Live imaging of rapid chromosome movements in meiotic prophase I in maize

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The ability of chromosomes to move across the nuclear space is essential for the reorganization of the nucleus that takes place in early meiotic prophase. Chromosome dynamics of prophase I have been studied in budding and fission yeasts, but little is known about this process in higher eukaryotes, where genomes and chromosomes are much larger and meiosis takes a longer time to complete. This knowledge gap has been mainly caused by difficulties in culturing isolated live meiocytes of multicellular eukaryotes. To study the nuclear dynamics during meiotic prophase in maize, we established a system to observe live meiocytes inside intact anthers. We found that maize chromosomes exhibited extremely dynamic and complex motility in zygonema and pachynema. The movement patterns differed dramatically between the two stages. Chromosome movements included rotations of the entire chromatin and movements of individual chromosome segments, which were mostly telomere-led. Chromosome motility was coincident with dynamic deformations of the nuclear envelope. Both, chromosome and nuclear envelope motility depended on actin microfilaments as well as tubulin. The complexity of the nuclear movements implies that several different mechanisms affect chromosome motility in early meiotic prophase in maize. We propose that the vigorous nuclear motility provides a mechanism for homologous loci to find each other during zygonema.

chromosome dynamics | cytogenetics | meiosis | cell biology

In early meiotic prophase, the nucleus undergoes a major spatial reorganization, which includes a general repositioning of chromatin and juxtaposition of homologous chromosomes (1). In many species, including maize, the nucleolus is located in the center of the nucleus during leptonema, and at the onset of zygonema, moves to a peripheral position (1, 2). Concurrently with the nucleus migration, all chromosome ends attach to the nuclear envelope (NE) and cluster on a single site forming the "telomere bouquet" (3, 4), which has been observed in most plants, animals, and fungi, including budding and fission yeasts, mouse, and maize. The telomeres remain clustered throughout zygonema. When the telomeres are clustered, centromeres are oriented in the opposite direction than the telomeres, resulting in a telomere–centromere polarization of the meiocyte nucleus. The presence of the bouquet coincides with pairing of homologous chromosomes (3, 5). In plants, mammals, and fungi, chromosome pairing depends upon the progression of meiotic recombination (5–7). However, a recombination-driven homology recognition mechanism can only operate across a relatively short distance, probably ≈1.2 μm (6). In large-genome species, such as maize, where the zygotene nucleus is ≈20 μm in diameter, this mechanism may not be sufficient to reach across the chromatin mass in the nucleus, even when the chromosomes are brought together by the bouquet (6). These constraints suggest that homologous chromosome segments must first be positioned close to each other, before the homology search can take place.

The ability of chromosomes to move across the nuclear space is essential for both the bouquet formation and chromosome pairing. Observations of live meiocytes in budding and fission yeasts have shown that during meiotic prophase chromosomes exhibit unexpectedly dynamic motility: the "horse-tail" chromosome movements in fission yeast and the rapid prophase movements (RPMs) in budding yeast (8–11). In fission yeast, these movements are particularly dramatic, with the entire nucleus moving violently back and forth (11, 12). However, it has been unclear whether these dynamic motility patterns are specific to small-genome unicellular organisms where meiotic prophase is fairly short, or whether they are also present in species with large genomes and slowly progressing meiotic prophase. Until now, in-depth examinations of meiotic prophase dynamics in live meiocytes have not been performed in multicellular eukaryotes. Only fragmentary observations have been conducted in mouse and rats, and they have shown only limited extent of chromosome motility (13, 14).

In maize, efforts to culture isolated meiocytes to observe chromosome motility have been largely unsuccessful. Although metaphase I and later meiocytes develop properly in culture (15), attempts to culture isolated meiocytes in early stages of meiotic prophase have failed (16). To circumvent this problem, we developed a system to observe meiotic prophase in meiocytes inside intact live anthers, which, in contrast to isolated meiocytes, can be cultured in grasses during prophase I (17). Maize meiocytes develop in the center of the anther locule, which lies ≈70 to 100 μm from the outside surface of the anther (Fig. S1). This depth is beyond the capabilities of confocal microscopy, but within the range of ≈200 μm of multiphoton excitation (MPE) microscopy (18). Using this approach, we found that maize chromosomes in zygonema and pachynema exhibit very dynamic and complex motility patterns. Several classes of movements can be distinguished, including rotations of the entire chromatin mass and movements of individual chromosome segments. Based on our data, we propose that several different mechanisms contribute to meiotic chromosome motility in maize. We also postulate that the purpose of the chromosome motility during zygonema is to allow homologous loci to find each other.

Results

Developing a Live Observation System for Tracking Chromosome Dynamics in Meiotic Prophase I. To monitor chromosome dynamics in live prophase I meiocytes, we developed a system that allows microscopic observation of meiocytes inside intact live anthers using MPE microscopy (19). To visualize chromosomes, anthers were stained with DAPI, which we found to provide good vital staining and be relatively resistant to photobleaching. Also, previous experiments showed that DAPI does not affect chromosome movements (20). Using the anther culturing system, we were able to maintain the viability of the meiocytes for >30 h, as visualized by mitochondrial viability staining (21) and the progression of meiosis. Over a 24-h period, we observed zygotene meiocytes that progressed to pachynema, and pachytene cells that progressed to dyads. Both progression patterns are consistent with the timing of meiosis observed *in planta* (22).

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To dissect nuclear motility patterns, we traced movements of individual chromosome segments, concerted movements of the entire chromatin in the nucleus, and movements of the NE. Our ability to distinguish and trace individual chromosomes in live meiocytes varied with the stage of meiosis. Individual chromosomes were apparent in pachynema. In early zygonema, however, individual chromatin threads were not always clearly visible in unfixed meiocytes. In such cases, the visibility of chromatin masses could be improved by finding edges, i.e., identifying threshold lines between the stained and unstained areas in the nucleus (Fig. S2). We found that many anonymous chromosome marks could be efficiently tracked over time. In some cases, we were also able to identify and track specific chromosome landmarks, such as heterochromatic knobs (Fig. S2) (23) and chromosome ends. In addition to following individual chromosomes, we tracked movements of the entire chromatin mass in the nucleus by manually delineating specific regions of interest and following the movements of these marks.

Chromosomes Exhibit Dynamic Motility During Zygonema and Pachynema. To elucidate chromosome dynamics in maize meiocytes, we collected time-lapse movies from all prophase I substages. Because chromosome appearance in unfixed cells does not always allow unequivocal determination of their stage, particularly in leptotene and zygotene cells, one of the three synchronously developing anthers in each maize flower was always fixed for precise staging, whereas the other two anthers were used for live observations. Our analyses showed that chromosomes in live meiocytes in zygonema and pachynema exhibited very dynamic and complex patterns of motility. In zygonema, we registered chromosome velocities averaging 400 nm s^{-1}, which is within the same order of magnitude as the speed of the RPMs in budding yeast (10). In pachynema, chromosome movements were slower, reaching an average maximum velocity of 148 nm s^{-1}. In contrast, we did not see chromosome movements in leptotene or diplotene nuclei. In diakinesis meiocytes and anther epidermal cells, we saw only minor chromatin movements that were not of the same magnitude as the zygotene or pachytene movements.

Three Distinct Chromosome Movement Classes Can Be Identified in Zygotene Meiocytes. To understand zygotene chromosome dynamics, we dissected movements of anonymous chromatin marks as well as specific landmarks (chromosome ends and knobs) in short (1–3 min) and longer (up to 20 min) time-lapse movies. These analyses indicated that zygotene chromosomes exhibited three distinct movement classes that often occurred concurrently in the same cell: (*i*) coordinated rotational movements of the entire chromatin, (*ii*) rapid, short-distance oscillations of small individual chromosome segments extending from the main chromatin mass into the nuclear space, and (*iii*) slower-paced movements of other chromosome segments mostly located inside the chromatin mass.

(*i*) Rotational movements of the entire chromatin were observed in nearly all zygotene meiocytes. The most common type of these movements was the chromatin randomly oscillating back and forth at angles ranging from 7 to 10° over periods of time as short as 5 s (Fig. 1; Movies S1–S3). The second type consisted of the chromatin mass sliding along the NE while rotating up to 45° in a single direction (Fig. 2 *A* and *B*; Movies S4–S6). The third, most dramatic and least frequently observed type, was large rotation (as much as 90°) of the chromatin mass that displaced the nucleolus in the process (Fig. 2 *C* and *D*; Movies S7–S11).

(*ii*) Concurrent with the rotational movements of the entire chromatin, we observed rapid movements of small chromosome segments extending from the chromatin mass toward the NE (Fig. 1; Movies S1–S3). Although the actual NE attachment was not always clearly visible in unfixed zygotene cells, analyses of fixed meiocytes indicated that they were at the bouquet stage, when chromosome ends are attached to the NE (24). These movements appeared to be telomere-led: Chromosome marks located close to the chromatin mass were more confined in their movement than the marks close to the chromosome end and the NE. Marks located near each other on the fast-moving chromosome segments showed similar movement trajectories, indicating that their movements were coordinated. In some cells, there were as many as five fast-moving chromosome ends visible in a single Z-section in early- and mid-zygotene cells and as few as one in late-zygotene meiocytes. The movements were generally abrupt with rapid accelerations and decelerations following each other within a short period, and exhibited velocities varying from 270 to 1,000 nm s^{-1}. The examination of several zygotene cells in the same anther showed that the scale of these movements was similar in all cells (Movies S1–S3).

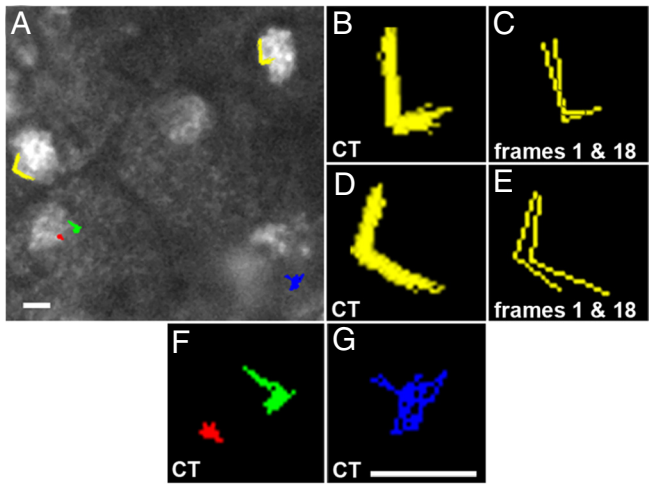

Fig. 1. Chromosome motility in zygonema. (*A*) Three chromosome movement classes observed in a group of five zygotene meiocytes: rotational movements of the entire chromatin (yellow lines marking chromatin mass edges), rapid, short-distance movements of small chromosome segments (blue and green), and slower-paced movements of chromosome segments inside the chromatin mass (red). (*B* and *D*) Cumulative tracks marking rotational movements of the entire chromatin in the two meiocytes traced in *A* after 145 s. (*C* and *E*) Starting and ending positions of the rotating chromatin in the nuclei from B and D. (*F* and *G*) Cumulative tracks of the small chromosome segments tracked in the two meiocytes in *A* after 145 s. (Scale bar, 5 μm.)

(*iii*) In addition to the fast abrupt movements of chromosome segments extending from the chromatin mass, we observed many other chromosome segments, mostly within the large brightly staining chromatin mass, that showed slower movements with smaller amplitudes (Fig. 1; Movies S1–S3). The average velocity of these movements was 28 nm s^{-1} with a maximum at 64 nm s^{-1}. Chromosome marks located in regions adjacent to each other showed similar movements trajectories suggesting that these movements were coordinated.

To examine the physical impediments that chromosomes must face when moving inside the nucleus, we measured the space occupied by chromatin and the nucleolus. Measurements of equatorial cross-sections of several live meiocyte nuclei showed that only ≈20% of the nuclear space was taken by the chromatin, whereas the nucleolus occupied ≈13% (Fig. S3). These calculations leave ≈60% of the total nucleus volume unoccupied by either chromatin or the nucleolus, indicating that there is ample space in the nucleus that chromosomes can use to rearrange their positions.

Chromatin Movements in Zygonema Are Concurrent with Transient NE Deformations. In addition to tracking chromosomes, we examined the morphology of the nucleus during zygonema. In live images, we could delineate the location of the NE by using cytoplasm-staining viability stains and the fact that DAPI also stains DNA-containing organelles in the cytoplasm. These observations showed that the

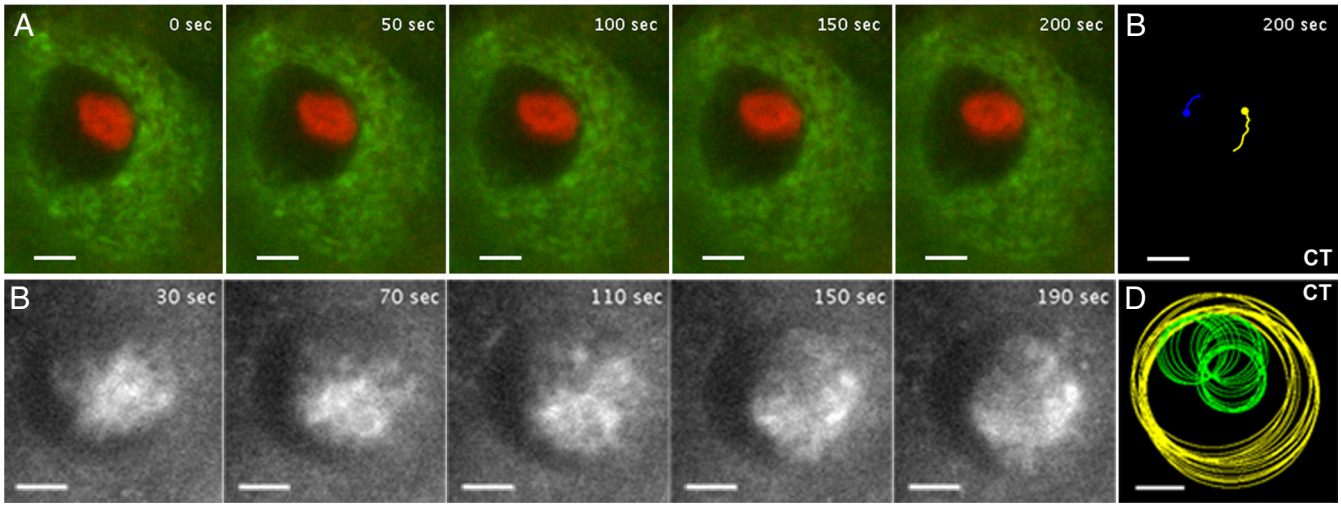

Fig. 2. Large-magnitude rotational motions in zygonema. (*A*) A zygotene nucleus showing sliding rotation of the entire chromatin (red) along the NE. Green, cytoplasm stained with Rhodamine 123. (*B*) Cumulative tracks of two anonymous chromosome marks located at the nuclear periphery in the nucleus shown in A after 200 s. (*C*) A zygotene nucleus exhibiting rotational motion that displaces the nucleolus. (*D*) The cumulative tracks of the NE (yellow) and nucleolus (green) in the nucleus in *B* after 190 s. (Scale bar, 5 μm.)

NE during zygonema was neither round nor static. The NE changed shape and deformed as the chromatin mass moved (Fig. 3; Movies S12–S14). The most obvious manifestations of these shape changes were protrusions of the NE into the cytoplasm as well as indentations of the NE into the nuclear space. These deformations could alter the diameter of the nucleus by up to 3 μm and were ephemeral, rarely lasting longer than 30–60 s. Although chromosome segments extending from the chromatin mass frequently came into contact with the NE, the NE protrusions into the cytoplasm were often not associated with chromosome segments, indicating that the chromosomes themselves were not the cause of these protrusions. On the contrary, the NE motility could be the source of chromatin motility, because movements of the chromatin often appeared to be coordinated with the overall changes of the nuclear topology (Movies S15–S21).

Chromosome Movements Slow Down at the Zygonema to Pachynema Transition. At the transition from zygonema to pachynema, we observed a period of quiescence, when only small-angle rotations of the entire chromatin mass were observed (Movie S22). Only a few chromosome segments were moving, and the magnitude and speed of these movements was reduced compared with zygonema.

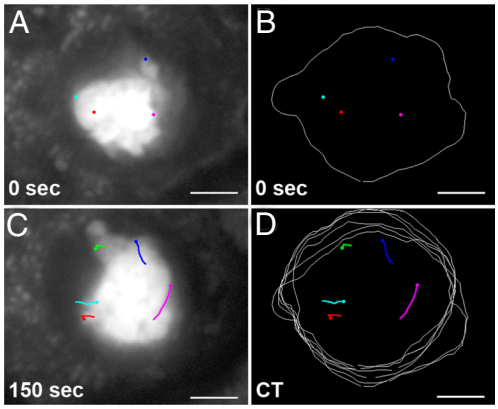

Fig. 3. Dynamic changes of the NE shape in zygonema. A zygotene nucleus was imaged every 60 s for 300 s. Overexposing the images enhanced the contrast between the nucleus and cytoplasm and allowed the delineation of the NE. Four chromosome marks (red, blue, purple, and turquoise) were also tracked for comparison. (*A*) The nucleus at 0 s. (*B*) The same nucleus at 300 s. (*C*) The NE and the chromosome marks at 0 s. (*D*) Cumulative tracks of the NE and chromosome marks after 300 s. (Scale bar, 5 μm.)

Long-Distance Sweeping Motions of Whole Chromosome Arms Are Characteristic for Pachytene Chromosome Movements. In pachynema, we observed stage-specific chromosome movements distinct from those observed in zygonema. However, as in zygonema, three chromosome movement classes could be distinguished: (*i*) rotational movement of the entire chromatin, (*ii*) movements of chromosome segments extending from the chromatin mass, and (*iii*) more restrained movements of chromosome segments inside the chromatin mass.

(*i*) Although the rotational movements of the entire chromatin in pachynema were similar to the zygotene rotational movements, the magnitude of these movements was, overall, greater than in zygonema. For example, in Fig. 4 *A* and *B* and Movies S23–S25, the chromatin mass rotated >90° while shifting ≈5 μm across the nucleus.

(*ii*) In pachynema, the chromatin mass was denser than in zygonema, and the chromosome segments extending from the chromatin mass into the nuclear space were much longer. These chromosome segments performed sweeping motions, where large chromosome regions moved slowly, and in a single direction or back and forth, across a large extent of the nucleus (Fig. 4 *C* and *D*; Movies S26–S28). These movements were much slower than the movements of chromosome segments extending from the chromatin mass in zygonema. Ends of the moving chromosomes sometimes appeared near the NE. In other cases, however, they were undoubtedly inside the nuclear space and some distance away from the NE. Chromosome ends traveled faster than interstitial regions of the same chromosome, suggesting that the movements originated at the chromosome ends. For example, in Movies S26–S28, the chromosome end traveled with a velocity of 60 nm s^{-1}, whereas an interstitial knob traveled half as fast. Both chromosome landmarks showed similar trajectories, indicating that their movements were coordinated (Fig. 4 *C* and *D*; Movies S26–S28). The telomeric origin of the individual chromosome motions in pachynema was further substantiated by the behavior of chromosome loops whose ends were embedded within the chromatin mass. The outer-most regions of these loops that extended into the unoccupied nucleus space showed limited movements, which resembled the motility of

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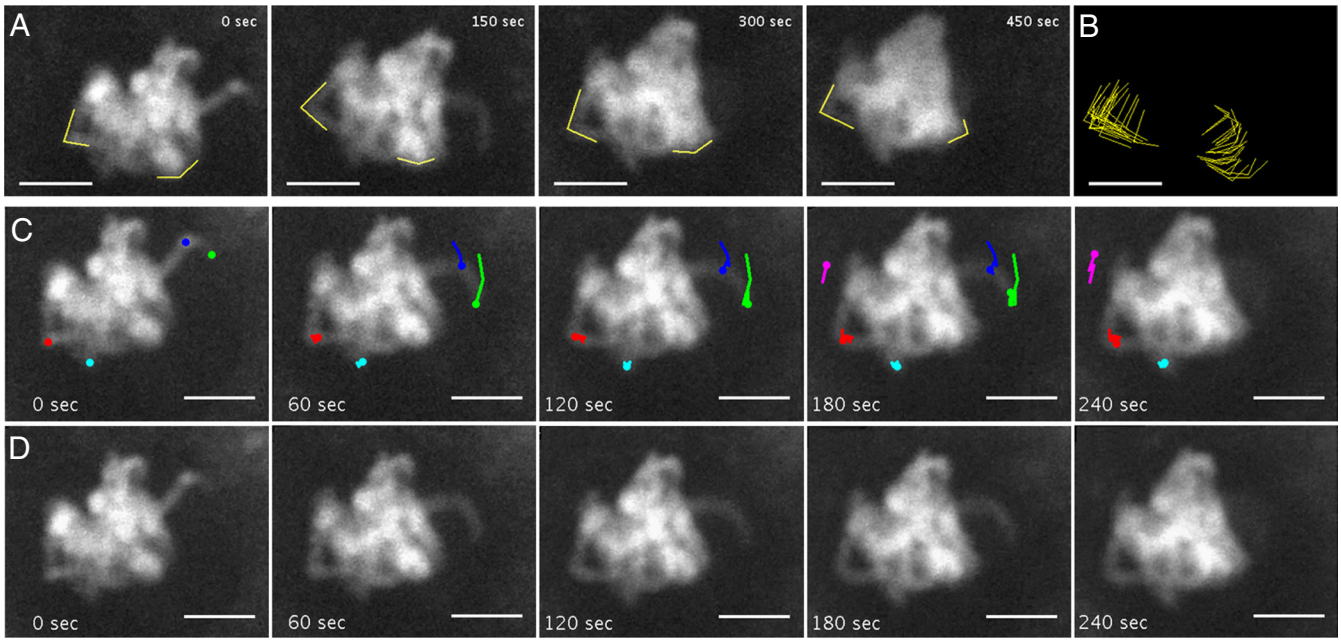

Fig. 4. Chromosome movements in pachynema. (*A*) Rotational movements of the entire chromatin in a pachytene nucleus. Yellow lines mark chromatin mass edges. (*B*) Cumulative tracks from A after 570 s, but without chromatin shown. (*C*) A pachytene nucleus overlaid with trajectories of chromosome marks shown every 60 s for 240 s. Green and blue mark the chromosome end and an interstitial knob, respectively, of a chromosome arm, which exhibits long-distance sweeping movements. Red: a chromosome loop whose both ends are embedded in the chromatin mass; cyan: a stationary chromosome region on the periphery of the chromatin mass; magenta: a free, fast moving chromosome end. (*D*) Same as *C*, but without tracking overlay. (Scale bar, 5 μm.)

chromosome segments inside the chromatin mass rather than the less constrained motility of free chromosome ends (Fig. 4 *C* and *D*; Movies S26–S28).

(*iii*) Chromosome marks located in the center of the chromatin mass also moved more slowly in pachynema than in zygonema and did not travel far from their origin (Fig. 4 *C* and *D*; Movies S26–S28).

Actin and Tubulin Are Both Required for Meiotic Prophase Chromosome Movements. To examine whether components of the cytoskeleton are required for chromosome movements in meiotic prophase in maize, we tested the effects of an actin-depolymerizing drug latrunculin B and a tubulin-depolymerizing drug colchicine (9, 17). An hour-long incubation of anthers in latrunculin B (at concentrations 0.5 or 1 μM) or colchicine (1 or 5 mM) resulted in cessation of all chromosome movements as well as NE deformations in both zygonema and pachynema (Movies S29 and S30). These results suggest that chromosome motility in maize meiocytes requires both actin and tubulin. It is worth noting that Cowan and Cande (17) demonstrated that, in rye, the telomere clustering at the onset of zygonema is sensitive to colchicine, but does not require cytoplasmic microtubules, and suggested that membrane-associated tubulin or tubulins other than β-tubulin may be the colchicine target in this case. We used colchicine concentrations that were higher than those found by Cowan and Cande (17) to specifically disrupt the telomere clustering and that would also disrupt the cytoplasmic microtubules. However, if colchicine has the same effect on chromosome motility in zygonema and pachynema as it does on the prezygotene telomere clustering, it is possible that the nuclear movement cessation after colchicine treatment in our experiments was a result of colchicine disruption of these other tubulins rather than disruption of cytoplasmic microtubules.

Chromatin Movements Do Not Show a Clear Correlation with Cytoplasmic Motility. To investigate how the meiocyte cytoskeleton directs chromosome movements, we compared chromosome motility patterns with the patterns of movement of organelles in the meiocyte cytoplasm, which were visualized with cytoplasmic viability stains. We found that in zygotene meiocytes, cytoplasmic organelles moved at velocities of up to 111 nm s^{-1} over short periods of time and ≈45 nm s^{-1} on average (Fig. S4 and Movies S31–S33). These speeds were much higher than the average 30 nm s^{-1} velocity of chromosome marks in zygotene nuclei. We rarely saw paired movement of two or more organelles, i.e., movement where the velocity and trajectory were similar for all particles involved. When present, these paired movements were short lived, lasting only 20–30 s. In contrast, chromosome marks often moved in coordinated ways. Also, we found no instances where the motility of cytoplasmic organelles was clearly mirrored by movements of chromosome marks (Fig. S4 and Movies S31–S33), suggesting that movements within the nucleus are not a simple reflection of cytoplasmic motility.

Discussion

Monitoring Meiosis in Live Meiocytes. We established a system to track meiosis using MPE microscopy, which permits observation of meiocytes inside intact live maize anthers. This approach has allowed a detailed dissection of chromosome dynamics during meiotic prophase I in a species with a large and complex genome. Keeping meiocytes in their native environment inside the intact anther limits the impact of in vitro culturing and microscopic observations on the progression of meiosis. Several lines of evidence suggest that the chromosome dynamics that we observed reflected normal chromosome behavior and were not results of cellular damage, including: (*i*) cytoplasmic viability staining, (*ii*) proper meiosis progression 24 h after the microscopic observations, (*iii*) stage-specificity of chromosome movements, and (*iv*) cessation of chromosome movements after treatment with cytoskeleton-disrupting drugs while the meiocytes stayed alive (as indicated by cytoplasmic viability stains). To further minimize the potential for photodamage-induced artifacts, we limited the number of consecutive time-lapse exposures of each cell.

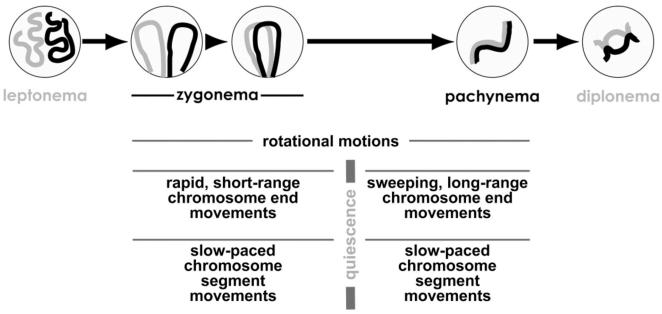

Fig. 5. Comparison of chromosome movement patterns in maize meiocytes in zygonema and pachynema.

Chromosome Movement Patterns During Meiotic Prophase Are Meiosis Stage-Specific. Although both the entire chromatin in the nucleus and individual chromosome segments exhibited dynamic motility during zygonema as well as pachynema, the patterns of movement clearly differed between the two stages (Fig. 5). In zygonema, short chromosome segments extending from the chromatin mass exhibited rapid, abrupt movements. After a period of quiescence at the zygonema-pachynema transition, these movements were supplanted by pachynema-specific motility, where long chromosome segments performed slow sweeping motions across large extents of the nuclear space. Rotational movements of the entire chromatin persisted throughout zygonema and pachynema, including the quiescence period. However, the patterns of these movements also were different in zygonema than in pachynema, with the pachytene movements exhibiting an overall greater magnitude than the zygotene movements.

Multiple Mechanisms Direct Chromosome Motility During Meiotic Prophase in Maize. The presence of the different classes of chromosome movements in maize meiocytes suggests existence of several mechanisms affecting nuclear motility. The rapid zygotene movements of short chromosome segments extending from the chromatin mass were led by the telomeres attached to the NE (24). At the same time, the motility of the NE in zygonema appeared to be caused by forces acting in the cytoplasm. These observations imply that the telomere-led chromosome movements in zygonema originated in the cytoplasmic cytoskeleton, and were transmitted through the NE onto the attached telomeres (4, 12, 20).

The telomere-led slow sweeping motions of large chromosome segments in pachynema were likely generated in a different way than the telomere-led zygotene movements, because by mid-pachynema maize telomeres are no longer attached to the NE after the resolution of the bouquet (24), and in many cases, we could identify that the ends of the moving chromosome arms were inside the nucleoplasm and not on the NE. Consequently, direct telomere attachment to intranuclear cytoskeleton may be involved in these movements. The forces causing these movements may be of intranuclear origin or they may be ultimately of cytoplasmic origin and transmitted through the NE.

Movements of small chromosome segments located inside or near the chromatin mass were more subtle than the motility of chromosome ends. Also, trajectories of adjacent chromosome marks within the chromatin mass were similar. These observations suggest that the movements inside the chromatin mass result from superimposition of forces generated by the movement of chromosome ends and the rotational movements of the entire chromatin, as well as the resistance imposed by inert chromosome segments within the chromatin mass.

Although the motility of short chromosome segments may only need simple "pull and push" forces, the rotational movements of the entire chromatin require more coordination. Although we cannot trace markers directly on the NE, we observed that cytoplasmic organelles immediately adjacent to the NE appeared relatively stationary during the rotational movements, suggesting that only chromatin participates in the rotations, rather than the entire nucleus together with the NE. During zygonema, the source of the rotational movements could be concerted sliding of telomeres along the NE. However, forces responsible for the rotational movements must be distinct from the forces generating the movements of individual chromosome ends because: (*i*) the two movement classes are not coordinated when they cooccur in zygonema, (*ii*) during the quiescence period, only the rotational oscillations are present, and (*iii*) chromosome end attachment to the NE terminates at the end of zygonema, whereas the rotational movements persist through pachynema.

Overall, we postulate that in maize, there are several sources of chromosome movements during the zygonema–pachynema period, in contrast to budding and fission yeasts, where chromosome motility has been suggested to use a single predominant mechanism (20, 25). These sources include (*i*) forces causing chromosome end movements, and (*ii*) forces responsible for the rotational movements of the entire chromatin. The forces generating the chromosome end movements in zygonema are almost certainly of cytoplasmic origin. In pachynema, they may be of cytoplasmic origin as well, although an intranuclear component to these movements cannot be excluded. The forces responsible for the rotational movements may also be of cytoplasmic origin, but they are likely different from the forces causing chromosome end movements. Our observation that chromatin movements do not simply mirror cytoplasmic motility supports the notion of a complex origin of the forces behind chromosome movements, and suggests that different regions of the cytoskeleton affect nuclear movements and cytoplasmic organelle movements. Future investigations will likely identify the specific elements of the cytoskeleton that are involved in chromosome motility. Future studies should also address the regulation of meiotic chromosome movements. Several characteristics of chromosome movements in maize, for example, the back and forth patterns exhibited by some of the movements, suggest that these movements are not random but rather regulated and directional.

Diversity of Meiotic Chromosome Dynamics Patterns Among Species. The detailed analyses of nuclear motility in early meiotic prophase in yeasts (8–12, 20) and maize (this study), and the more fragmentary studies in mouse (14) and rat (13), have shown that patterns of meiotic chromosome movements exhibit immense diversity among species. In fission yeast, nuclear motility was highly dynamic throughout prophase and movements of all chromosomes were coordinated (11). In budding yeast, in contrast, chromosomes moved independently from each other (9, 10, 26), and although the chromosome movements were also observed throughout prophase, they were most complex in early prophase when two distinct classes of chromosome velocity could be identified (10). In mouse spermatocytes, rotational chromatin movements and more subtle movements of individual chromosome segments were observed in zygonema and pachynema (14). The zygotene movements were faster than the pachytene movements, but even the zygotene movements were less dynamic than the movements in yeasts or maize. Rotational movements of the entire chromatin have not been reported in yeast. In contrast, in rat, only rotational movements were reported and only during zygonema (13). Compared with yeasts, rat, and mouse, meiotic prophase chromosome motility patterns in maize seem more complex, exhibiting both concerted rotational movements of the entire chromatin as well as dynamic movements of individual chromosome segments. At least some of these differences may stem from differences in the cytoskeletal forces that are responsible for prophase movements in each species. In fission yeast and rat, the microtubule cytoskeleton is involved in chromosome motility (25, 27), whereas in budding yeast, the actin cytoskeleton is used, but microtubules are not essential (9, 20). In maize, both

actin and tubulin appear to be involved in prophase chromosome movements.

What Is the Significance of Meiotic Prophase Chromosome Movements? The dramatic prophase I nuclear dynamics coincide with several major meiotic processes. Chromosome pairing and most steps of meiotic recombination take place in zygonema. By pachynema, pairing is complete, but chromosomes show frequent entanglements (interlocks) that must be removed (28, 36). Analyses of prophase I chromosome motility shed light on how chromosome mechanics may aid these processes. Chromosome movements have been suggested to facilitate chromosome pairing and disrupt ectopic recombination interactions (10, 13, 20). We propose that the dynamic nature of the zygotene chromosome motility in maize provides an attractive explanation on how homologous loci get into a close vicinity of each other in large and complex genomes so that the recombination-dependent homology search process can take place. The vigorous movements may allow many pairing combinations to be tried until a proper homologous interaction is found. Even though chromosome movements in maize exhibit similar velocities to chromosome movements in budding yeast, meiotic prophase in maize is several fold-longer (22, 29, 30), which could be a reflection of the fact that maize chromosomes are much longer than yeast chromosomes and, consequently, require longer time to find their correct pairing partners. Also, colchicine, which stops nuclear motility in maize, has been shown to cause homologous pairing defects in plant meiocytes (31). The nuclear movements during pachynema in maize show different patterns than the zygotene movements, suggesting that they have a different role. The relatively slow pachytene movements may, for example, aid resolving chromosome interlocks (20). Further functional studies should show whether the different chromosome movement classes in maize meiocytes serve different purposes.

Materials and Methods

Culture Conditions and Solutions. For live microscopy observations, anthers were placed in an eight-chamber culture slide (Nunc-155411, Thermo Fisher Scientific) in the artificial pond water (APW) buffer (32) devoid of growth regulators (18). The medium was supplemented with 50 μg mL^{-1} DAPI, a vital mitochondrial stain (see the Meiocyte viability section below), and DMSO at a concentration of up to 1%. After 1 h, the solution was removed and replaced with the APW buffer to remove unincorporated dyes before imaging. In addition to DAPI, we tested several other vital chromatin stains, including SYTO11, SYTO12, SYTO13, SYTO14, SYTO15, and SYTO16 (Invitrogen), but none of these dyes produced adequate chromosome staining in our system. DMSO was used to aid the penetration of stains and the cytoskeleton-disrupting drugs. Although DMSO is known to polymerize microtubules in vitro, it does so at much higher concentrations than the concentrations we used (33, 34). Also, we did not observe any differences in either chromosome or cytoplasmic motility using DMSO concentrations ranging from 0.1 to 5%.

Meiocyte Viability. To monitor cell viability, we tested three dyes that specifically stain live mitochondria, Rhodamine 123, $DiOC_7$ (3), and Mitotracker Green FM (Invitrogen). Rhodamine 123 at a concentration of 20 μM (21) showed the best penetration of the inside of the anther of the three stains tested and was used for the majority of our experiments.

Cytoskeleton-Disrupting Drugs. Latrunculin B (Sigma–Aldrich) (35) was used at concentrations of 500 nM and 1 μM in the APW medium. Colchicine (Sigma–Aldrich) (17) was used at concentrations of 1 and 5 mM. In the drug treatment experiments, anthers were incubated in the APW medium containing DAPI, DMSO, and colchicine or latrunculin B for 1 h. After DAPI staining, this solution was replaced with a wash solution comprised of APW medium supplemented with the same concentration of colchicine or latrunculin B as used during the 1-h DAPI incubation.

Live Imaging. Anthers were imaged in the slide chambers on a Bio-Rad MR1024MP workstation equipped with an Olympus IX70 microscope. A tunable Ti:Sapphire laser (Coherent) was set to 780–800 nm, and the pump laser was set to 4.5 to 5.5 W. These settings produced laser power of 525 - 900 mW. The 20× UAPO water (0.7 WD 0.4 mm) and 40× UAPO water (1.15 WD 0.4 mm) lenses with up to 10× additional digital zoom were used to visualize meiocytes. For most images, a normal speed scan of 488 lines per second was used with three scans of Kalman filter averaging to improve image signal to noise ratio. Images were collected using the LaserSharp image acquisition software (Bio-Rad). Image analyses were performed in ImageJ (National Institutes of Health) and Imaris (Bitplane).

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