, making it difficult to resolve each of the sixteen bivalents. (*f*) Diplotene nucleus showing desynapsis in some bivalents, a duplicated but unseparated spindle pole body, and multiple dense bodies in the nucleolus. (*g*) Postdiplotene nucleus with some remaining indications of linear, silver-stained structures and well-separated spindle pole bodies. (*h*) Two nuclei from a binucleate cell showing separation of the spindle pole bodies in each, in preparation for the second meiotic division. Bar, 5 μm.

Nucleoli are densely stained with silver (Figs. 1 and 2) and fluoresce orange-red after staining with acridine orange (Fig. 3). Spindle pole bodies (SPBs) and dense bodies in the nucleolus are also evident after silver staining (Figs. 1 and 2). Morphological changes in these structures correlate with the extent of SC formation and dissolution (13, 35) allowing identification of the leptotene, zygotene, pachytene, and diplotene stages of meiosis.

Stages of Meiotic Prophase

Leptotene (Presynapsis). The earliest identifiable stage of meiotic prophase is leptotene, when threads of chromatin first become evident (compare Fig. 3 *b* with 3 *a*) and silver-stained axial elements are observed (Fig. 2 *b*). The axial elements first form in short, discontinuous lengths which subsequently, probably by linking together, form continuous

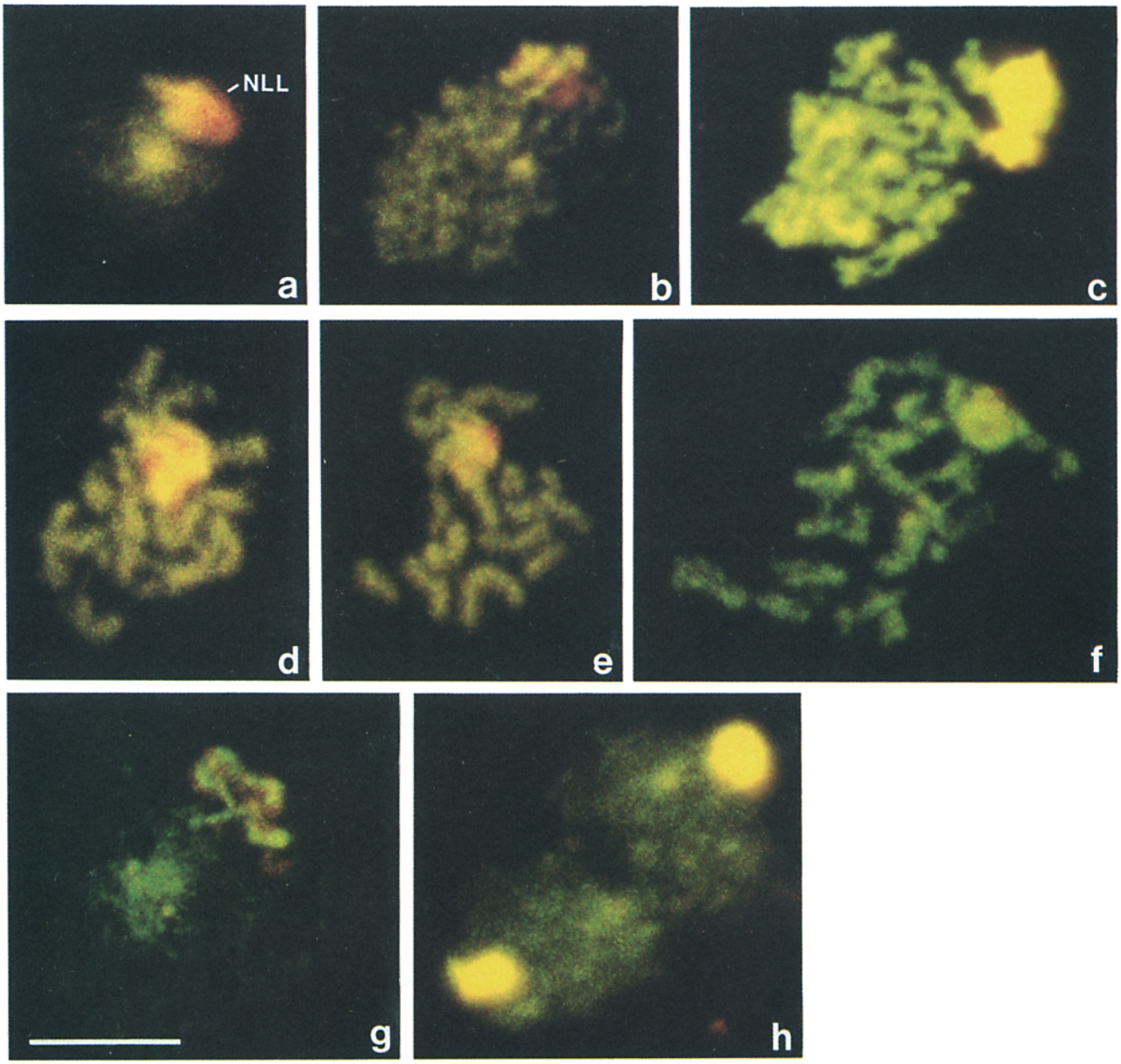

Figure 3. Light micrographs of acridine orange-stained nuclei illustrating the series of changes in nuclear morphology observed in cells undergoing sporulation, beginning with an early meiotic (or vegetative) cell and ending with a binucleate cell. (*a*) Early meiotic (or vegetative) nucleus showing orange-red fluorescence of the nucleolus (*NLL*), yellow fluorescence of the nucleolus organizing regions, and dim yellow-green fluorescence of the chromatin. (*b*) Leptotene nucleus showing condensation of the chromatin into thin threads. (*c*) Zygotene nucleus showing chromosomes as single threads that in some areas are paired and synapsed. In the corresponding phase image (not shown) the spindle pole body appears single. (*d*) Late zygotene nucleus where bivalent formation is nearly complete, but some ends are unsynapsed. (*e*) Pachytene nucleus where synapsis is complete. (*f*) Diplotene nucleus where the bivalents are partially desynapsed and are less condensed. In the corresponding phase image (not shown) there are two clearly separate spindle pole bodies. (*g*) Postdiplotene nucleus where the fluorescence of the chromatin is dim and appears to be less condensed than in previous stages. The spindle pole bodies are visible as small dots near the center of the image (the basis for their fluorescence is not known). (*h*) Nuclei from a binucleate cell, i.e., after the first meiotic division. Bar, 5 μm.

filaments that define the chromosome axes. The nucleolus contains a single nuclear dense body (NDB) (Fig. 2; 13, 35), and the SPB, having begun duplication, has an adjacent silver-stained daughter that typically is less than half the diameter of the mother.

Zygotene (Incomplete Synapsis). Chromosome pairing (alignment of homologues) is evident (Fig. 3 *c* and *d*), coincident with synapsis (assembly of the SC) (Fig. 2, *c* and *d*). Synapsis can initiate interstitially or at the ends of the chromosomes, although at late zygotene it is typical for only the ends of one or more bivalents to remain unsynapsed (Figs. 2 *d* and 3 *d*). Axial elements and SCs frequently associate end-to-end (Fig. 2 *c*), giving rise to the chromosomal "bouquet" described in many large eukaryotes (33) but not previ-

ously observed in yeast. Chromosome condensation is extensive and allows resolution of regions of individual homologues that are unsynapsed (Fig. 3 *c*).

Pachytene (Complete Synapsis). The bivalents are completely synapsed and are most easily (as compared with other stages) distinguished from one another at the LM during pachytene (Figs. 1, 2 *e*, 3 *e*). Overly spread nuclei are apparent in these preparations as nuclei that cover a relatively large area and contain obviously stretched SCs and nucleoli (not shown here). In spite of the spreading forces, the distance between synapsed LEs is equal in overly spread and less dispersed nuclei, indicating that the synaptic association is quite stable. In practical terms, this means that misinterpretation of stages due to "artifactual asynapsis" is unlikely. End-to-end associations are frequent and probably reflect the persistence of associations formed in the bouquet. Recombination nodules, reported for yeast from sectioned material (1), are not obvious in these preparations. No axial element formation has been detected in the body of the nucleolus at any stage (Figs. 1 and 2), consistent with the reported absence of detectable SC in the nucleolus (1, 20). In addition, the regions immediately adjacent to the nucleolus frequently are unsynapsed when interstitial regions elsewhere in the genome are completely synapsed (data not shown).

In well-preserved, well-spread nuclei, 16 bivalents are resolved, the two SC segments associated with the nucleolus representing a single, continuous bivalent. As measured in the spread preparations, the total length of the SCs in 10 nuclei range from 21.2 to 35.7 μm for the whole complement (data available on request) and correspond well with the lengths measured in sections, which range from 22.4 to 33.1 μm in one report (1) and from 21.8 to 29.3 μm in another (20; where the values are for several different strains and exclude a strain with "short" SCs). These results indicate that there is relatively little or no stretching of the SCs during spreading.

In the absence of cytological markers that distinguish different chromosomes (e.g., centromere regions, which would allow measurement of arm ratios), only the nucleolus-organizing bivalent, which corresponds to genetic linkage group XII (24), can be identified unambiguously. The relative length of SC XII, calculated by adding its two SC segments together, ranges from the first to the fifth largest of the bivalents and averages to the third largest, the same position reported for two different strains from sectioned material (20). The nonribosomal DNA of chromosome XII (~1,100 kb; Link, A., G. F. Carle, and M. V. Olson, Washington University School of Medicine, St. Louis, MO, personal communication) is equal to 9.6% of the total DNA content of the remaining chromosomes, which corresponds well with the relative SC length of 9.8% measured here.

Diplotene (Desynapsis). Concurrent with desynapsis during diplotene, the LEs disassemble and the chromatin decondenses (Figs. 2 *f* and 3 *f*). Fewer end-to-end associations are seen than in pachytene. The orange-red fluorescence of the nucleolus becomes less intense and covers a broader area than in earlier stages. Multiple silver-dense bodies appear in the nucleolus and may correspond to the polycomplexes described in sectioned material (13, 21, 35). At this stage, the two SPBs are typically equal in size.

At the end of diplotene the SPBs are clearly separate, and only traces of the linear condensations of chromosomes (Fig. 3 *g*) and of the LEs (Fig. 2 *g*) can be observed. The nucleolus typically is homogeneous in structure. The SPBs fluoresce in acridine orange stained preparations but it is not clear whether this is from an intrinsic uptake of stain or from a relatively high localized concentration of chromatin (Fig. 3 *g*).

Postdivision. After the first meiotic division the two chromatin masses are still contained within a single nuclear membrane (22). The nuclei from these "binucleate" cells are evident (Figs. 2 *h* and 3 *h*) as adjacent masses of chromatin that are similar in size, contain discrete small nucleoli, and can have duplicated and enlarged SPBs characteristic of metaphase II nuclei (Fig. 2 *h*; reference 22). Marking the completion of meiotic prophase, these "binucleates" are present only in transit to the "tetranucleate" stage that leads to ascosporogenesis. The chromosomes are not condensed in these nuclei, and there is no acridine orange fluorescence of (or adjacent to) the SPBs.

Discussion

To establish a connection between the developing molecular biology of yeast meiosis and the existing structural biology of the meiotic nucleus in yeast and in large eukaryotes, we have developed surface spreading methods that allow the contents of large numbers of yeast meiotic nuclei to be visualized by both LM and EM (Figs. 1–3). Chromosome condensation and pairing, SC morphogenesis, SPB duplication, and changes in nucleolar morphology are evident in these preparations, providing internal markers for the progression of individual nuclei through meiosis (Figs. 2 and 3).

Meiotic Chromosome Behavior in Yeast

Meiotic Chromosome Condensation. Previous reports suggesting the absence of chromosome condensation during the yeast vegetative cell cycle (for example, see reference 12) have questioned the general applicability of yeast data to the study of eukaryote chromosome organization. The problem has been that the outlines of the individual chromosomes are difficult to visualize in plastic-embedded, sectioned material, both in vegetative and in meiotic nuclei (for example, see reference 35). In LM preparations demonstrating individual bivalents, the other nuclear structures have not been evident, so that staging of meiotic development has been ambiguous and condensation has been interpreted to take place during metaphase I division (18). In the preparations reported here, condensation and decondensation are seen to parallel assembly and dissolution of the synaptonemal complex, so that chromosome condensation appears maximal in midmeiotic prophase. While in large eukaryotes condensation typically is maximal in metaphase, decondensation of the chromatin often occurs between midmeiotic prophase and metaphase I, during the "diffuse" stage (33) which is followed by a second round of condensation that compacts the chromatin further. Yeast clearly has in common the first, or "synaptic," pattern of condensation but the second, perhaps "segregational," pattern of condensation has not been observed. Yeast chromosomes are small and may not require the extensive condensation that is necessary to physically separate larger eukaryotic chromosomes during metaphase segregation.

Bouquet Formation and Telomeres. Nonhomologous

chromosomes associate end-to-end early in meiotic prophase, leading to the formation of a chromosomal "bouquet" (Fig. 2 *c*) not previously described in yeast. The bouquet arrangement, like the diffuse stage, is found in classical descriptions of meiotic chromosome behavior in many organisms (33). The telomeres of yeast chromosomes contain a common structural arrangement of conserved sequence elements which have been observed to undergo frequent genetic exchange (2, 3, 14). The end-to-end associations may reflect or lead to this exchange, may relate to recombination associated with the completion of DNA synthesis at the ends (31), or could result from telomere attachment to the nuclear envelope (22, 35).

SC Formation and Meiotic Recombination. The absence of detectable SC formation in the nucleolus (Figs. 1 and 2; also reported from sectioned material, references 1, 20) correlates with a marked deficiency in meiotic recombination between homologs in the rDNA repeat cluster (which lies within the nucleolus). Whereas levels of reciprocal exchange are comparable for the rDNA and other regions of the genome during the vegetative cell cycle, the rDNA is distinct from the other regions in that it does not exhibit the elevated levels of reciprocal exchange between homologs characteristic of meiosis (29, 34). It is interesting that only meiotic recombination between homologs is reduced in the rDNA array; meiotic unequal sister chromatid exchange is elevated (28). This difference suggests a specific defect in the opportunity for exchange between homologs in this region as opposed to a specific defect in the molecular mechanisms of recombination, and further suggests the dependence of homolog recombination on synapsis via SC formation. Recent reports of meiotic reciprocal exchange events between nonhomologous chromosomes (16, 19) have suggested the independence of these events from SC formation but there may be a transient or uniquely labile synapsis at the site of exchange between these nonhomologous chromosomes. The well-defined genetics of yeast allows comparisons such as these to be made between chromosome organization and meiotic recombination which would be difficult in most other organisms.

Future Directions. Using the methods described in this report, nuclear structure morphogenesis in meiotic mutants can be readily characterized, e.g., preliminary results with mutants of the meiosis-specific gene SP011 (9). In addition, the nuclear localization of specific proteins can be determined using immunocytological techniques, as previously demonstrated on similar mammalian preparations (5, 23; Dresser, M. E., and C. N. Giroux, unpublished observations). The combination of these abilities will facilitate the description of meiosis at the molecular level in yeast.

We dedicate this paper to Professor Montrose J. Moses, our friend and colleague, who 30 years ago initiated this work by his discovery of the synaptonemal complex.

We gratefully acknowledge Montrose J. Moses for the use of his laboratory's resources, for his ongoing encouragement and criticism, and for his participation in early pilot experiments. We thank Stephen Johnston and Jim Mason for critical comments on this manuscript, and Karen Cowardin for help in preparing this manuscript.

Supported by National Institutes of Health intramural research project No. Z01 ES 21035 CGTB (C. N. G., principal investigator), by National Research Council Research Associate Award (M. E. D.), and by National Science Foundation Grant No. PCM 8308651 (to Montrose J. Moses).

Received for publication 6 August 1987, and in revised form 10 November 1987.

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