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Oscillatory nuclear movement in fission yeast meiotic prophase is driven by astral microtubules, as revealed by continuous observation of chromosomes and microtubules in living cells

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SUMMARY

Using a computerized fluorescence microscope system to observe fluorescently stained cellular structures in vivo, we have examined the dynamics of chromosomes and microtubules during the process of meiosis in the fission yeast *Schizosaccharomyces pombe*. Fission yeast meiotic prophase is characterized by a distinctive type of nuclear movement that is led by telomeres clustered at the spindle-pole body (the centrosome-equivalent structure in fungi): the nucleus oscillates back and forth along the cell axis, moving continuously between the two ends of the cell for some hours prior to the meiotic divisions. To obtain a dynamic view of this oscillatory nuclear movement in meiotic prophase, we visualized microtubules and chromosomes in living cells using jellyfish green fluorescent protein fused with α-tubulin and a DNA-specific fluorescent dye, Hoechst 33342, respectively. Continuous observation of chromosomes and microtubules in these cells demonstrated that the oscillatory nuclear movement is mediated by dynamic reorganization of astral microtubules originating from the spindle-pole body. During each half-oscillatory period, the microtubules extending rearward from the leading edge of the nucleus elongate to drive the nucleus to one end of the cell. When the nucleus reversed direction, its motion during the second half of the oscillation was not driven by the same microtubules that drove its motion during the first half, but rather by newly assembled microtubules. Reversible inhibition of nuclear movement by an inhibitor of microtubule polymerization, thiabendazole, confirmed the involvement of astral microtubules in oscillatory nuclear movement. The speed of the movement fluctuated within a range 0 to 15 μm/minute, with an average of about 5 μm/minute. We propose a model in which the oscillatory nuclear movement is mediated by dynamic instability and selective stabilization of astral microtubules.

Key words: *Schizosaccharomyces pombe*, Meiosis, Microtubule, Nuclear movement, GFP

INTRODUCTION

Microtubule-mediated nuclear migration has been observed in a wide variety of organisms. During early embryogenesis in *Drosophila*, nuclear migration from the interior of the embryo to the embryo cortex requires the presence of functional microtubules (Zalokar and Erk, 1976; Baker et al., 1993; von Dassow and Schubiger, 1994). At the two-cell stage of *C. elegans*, the oriented nuclear rotation that determines the cell division axis also requires microtubules (Hyman and White, 1987; Hyman, 1989). In the fertilized eggs of many species, migration of the male pronucleus is driven by asters of microtubules nucleated by the sperm-derived centrosome (Schatten, 1982; Briedis and Elinson 1982; Hamaguchi and Hiramoto, 1986; Navara et al., 1994; Rouviere et al., 1994). During plant morphogenesis, premitotic nuclear migration mediated by microtubules and/or actin filaments generates the cell polarity leading to asymmetrical differential cell divisions (Galatis et al., 1986; Kennard and Cleary, 1997). In the multinuclear filamentous fungus *Aspergillus nidulans*, nuclei migrate into the germ tube after germination in a microtubule-dependent fashion (Oakley and Morris, 1980, 1981; Xiang et al., 1994). In the budding yeast *Saccharomyces cerevisiae*, migration of the short mitotic spindle to the bud neck is dependent on the astral microtubules, which radiate from the spindle-pole body (SPB) (Sullivan and Huffaker, 1992; Palmer et al., 1992; Carminati and Stearns, 1997).

In these examples of nuclear migration, the nuclei move to their proper positions, where movement subsequently ceases. On the other hand, a unique type of nuclear movement has been observed during meiotic prophase in the fission yeast *Schizosaccharomyces pombe*: the nucleus migrates back and forth along the cell axis, oscillating between the two ends of the cell continuously for some hours prior to the meiotic divisions (Chikashige et al., 1994). This continuous oscillatory movement is a striking feature of *S. pombe* nuclear movement

and contrasts with the simple nuclear repositioning type of movement exhibited by other organisms. During the period of oscillatory nuclear movement in *S. pombe*, the nucleus shows a characteristic elongated morphology. Due to its shape, this elongated nucleus is generally called the 'horse-tail' nucleus (Robinow, 1977; Robinow and Hyams, 1989). Analysis by in situ hybridization revealed that the telomeres are clustered at a single locus near the SPB, which is located at the leading edge of the nucleus during oscillatory nuclear movement while centromeres are separated from the SPB; once meiotic chromosome segregation starts, however, centromeres resume the position near the SPB, as they do in mitosis (Chikashige et al., 1994, 1997).

Cytological observations of microtubule arrangement have been obtained in fixed specimens of both mitotic and meiotic cells of fission yeast (Hagan and Hyams, 1988; Svoboda et al., 1995; Hagan and Yanagida, 1995). In mitotic interphase, several arrays of cytoplasmic microtubules extend along the length of the cell and the SPB is usually located on one of these microtubule bundles, most of the cytoplasmic microtubules not being associated with the SPB. Unlike the interphase cytoplasmic microtubules, mitotic nuclear microtubules do associate with SPBs forming a spindle within the mitotic nucleus (Hagan and Hyams, 1988). When fission yeast cells are induced to undergo meiosis the cytoplasmic microtubules significantly change their organization and originate exclusively from the SPB, both during karyogamy and meiotic prophase (Svoboda et al., 1995; Hagan and Yanagida, 1995). From these observations of microtubule arrangement in fixed specimens, it has been inferred that astral microtubules are involved in the oscillatory movement of the nucleus during meiotic prophase. However, direct observations of microtubule dynamics over time in living cells undergoing meiosis have not been done.

In this study, we examined the behavior of microtubules in individual living cells and obtained a dynamic view of microtubule rearrangement during the process of meiosis. In living meiotic cells, microtubules were stained by the use of jellyfish green fluorescent protein (GFP) fused with tubulin and chromosomes were stained with a DNA-specific fluorescent dye, Hoechst 33342. Fluorescently stained living cells were observed using a fluorescence microscope system with a computerized wavelength control to record multiple-color images. Simultaneous observation of chromosomes and microtubules in living cells revealed that the oscillatory nuclear movement characteristic of fission yeast meiotic prophase was driven by a continual reorganization of the astral microtubules originating from the SPB. Live observations also demonstrated that nuclear movement was reversibly inhibited by the addition of a microtubule polymerization inhibitor. From our results, we propose a molecular mechanism by which oscillatory movement of the horse-tail nucleus during meiotic prophase is directed by reorganization of astral microtubules.

MATERIALS AND METHODS

Strains and culture conditions

S. pombe strains used in this study were HM143 (*h*^{90} *ade6-210*) and CRL126 (*h*^{90} *leu1-32 ura4*). Complete medium YEade (YE containing 75 μg/ml adenine sulphate) and minimum medium EMM2 were used for routine culture of the strain HM143 (Moreno et al., 1991). The strain CRL126 was used for transformation with the GFP fusion plasmid pDQ105 (see below); transformed cells were cultured in EMM2 supplemented with 75 μg/ml uracil and 2 μM thiamine. For observation of mating and meiosis, *h*^{90} strains were cultured in EMM2 supplemented with appropriate nutrients, washed in EMM2-N (EMM2 deprived of nitrogen) and incubated in EMM2-N at 25°C.

Preparation of cell extracts and western immunoblotting

Cell extracts for western immunoblotting were prepared according to Moreno et al. (1991) and Tange et al. (1998) with modifications. 3×10^7 cells were harvested and washed once with the ice-cold stop buffer (150 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM NaN_3, pH 8.0). Cells were resuspended in 200 μl of disruption buffer (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 10% glycerol, 30 mM NaCl, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 μg/ml pepstatinA, 20 μg/ml leupeptin, 40 μg/ml aprotinin and 0.1 mg/ml L-1-chloro-3-(4-tosylamido)-4-phenyl-2-butanone (TPCK)), and disrupted with 0.3 g glass beads (425-600 μm diameter, Sigma) by vortexing 10 times each for 1 minute at 4°C. 50 μl of loading buffer (0.25 M Tris-HCl, pH 6.8, 40% glycerol, 4% SDS and 40 μg/ml Bromophenol Blue) were added to the disrupted cell suspensions, which were then boiled for 5 minutes. The cell disruptions were then centrifuged at 15000 ***g*** for 10 minutes. The supernatants were electrophoresed on 10% SDS-polyacrylamide gels and transferred to a polyvinylidene fluoride membrane Immobilon-P (Millipore). Monoclonal anti-α-tubulin antibody DM1A (Sigma) was used to detect α-tubulin, and polyclonal anti-GFP antibody (Clontech) was used to detect the GFP fusion protein. Protein detection was performed by using an immunoblotting ABC-POD(M) kit (Wako).

Microscope system setup

Fluorescence images were obtained on a cooled, charge-coupled device (CCD) as an image detector using a computer-controlled, fluorescence microscope system. In our microscope system, a Peltier-cooled CCD camera (Photometrics Ltd, Tucson, Arizona) with a 1340×1037 pixel CCD chip (KAF1400) coated to improve short-wavelength sensitivity (Metachrome II coating, Photometrics Ltd), is attached to an Olympus inverted microscope IMT-2; microscope lamp shutter, focus movement, CCD data collection and filter combinations are controlled by a Silicon Graphics Personal Iris 4D35/TG. For wavelength switching during data collection, excitation and barrier filters are mounted on revolving wheels controlled by the Silicon Graphics workstation. Details of the microscope system setup are described in Hiraoka et al. (1991), except that the MicroVAX was replaced by the Silicon Graphic workstation.

Immunofluorescence microscopy

Cells were fixed with 3% formaldehyde (freshly prepared from paraformaldehyde) and 0.2% glutaraldehyde in PEM buffer (100 mM Pipes, pH 6.9, 1 mM EGTA, 1 mM $MgCl_2$) for 10 minutes at room temperature. After fixation, cells were permeabilized as described in Funabiki et al. (1993). The position of the SPB was determined by indirect immunofluorescence microscopy using an antibody against the fission yeast *sad1* gene product, a well-characterized marker of the SPB in fission yeast mitotic cells (Hagan and Yanagida, 1995), and a Texas Red-conjugated second antibody. TAT1 anti-α-tubulin antibody (Woods et al., 1989) and a fluorescein-conjugated second antibody were used to visualize microtubules. Immunofluorescence labeling was carried out in PEM buffer as described in Hagan and Hyams (1988). Nuclear DNA was counterstained with 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI). Triple-color fluorescent images of DAPI, fluorescein and Texas Red were obtained on the computerized CCD microscope using an Olympus oil immersion objective lens (DPlan Apo 100/NA=1.3). High-selectivity excitation and barrier filter combinations (Chroma Technology, Brattleboro, Vermont) for DAPI, fluorescein and Texas Red were used on revolving wheels

under computer control. A single dichroic mirror with triple-band pass properties designed for wavelengths of DAPI, fluorescein and Texas Red (Chroma Technology, Brattleboro, Vermont) was used to eliminate significant displacement of images during wavelength switching, and thus no further alignment was necessary (Hiraoka et al., 1991).

Green fluorescent protein fusion

The *S. pombe* expression vector pREP1 with the thiamine-regulatable, wild-type *nmt1* promoter (Maundrell, 1993) was employed to construct a plasmid bearing the GFP-α2-tubulin fusion gene. The plasmid pREP1 contains the *S. pombe ars1* and the *S. cerevisiae LEU2* gene as the selection marker. The coding sequence of GFP-S65T (Heim et al., 1995) was amplified by PCR using GFP-S65T cloned in pRSET-B (a gift of Dr Tsien) as a template, with a 5′ primer TACCGCTCGAGTATGAGTAAAGGAGAAGAAACTT and a 3′ primer CGCGGATCCTTTGTATAGTTCATCCATGCC. The PCR product, which contains the full-length GFP open reading frame and no termination codon, was digested with *Xho*I and *Bam*H1, and cloned into pREP1 digested with *Sal*I and *Bam*H1, constructing a GFP expression vector driven by the *nmt1* promoter; this vector is referred to as pEG5. The coding region of the α2-tubulin gene (NDA2-2) was amplified by PCR using pDB(NDA2-2) (Toda et al., 1984) as a template, with a 5′ primer GGGCCCGGATCCATGAGAGAGATC-ATTTCC and a 3′ primer GTGCACGATATCTTAGTACTCTT-CTTCCAT. The PCR product of α2-tubulin was digested with *Bam*H1 and *Eco*RV, and cloned into the pEG5 vector digested with *Bam*H1 and *Sma*I. The resulting plasmid pDQ105 expresses GFP fused to the N-terminus of α2-tubulin, GFP-α2-tubulin, under the control of the *nmt1* promoter. A wild-type strain with an auxotrophic marker, CRL126, was transformed with the multicopy expression plasmid pDQ105. Determination of the nucleotide sequences of GFP-α2-tubulin verified that no base substitutions were introduced into the GFP or α2-tubulin genes during PCR cloning. During the verification process, we reexamined nucleotide sequences of the original α2-tubulin clone (Toda et al., 1984), and found errors in the published sequences at two amino acid residues: Asp (GAC) at residue 81 and Gly (GGC) at residue 126 should be corrected to Gly (GGC) and Ala (GCC), respectively. With these corrections, amino acid sequences of α2-tubulin turn out to be identical to those of α1-tubulin at the corresponding residues.

Fluorescence in vivo imaging of microtubules and chromosomes

Cells bearing the GFP-tubulin fusion construct pDQ105 were cultured in EMM2 supplemented with 75 μg/ml uracil and 2 μM thiamine, culture conditions which repress the *nmt1* promoter. Under conditions that induce transcription from the *nmt1* promoter, i.e. in the absence of thiamine, the induced high levels of GFP-α2-tubulin expression were lethal to the transformed cell. In the presence of 2 μM thiamine, the GFP-α2-tubulin fusion protein was expressed at levels which did not affect the mitotic growth or sporulation of the transformed cells but were sufficient to efficiently stain microtubules.

For staining chromosomes and microtubules simultaneously in living meiotic cells, cells producing GFP-α2-tubulin fusion protein were transferred to EMM2-N medium to induce meiosis. During induction of meiosis and microscopic observation, EMM2-N was not supplemented with uracil or thiamine. Meiotic cells were then stained with Hoechst 33342 (0.5 μg/ml) in distilled water for 10 minutes at a room temperature and then resuspended in EMM2-N. The living fluorescently stained cells were mounted either on a coverslip or in a 35 mm glass-bottom culture dish (MatTek Corp., Ashland, MA) coated with concanavalin A (1 mg/ml), and observed in EMM2-N at 25°C on the CCD microscope system using an Olympus oil immersion objective lens (SPlan Apo 60/NA=1.4). Images were obtained on the cooled CCD with an exposure time of 0.1-0.2 seconds under the illumination of a mercury arc lamp. An excitation filter with a narrow peak at 380 nm for Hoechst 33342 and a high-selectivity fluorescein excitation filter for GFP, in combination with high-selectivity barrier filters for DAPI and fluorescein (Chroma Technology, Brattleboro, Vermont), were used on revolving wheels under computer control. A single dichroic mirror with triple-band pass properties designed for wavelengths of DAPI, fluorescein and Texas Red (Chroma Technology) was used.

In experiments using thiabendazole (TBZ) treatment, fluorescently stained cells were mounted in a 35 mm glass-bottom culture dish (MatTek Corp., Ashland, MA) coated with concanavalin A (1 mg/ml), and TBZ was added to the culture medium at a final concentration of 40 μg/ml and removed by replacing the culture medium with fresh TBZ-free medium. By using a glass-bottom culture dish on an inverted microscope, the culture medium can be supplemented or replaced while the dish remains on the microscope stage throughout the period of microscopic observation.

RESULTS

Fluorescence imaging of microtubules by GFP-tubulin fusion protein in living fission yeast cells

In the fission yeast *Schizosaccharomyces pombe*, meiotic prophase is characterized by the repeated movement of the elongated horse-tail nucleus from one end of the cell to the other (Chikashige et al., 1994). Observations of microtubule arrangement in fixed specimens show that arrays of microtubules are associated with the horse-tail nucleus (Svoboda et al., 1995; Hagan and Yanagida, 1995). Moreover, it has been observed in synchronous populations of fixed meiotic cells that the horse-tail nuclei disappear in the presence of an inhibitor of microtubule polymerization, TBZ (Svoboda et al., 1995). These observations have suggested that microtubules are involved in the movement of the horse-tail nucleus. As demonstrated below (see Fig. 7), we confirmed this idea by examining the effects of TBZ treatment on horse-tail nuclear movement in living cells over time, during which nuclear movement was inhibited by addition of TBZ and was recovered after removal of TBZ.

Thus, to understand the mechanism by which microtubules mediate oscillatory nuclear movement, we wished to observe the behavior of microtubules in living fission yeast cells. To this end, we constructed a fusion protein in which GFP is fused with tubulin. The genome of *S. pombe* has two α-tubulin genes and one β-tubulin gene; one of the two α-tubulin genes (designated α1-tubulin) and the β-tubulin gene are essential for mitotic growth whereas the other α-tubulin gene (designated α2-tubulin) is non-essential (Toda et al., 1984; Hiraoka et al., 1984; Adachi et al., 1986). Also, it is known that transformation of *S. pombe* cells with the β-tubulin gene on a multicopy plasmid is lethal (Hiraoka et al., 1984). Thus, we selected the non-essential α2-tubulin to construct the GFP-tubulin fusion on a multicopy plasmid as a fluorescent marker of microtubules: GFP is fused to the N terminus of fission yeast α2-tubulin (designated GFP-α2-tubulin) and the GFP-α2-tubulin construct is under the regulation of the wild-type *nmt1* promoter.

Under conditions that repress the *nmt1* promoter, the GFP-α2-tubulin fusion protein was expressed at levels lower than authentic α2-tubulin, as estimated by western immunoblotting (Fig. 1; see legend); under these conditions, GFP-α2-tubulin brightly stained cytoplasmic microtubules as well as spindle

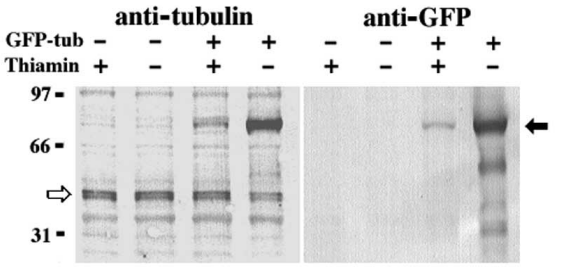

Fig. 1. Expression of the GFP-α2-tubulin fusion gene. Western immunoblotting with anti-α-tubulin antibody (left) and anti-GFP antibody (right). Positions of molecular mass markers (in kDa) estimated using a Bio-Rad molecular mass standard. The band of GFP-α2-tubulin (molecular mass 77,619; filled arrow) was detected only in cells bearing the GFP-α2-tubulin fusion construct (right panel). The corresponding band was also detected with anti-α-tubulin antibody (left), which also detected authentic α1-tubulin and α2-tubulin (molecular mass 51,200 and 50,600, respectively; open arrow) in addition to the GFP-α2-tubulin fusion protein.

microtubules in living cells without affecting the mitotic growth or sporulation (for details of culture conditions, see Materials and methods). Fig. 2 shows the mitotic behavior of the spindle microtubules stained with GFP-α2-tubulin in a single living cell, demonstrating the normal morphology and behavior of the mitotic spindle and the normal progression of mitosis. Also in meiotic cells expressing uninduced levels of GFP-α2-tubulin, the processes of meiosis proceeded normally and viable spores were formed. Moreover, the morphology of microtubules visualized with GFP-α2-tubulin in living cells was essentially identical to their morphology as visualized by indirect immunofluorescence staining using an anti-tubulin antibody (compare Fig. 3A with B-E). Thus, GFP-α2-tubulin acts as a fluorescent marker of microtubules in mitosis as well as in meiosis in living fission yeast cells under conditions in which the wild-type *nmt1* promoter is repressed.

Reorganization of microtubule arrays upon the onset of oscillatory movement of the horse-tail nucleus

Use of the GFP-α2-tubulin fusion construct allows observation of chromosomes and microtubules simultaneously in living cells. To investigate how the oscillatory movement is initiated, we examined the arrangement of microtubules during the transition from karyogamy to the horse-tail period in living and fixed cells. Fig. 3A shows the behavior of microtubules and chromosomes observed in a single living cell over time from karyogamy to meiotic prophase. Fig. 3B-E shows indirect immunofluorescence staining of cells fixed at meiotic stages corresponding to those observed in the living cell. In the living cell shown in Fig. 3A, two haploid nuclei can be observed approaching each other during early karyogamy; at this stage, a bundle of microtubules was seen between the approaching nuclei (Fig. 3A, frames at 0-2 minutes). This stage corresponds to the fixed cell shown in Fig. 3B, in which two separate SPBs are connected by a bundle of microtubules. Later, microtubules converged at the midpoint between the two nuclei to form an X-shaped array (Fig. 3A, frames at 3-10 minutes). Finally, the two haploid nuclei came together and stopped at the center of the zygote. At this stage, a single SPB was located at the center of the X-shaped microtubule array as observed in the fixed cell shown in Fig. 3C.

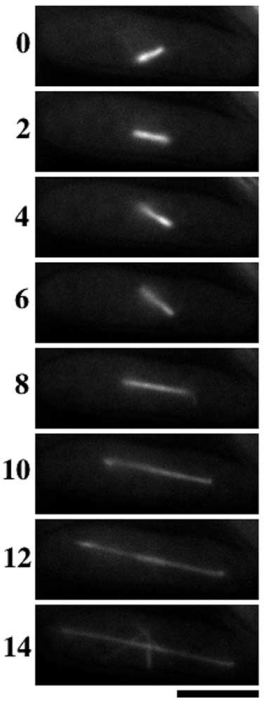

Fig. 2. Mitotic behavior of microtubules in a living fission yeast cell. Cells bearing the GFP-α2-tubulin fusion gene were observed in EMM2 medium. Numbers on the left represent time in minutes. Bar, 5 μm.

Microtubules retained the basically same overall X-shaped arrangement for some time (5-23 minutes in Fig. 3A). The SPB then started to move toward one end of the cell and the X-shaped array of microtubules centered at the SPB were transformed to the horse-tail arrays of microtubules typical of meiotic prophase (Fig. 3A, 24 minutes). This stage corresponds to the fixed cell shown in Fig. 3D. This figure also shows small portions of chromatin, one from each nucleus, being pulled together while the major portions of chromatin remain separated. The nuclei, joined at their tips, then began to oscillate back and forth (Fig. 3A, 24-31 minutes). After several oscillations, the remainder of the two nuclei fused, forming the horse-tail nucleus (Fig. 3A, 32-34 minutes). In the horse-tail period, bright staining of one or two bundles of microtubules extending from the leading edge of the nucleus rearward along the long axis of the cell can be seen (Fig. 3E). Microtubules extending forward from the nucleus exhibited dimmer staining (Fig. 3E). Similar observations in fixed specimens have been obtained in previous studies (Svoboda et al., 1995; Hagan and Yanagida, 1995). These results show that while microtubules radiate from the SPB during the entire process from karyogamy to meiotic prophase, karyogamy-type X-shaped arrays of microtubules are reorganized to the bundles of microtubules along the cell axis, typical of meiotic prophase, prior to the start of oscillatory nuclear movement.

Elongation of rearward-extending microtubules during meiotic prophase nuclear movement

During the oscillatory movement of the horse-tail nucleus from one end of the cell to the other, microtubules can be seen extending from the leading edge of the nucleus both rearward, in the direction opposite that of nuclear movement, and forward,

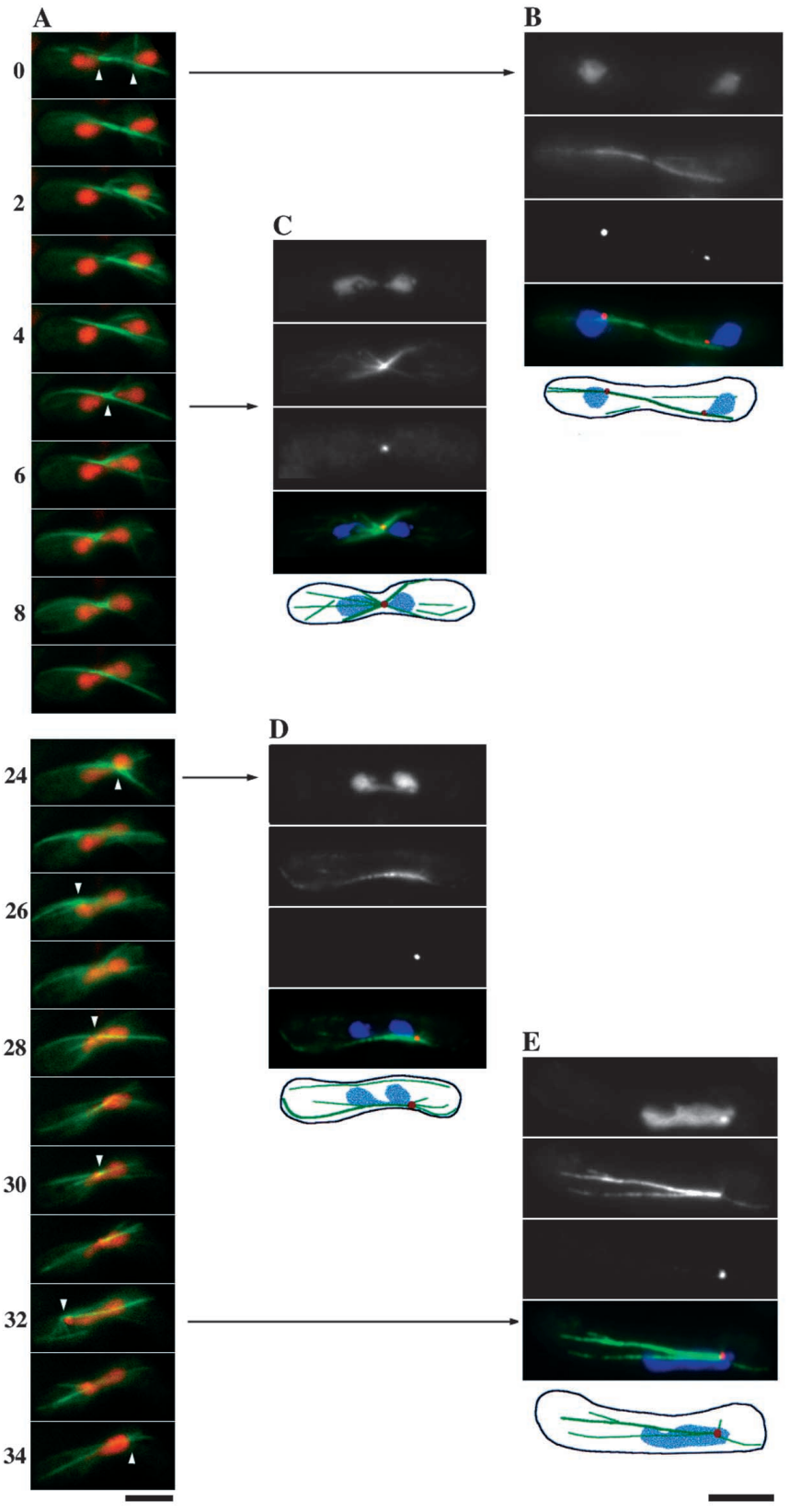

Fig. 3. Arrangement of microtubules in meiotic cells. (A) Behavior of chromosomes and microtubules in a living meiotic cell. Microtubules were visualized with GFP-α2-tubulin (green) and the nuclei were counterstained with Hoechst 33342 (red). Images of a time-lapse series obtained from a single cell are shown. Numbers at the left side of each column are time in minutes. Images from 10 to 24 minutes are not shown because the positions of the nucleus during this period were almost the same as at 9 minutes. All of the images were taken in a single focus plane. Arrowheads indicate the positions of the SPBs. Bar, 5 μm.
(B-E) Immunofluorescence staining of microtubules in fixed meiotic cells. The five images each in panels B-E show in order (from top to bottom): DAPI staining of chromatin; TAT1 antibody staining of microtubules; anti-Sad1 antibody staining of the SPB; a merged image of DAPI (blue), microtubule (green) and SPB (red); and a cartoon. The image of the microtubules is a projection of images at the different focus planes that contained most of the fluorescent signals. The other images are from a single focus plane. Bar, 5 μm.

in the direction of nuclear movement (Fig. 3E). One might imagine that the nucleus is sliding on a single continuous bundle of microtubules along the cell axis. To obtain dynamic views of these microtubules during oscillatory nuclear movement, we examined the behavior of microtubules during meiotic prophase in living cells using the GFP-α2-tubulin fusion protein. The behavior of microtubules during approx. one full period of oscillatory nuclear movement is shown in Fig. 4.

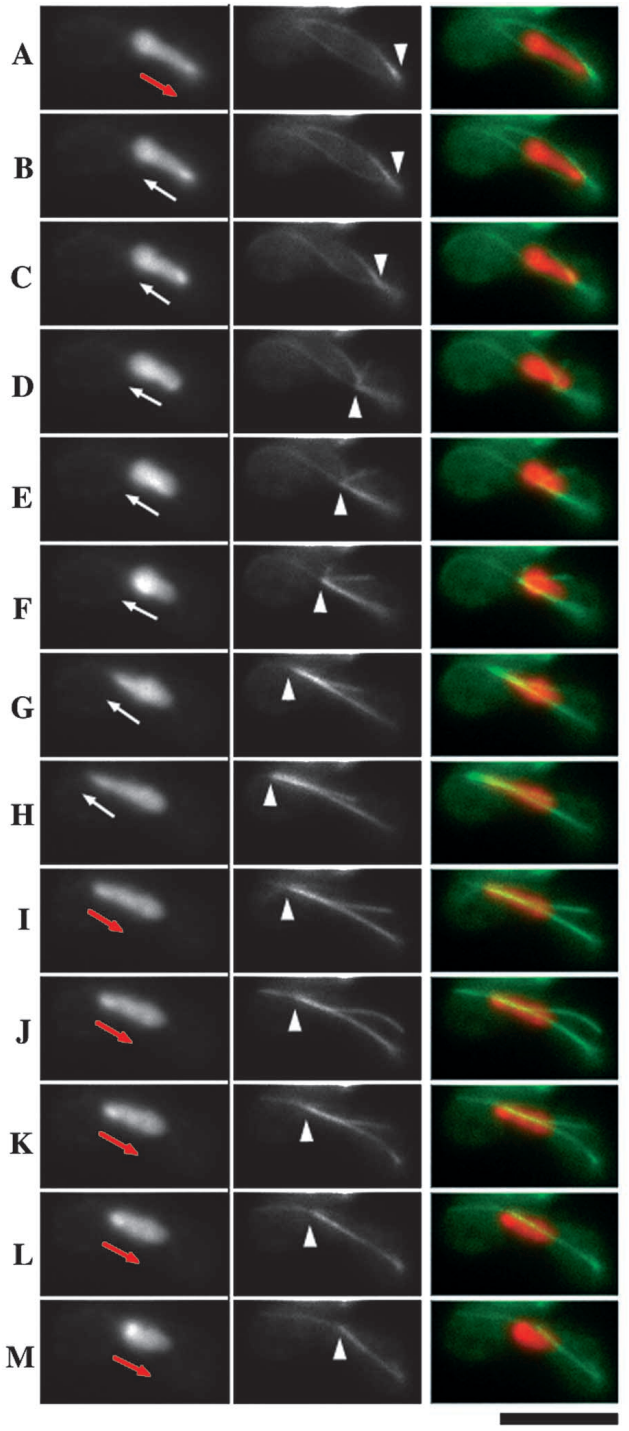

Fig. 4. Dynamics of microtubules during oscillatory movement of the horse-tail nucleus in a living cell. A nucleus was stained with Hoechst 33342 (left) and microtubules were stained with GFP-α2-tubulin (middle). In merged images (right), microtubules and chromosomes are displayed in green and red, respectively. Time-lapse images (A-M) were taken every 10 seconds. The leading edge of the moving nucleus is indicated by an arrowhead while the direction of movement is indicated by white or red arrows. Bar, 5 μm.

Analysis of microtubule dynamics in living cells revealed that the microtubules were not static, but instead were being dynamically reorganized for each half oscillation of the nucleus. A bundle of microtubules was newly formed rearward of the oscillating nucleus each time the nucleus reversed direction. The position of the leading edge of the moving nucleus (arrowheads in Fig. 4) moves in accompaniment with elongation of the rearward-extending microtubule bundles (Fig. 4C-G; also see Fig. 5). Throughout each half oscillation, while the nucleus is moving toward one end of the cell, the rear ends of the elongating microtubule bundles remained fixed in position (white asterisk in Fig. 5; also see Fig. 4G,H). Thus, it seems that the rear ends of these microtubule bundles are anchored at the cell cortex; when these anchored bundles of microtubules elongate, the nucleus is pushed forward (Fig. 9A; see Discussion). In this report, hereafter, it is convenient to use the term ‘anchors’ to refer to the stationary rear ends of microtubules, but it should be mentioned that such microtubule anchors are putative structures and their existence has not been proved.

The horse-tail nucleus reverses its direction of movement after microtubule anchors are disengaged

During oscillatory nuclear movement, arrays of astral microtubules were seen extending both forward and rearward from the leading edge of the nucleus. When the approaching nucleus neared the end of the cell, the nucleus often paused before reversing direction. The pause is presumably caused by a balance of forces between the forward- and rearward-extending arrays of microtubules (Fig. 9B; see Discussion). Before the nucleus restarted movement toward the opposite end of the cell, abrupt elongation of the rearward-extending microtubule bundles was observed (red asterisk in Fig. 5; also see Fig. 4H,I). It should be noted that the position of the rear end of these microtubules was displaced (white and red asterisks in Fig. 5) while the leading edge of the nucleus remained stationary (arrowhead in Fig. 5). This observation suggests that at this point in the oscillation of the horse-tail nucleus, the rearward-extending microtubules become disengaged from the cell cortex and consequently they elongate; however this elongation no longer forces the nucleus to move, but rather displaces the free ends of the rearward-extending microtubules. Contrary to the rearward-extending microtubules, the forward-extending microtubules become anchored at the cell cortex at this time; as these forward-extending microtubules stably elongate, the leading edge of the nucleus turns and begins moving toward the opposite end of the cell (Fig. 9C; see Discussion).

After the nucleus reversed direction, the microtubule bundles that had been pushing the nucleus in the preceding half oscillation remained at the front side, in the direction of movement of the nucleus (Fig. 4I-M; also see Figs 5 and 6). As the nucleus moved forward, the forces generated by the rearward-extending bundles of microtubules often caused the forward-extending bundles of microtubules to curve at their far ends (asterisk in Fig. 6). Thus, the forward-extending bundles of microtubules do not seem to generate the forces which direct nuclear movement. These forward-extending bundles of microtubules seemed to be relatively stable but did gradually disassemble during the period of the ongoing half oscillation.

During nuclear movement in the new direction, the radial arrays of short rearward-extending microtubules elongated and became bundled (Figs 4F-G, 5B and 6B). This bundle of

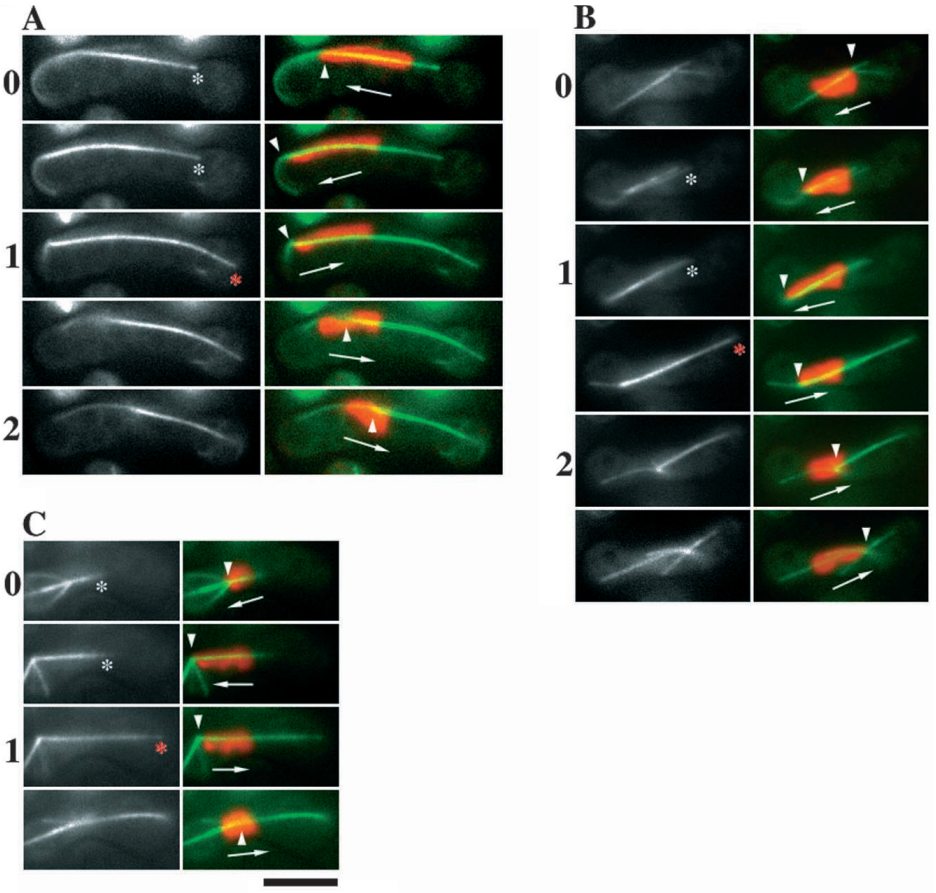

Fig. 5. Elongation of rearward-extending microtubules just prior to reversal of nuclear motion. Microtubules were visualized with GFP-α2-tubulin (green), and the nucleus was counterstained with Hoechst 33342 (red). Three separate series of time-lapse images (A-C) were taken every 30 seconds. The examples show the abrupt elongation of rearward extending microtubule bundles (red asterisks) immediately before the nucleus reversed direction. Arrowheads show the position of the leading edge of the nucleus. Bar, 5 μm.

rearward-extending microtubules continued to elongate until the nucleus reached the other end of the cell, as described above for the first half of the oscillation period.

Force-generating microtubules originate exclusively from the SPB

Throughout the period of horse-tail nuclear movement, microtubules originated exclusively from the SPB. To confirm that the SPB acted as the microtubule-organizing center, we examined the behavior of microtubules during the recovery of nuclear movement following treatment of living cells with the microtubule polymerization inhibitor, TBZ. Addition and removal of TBZ was carried out on the microscope stage during microscopic observation of the microtubules and chromosomes in individual living cells over time (Fig. 7). Before the addition of TBZ, the elongated nucleus was oscillating back and forth within the cell. While this movement was being observed under the fluorescence microscope, TBZ was added to the culture medium at a final concentration of 40 μg/ml. Nuclear movement stopped shortly after TBZ was added to the culture medium, and soon thereafter microtubule disassembly occurred. After a few minutes in TBZ, only residual short microtubules were observed radiating from the SPB (Fig. 7, 6-10 minutes). After most of the microtubules were disassembled, TBZ was removed by replacement with fresh culture medium. After removal of TBZ, microtubules reassembled at the SPB and nuclear movement restarted as the microtubules begin to elongate (Fig. 7, 12-19 minutes). These results confirm that the horse-tail nuclear movement in meiotic prophase is mediated by microtubules originating from the SPB.

Paths of the horse-tail nuclear movement traced in living cells

Our live observations have demonstrated that the horse-tail nucleus repeats its back-and-forth oscillations at an irregular speed. To obtain kinetic data on the movement of the horse-tail nucleus, we traced the path of the leading edge of the nucleus on microscopic images collected every 10 seconds. Examples of several path tracings are shown in Fig. 8: the horse-tail nucleus was oscillating back and forth, moving from one end of the cell to the other, and the position of the leading edge of the nucleus within the cell was plotted separately for each half oscillation (Fig. 8A and B, left). Superimposition of the paths of the oscillations shows that the leading edge moved on a different path during each traverse of the cell (Fig. 8A and B, bottom). These observations are consistent with the result that microtubules are reorganized each time the nucleus reverses its direction of movement.

The velocity of movement, measured for several 10-second periods, was plotted as a function of time (Fig. 8A and B, right). Motion analysis shows that the speed of movement of the horse-tail nucleus was quite variable, but overall it was relatively rapid; most of the time its speed was significantly faster than that of mitotic nuclear division, in which nuclei separate from each other at a constant speed of about 1 μm/minute in fission yeast (Hiraoka et al., 1984). In measurements from five cells, the average speed over the entire period of observation, including the period of direction reversal, was 4.6 μm/minute with a maximum instantaneous velocity of 15.6 μm/minute (Fig. 8C). 78% of the time, the leading edge of the horse-tail

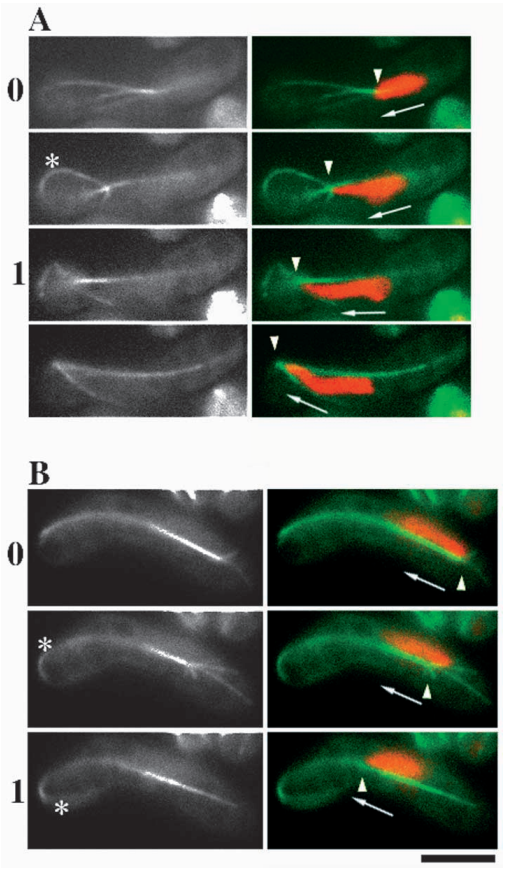

Fig. 6. Bending of forward-extending microtubules after reversal of nuclear motion. Microtubules were visualized with GFP-α2-tubulin (green), and the nucleus was counterstained with Hoechst 33342 (red). Two series of time-lapse images (A,B), taken every 30 seconds. Asterisks indicates a bundle of microtubules curved as if it was pushed. Arrowheads show the position of the leading edge of the nucleus. Bar, 5 μm.

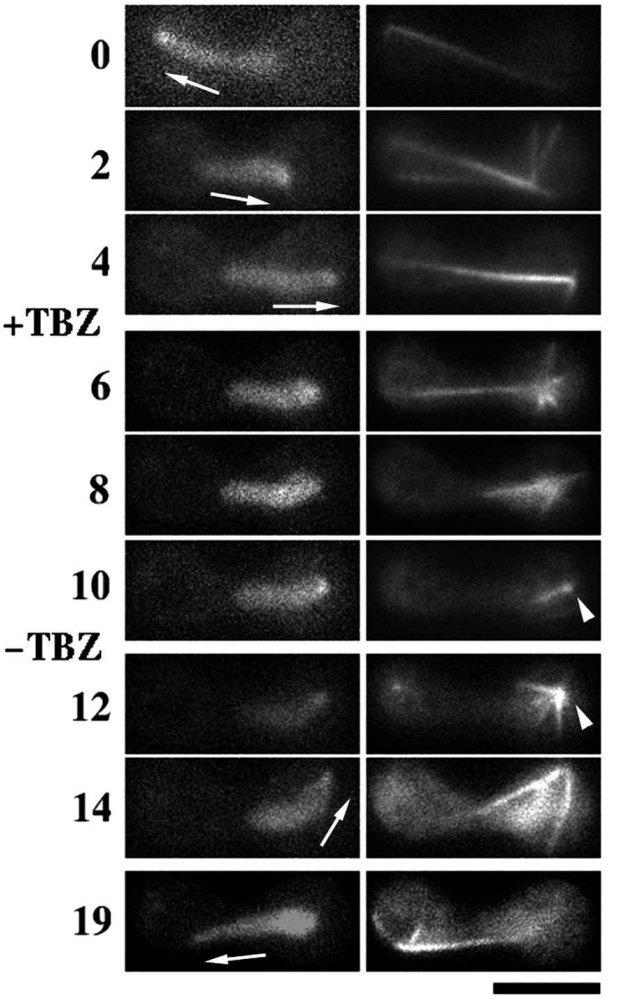

Fig. 7. Microtubule assembly after recovery from the TBZ inhibition. Microtubules were visualized with GFP-α2-tubulin (right) and the nucleus was counterstained with Hoechst 33342 (left). Addition and removal of TBZ was carried out on the microscope stage during microscopic observation of the microtubules and chromosomes in individual living cells over time. Selected frames of time-lapse images at 2-minute intervals are shown. TBZ (40 μg/ml) was added to the culture medium at 6 minutes and was removed from the medium at 12 minutes. Arrowheads indicate the position of the SPB where microtubules regenerated after removal of TBZ. Bar, 5 μm.

nucleus was moving at a speed above 2 μm/minute, the average speed in this population being 5.3 μm/minute. During the rest of the time, the leading edge of nucleus was moving below 2 μm/minute or pausing. Pauses occurred predominantly when the nucleus reversed its direction of movement. Pauses were also variable in duration, ranging from less than 10 seconds to 60 seconds. Pause times were measured during 20 directional reversals; the average duration of these pauses was 37 seconds with a standard deviation of 18 seconds. Nuclear movement with this kind of fluctuating speed can be explained by the balanced forces generated by dynamic instability and selective stabilization of microtubules (see Discussion).

DISCUSSION

In this study, using a GFP-tubulin fusion protein, we effected fluorescence imaging of microtubules and chromosomes simultaneously in living cells of *S. pombe*. Observations of microtubules in vivo provided a dynamic view of the characteristic meiotic prophase nuclear movement exhibited by this organism, showing how assembly and disassembly of microtubules mediate the unique back-and-forth oscillatory movement of the horse-tail nucleus. We propose a model in which this oscillatory movement is driven by elongation of a subset of microtubules stabilized at the cortex near the cell pole and when the nucleus reverses direction a new set of microtubules is stabilized at the other pole. Repeating the alternating cycles of selective stabilization of microtubules at either end of the cell leads to an oscillating back and forth movement of the horse-tail nucleus between the cell poles.

GFP fusion with tubulin

We constructed a multicopy plasmid, pDQ105, which expressed GFP fused to the N terminus of the fission yeast α2-tubulin (GFP-α2-tubulin) under the regulation of the inducible *nmt1* promoter. The GFP-α2-tubulin fusion protein stained microtubules without affecting mitotic growth or sporulation

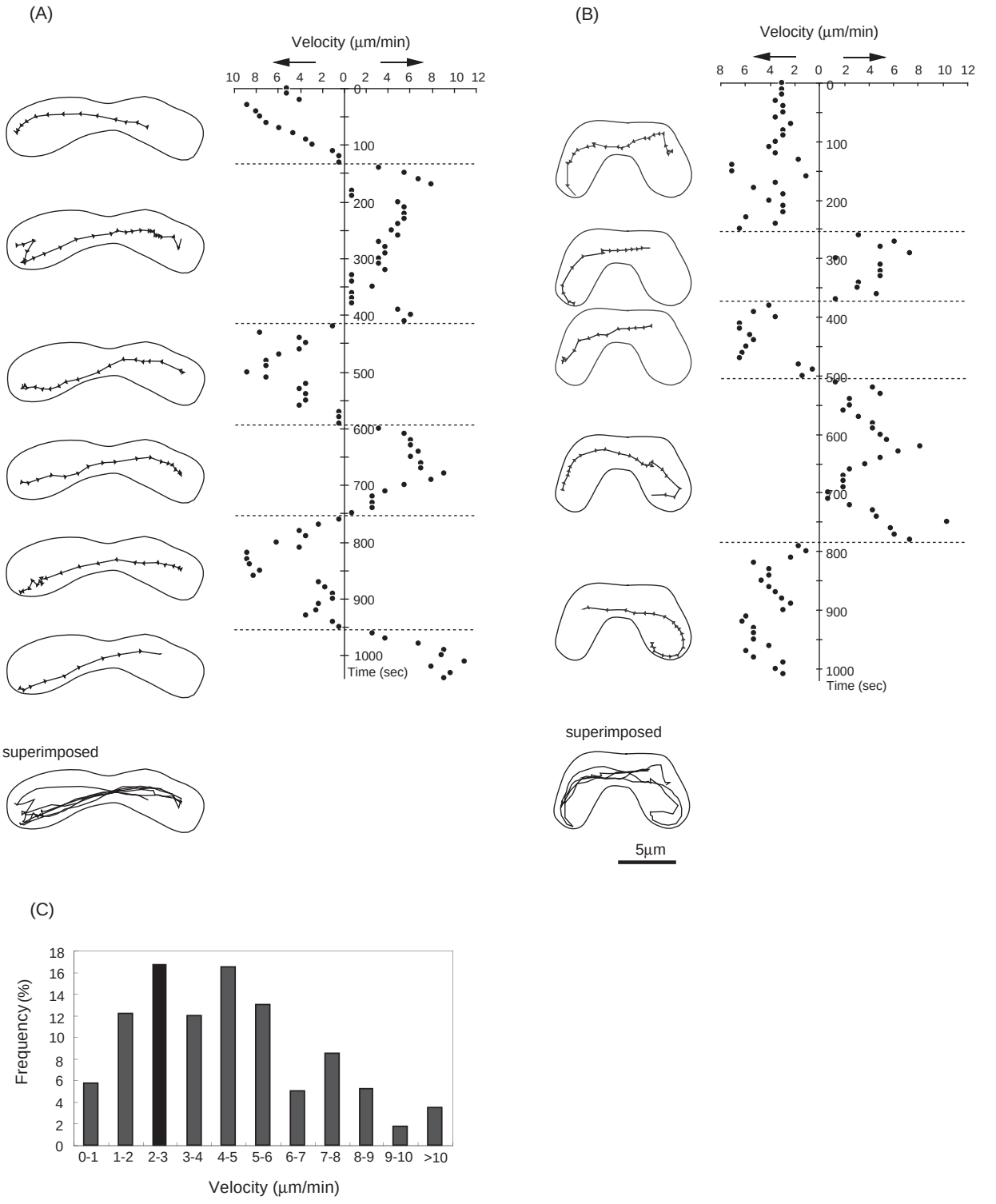

Fig. 8. Motion analysis of the horse-tail nuclear movement. (A,B; left) The position within the cell of the leading edge of the nucleus was plotted every 10 seconds; each panel (except for the bottom panel, labelled superimposed) represents the path of a one-half oscillation of the nucleus either to the left or the right. The bottom panel shows the superimposed paths for several oscillations of the nucleus. (A,B; right) The velocity measured for each 10 second period is plotted; velocity toward the right or left is plotted in the right or left side respectively, and each half oscillation is separated by a dotted line. (C) A histogram of the velocity of nuclear movement.

under conditions which repressed *nmt1* promoter activity. Microtubules stained with GFP-α2-tubulin behaved normally, cytoplasmic microtubules and the mitotic spindle exhibiting normal morphology and behavior as the cells moved through mitosis. In meiotic cells, microtubules visualized with GFP-α2-tubulin reproduced basically the same arrangement of

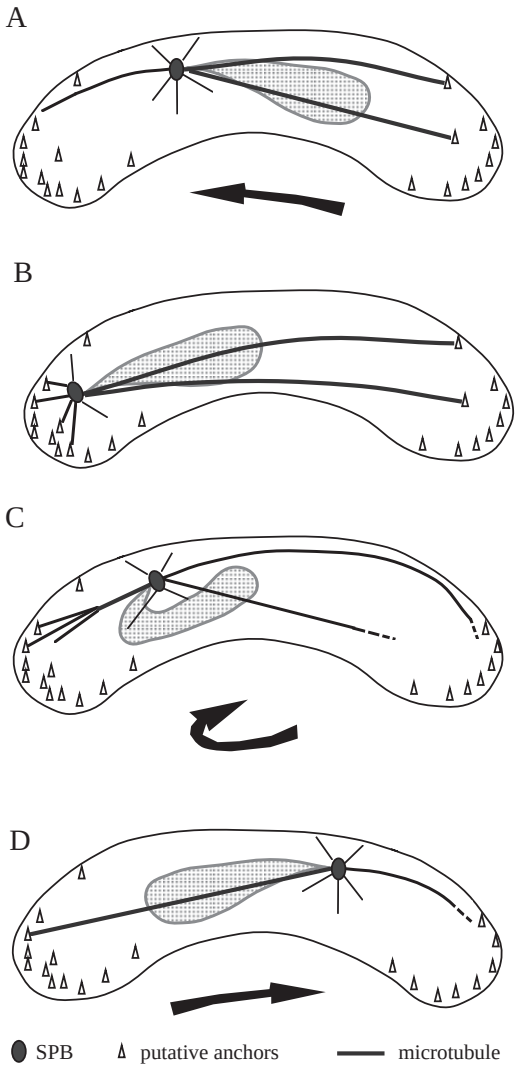

Fig. 9. A model by which nuclear movement is directed by selective stabilization of microtubules. (A) The horse-tail nucleus moves forward, accompanied by the elongation of the rearward-extending microtubule bundles that are anchored at the rear end of the cell. (B) When the nucleus reaches the end of a half-oscillation it often pauses, presumably due to the balanced forces between forward- and rearward-extending arrays of microtubules. (C) After the rearward-extending microtubules disengage from their cortical anchors, the nucleus reverses direction and begins to move toward the opposite end of the cell. The forces generated by the microtubules driving the nucleus to traverse the cell also cause the unanchored ends of the forward-extending microtubules to bend, curving along the cell cortex. (D) The horse-tail nucleus continues moving in the new direction. Repeated cycles of microtubule reorganization, as shown in A-D, result in oscillatory movement of the horse-tail nucleus during meiotic prophase, typically lasting for 2-3 hours.

microtubules as obtained from visualization by immunofluorescence staining of fixed specimens (Svoboda et al., 1995; Hagan and Yanagida, 1995; also see Fig. 3 in this paper). However, under conditions that repressed *nmt1* promoter activity, GFP-α2-tubulin did not complement the cold-sensitive growth of an α1-tubulin mutant, nda2-KM52. This is not because GFP-α2-tubulin itself is cold-sensitive. We observed that GFP-α2-tubulin assembled into microtubules at temperatures ranging from 19°C to 36°C in wild-type cells. Alternatively, the complementation of functions of α1-tubulin by α2-tubulin depends on the amount of α2-tubulin (Adachi et al., 1986). The cold-sensitive growth of nda2-KM52 is complemented by the α2-tubulin gene on a multicopy plasmid, but not on the chromosome (Toda et al., 1984). Thus, the multicopy plasmid pDQ105 without induction of the *nmt1* promoter is not producing levels of functional α2-tubulin high enough to complement the α1-tubulin mutation. This possibility was supported by our experiments of western immunoblotting. Conditions that induce high levels of transcription from the *nmt1* promoter did result in high-level expression of GFP-α2-tubulin; these levels of expression, though, were lethal to the transformed cells.

Microtubule nucleation activity at the activated meiotic SPB

Previous studies have shown that in *S. pombe*, arrangement of cytoplasmic microtubules in meiotic cells is quite different from that in mitotic cells (Hagan and Hyams, 1988; Svoboda et al., 1995; Hagan and Yanagida, 1995). Our observations in fixed and living cells reproduced these results. During mitosis, spindle microtubules are formed at the SPB only during nuclear division. In mitotic interphase cells, although the SPB is often located on one of the bundles of cytoplasmic microtubules extending along the cell length, astral microtubules radiating from the SPB are not observed; the interphase SPB acquires microtubule-nucleating activity after incubation with Xenopus oocyte mitotic extracts (Masuda et al., 1992). In contrast, cytoplasmic microtubules are organized exclusively at the SPB during meiosis. These observations suggest that SPBs change their properties of microtubule-nucleating activity upon entering meiosis. Our observation of microtubule assembly at the SPB after recovery from TBZ inhibition also shows that the meiotic SPB has microtubule-nucleating activity. The continuous reorganization of microtubules during oscillatory nuclear movement in meiotic prophase probably results from the increased dynamics of microtubules at the activated SPB.

Meiotic nuclear movement is mediated by selective stabilization of astral microtubules

The behavior of microtubules can be described by dynamic instability, where individual microtubules exist in persistent phases of growing or rapid shortening with abrupt transitions between the two phases (Mitchison and Kirschner, 1984a,b; reviewed in Kirschner and Mitchison, 1986). During morphogenesis, while free-end microtubules are repeatedly growing and shrinking in all directions, stabilization of selective microtubules by anchoring at the cell cortex, coupled with an asymmetrical distribution of the anchoring structures, leads to a polarized orientation of microtubule arrays, as discussed in Kirschner and Mitchison (1986).

Such a model of selective stabilization can explain how the oscillating movement of the horse-tail nucleus can be directed by astral microtubules. During meiotic nuclear movement in fission yeast, we assume the existence of putative microtubule-anchoring structures at each end of the cell. While free-end microtubules radiating from the SPB are dynamically unstable, repeatedly growing and shrinking, a subset of the microtubules are stabilized by engaging the putative anchoring structures.

These anchored microtubules are able to stably elongate, pushing the nucleus away from their anchored ends (Fig. 9A). Transient contact of the free-end astral microtubules with the cell cortex may cause the fluctuations of speed exhibited by the oscillating nucleus, but does not stop the movement entirely. When the nucleus approaches the end of the cell, a subset of the forward-extending microtubules engages the anchoring structures and nuclear movement is arrested due to the balanced forces exerted by the forward- and rearward-extending microtubule bundles (Fig. 9B). Shortly after the nucleus pauses, the rearward-extending microtubules disengage from their anchors and no longer oppose elongation of the forward-extending microtubules (Fig. 9C); the forward-extending microtubules, which have been stabilized by engaging with the putative cortical anchors, stably elongate, pushing the nucleus back in the opposite direction (Fig. 9D).

Similar interaction of astral microtubules with the cell cortex may occur in other examples of microtubule-mediated nuclear migration. It has been shown that the nuclear rotation at the two-cell stage in *C. elegans* is mediated by microtubules running from the centrosome to the cell cortex (Hyman and White, 1987; Hyman, 1989). In *S. cerevisiae*, nuclear positioning to the bud neck is mediated by astral microtubules radiating from the SPB (Sullivan and Huffaker, 1992; Palmer et al., 1992; Carminati and Stearns, 1997). Although the molecular basis of the anchoring structures is unknown, it is known that fission yeast cells have a polarity of growth and there are cytoskeletal structures that establish cell polarity, e.g. the *tea1* gene product is localized at the cell poles and determines polarity (Mata and Nurse, 1997).

Force generation for the horse-tail nuclear movement

An important key to understanding the mechanisms controlling the movement of the horse-tail nucleus is identification of the specific microtubule motor proteins driving the movement. It has been demonstrated that cytoplasmic dynein is required for microtubule-mediated nuclear migration in several cases, i.e. nuclear positioning at the bud neck in *S. cerevisiae* (Sullivan and Huffaker, 1992; Palmer et al., 1992; Carminati and Stearns, 1997) and nuclear migration to the germ tube in *A. nidulans* (Oakley and Morris, 1980, 1981; Xiang et al., 1994). Also in fission yeast, we recently found that disruption of a cytoplasmic dynein gene specifically inhibited the oscillatory movement of the horse-tail nucleus; cells with dynein disruption proceeded with meiotic nuclear divisions but with no horse-tail nuclear movement (A. Yamamoto, R. West, R. McIntosh and Y. Hiraoka, manuscript in preparation), indicating that the dynein microtubule motor is required for horse-tail nuclear movement. Our in vivo observations suggest that horse-tail nuclear movement is driven by the forces generated by dynein acting in combination with reorganization of astral microtubules. While cytoplasmic dynein is a minus end-directed motor (Paschal and Vallee, 1987; Vallee, 1993), the minus ends of astral microtubules are located at the SPB and the plus ends at the cell cortex. If the dynein is localized at the SPB, it would generate a force pushing the nucleus away from the cell pole in accompaniment with microtubule elongation; if localized on the cell cortex, it would generate a force pulling the nucleus toward the cell pole in accompaniment with microtubule shrinking or sliding. It has been shown in *S. cerevisiae* that cytoplasmic dynein is localized both at the SPB and the cell cortex (Yeh et al., 1995), suggesting the possibility of a combination of pushing and pulling forces controlling nuclear repositioning in this organism. Determination of the intracellular localization of cytoplasmic dynein in fission yeast is currently under way.

Biological significance of oscillatory nuclear movement during meiotic prophase

Unlike the examples of nuclear repositioning in which the nucleus moves to its destination and then stops, the horse-tail nucleus continues moving back and forth for some hours, as if the movement itself serves some sort of purpose. Although the biological role of the oscillatory nuclear movement in fission yeast is not understood, it is tempting to speculate that the movement may have a role in an initial step of searching for and aligning homologous chromosomes, as discussed in Chikashige et al. (1997). By examining the consequences of bypassing nuclear movement in the dynein-disrupted strain, it will be possible to ask whether oscillatory nuclear movement has any biological role in meiosis. Our unpublished observations suggest that oscillatory nuclear movement is needed for proper alignment of homologous chromosomes, and as a consequence, the lack of the nuclear movement results in decreased frequency of meiotic recombination (A. Yamamoto, R. West, R. McIntosh, and Y. Hiraoka, manuscript in preparation). Further analyses by molecular biological and cytological approaches will unveil mechanisms for and the biological roles of oscillatory movement of the horse-tail nucleus during meiotic prophase.

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