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Fission yeast Myo51 is a meiotic spindle pole body component with discrete roles during cell fusion and spore formation

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Summary

Class V myosins are dimeric actin-associated motor proteins that deliver cellular cargoes to discrete cellular locations. Fission yeast possess two class V myosins, Myo51 and Myo52. Although Myo52 has been shown to have roles in vacuole distribution, cytokinesis and cell growth, Myo51 has no as yet discernible function in the vegetative life cycle. Here, we uncover distinct functions for this motor protein during mating and meiosis. Not only does Myo51 transiently localise to a foci at the site of cell fusion upon conjugation, but overexpression of the Myo51 globular tail also leads to disruption of cell fusion. Upon completion of meiotic prophase Myo51 localises to the outside of the spindle pole bodies (SPBs), where it remains until completion of meiosis II. Association of Myo51 with SPBs is not dependent upon actin or the septation initiation network (SIN); however, it is dependent on a stable microtubule cytoskeleton and the presence of the Cdc2-CyclinB complex. We observe a rapid and dynamic exchange of Myo51 at the SPB during meiosis I but not meiosis II. Finally, we show that Myo51 has an important role in regulating spore formation upon completion of meiosis.

Supplementary material available online at
http://jcs.biologists.org/cgi/content/full/122/23/4330/DC1

Key words: *Schizosaccharomyces pombe*, Myosin V, Meiosis, SPB, Spore formation, Fission yeast, Myo51

Introduction

In response to specific stimuli, cells can undergo dramatic changes in their shape, allowing them to adapt to different extracellular environments. This activity has a crucial role in the determination of cell fate during metazoan development, and is an implicit requirement for the regulation of cell differentiation. These stimuli can bring about cytoskeleton reorganisation and thereby alter cellular morphology, in addition to halting cell cycle progression and triggering entry into a different growth pattern or life cycle.

In unicellular organisms such as yeast, the actin and microtubule cytoskeletons each have important and sometimes collaborative roles in modifying the growth pattern of a cell to allow it to respond to stress conditions such as nutrient starvation. The cytoskeletons bring about changes to the cell shape and intracellular organisation to promote entry into the sexual life cycle by facilitating conjugation, cell fusion, meiosis and subsequent spore formation. It is therefore crucial that the organisation of these two cytoskeletons is correctly coordinated.

In response to pheromones, nitrogen-starved fission yeast cells of opposite mating types grow towards each other from projecting tips, which extend from the end of the cell. The microtubule and F-actin cytoskeletons undergo a dramatic rearrangement to facilitate the subsequent conjugation and fusion between the two cells (Petersen et al., 1998a). Fus1, a sexual-cycle-specific formin, is targeted by its FH3 domain to the projection tip of agglutinating cells, where it is required to promote cell wall degradation at the site of cell fusion (Petersen et al., 1998c; Petersen et al., 1995). There, it colocalises with other regulators of actin dynamics (such as profilin and tropomyosin) to promote polymerisation of an actin-

based structure that is required for conjugation to occur (Kurahashi et al., 2002; Petersen et al., 1998b). Upon completion of cell fusion, nuclei fuse and rapidly enter into meiosis, marked by a period of telomere-led back and forth 'horsetail' movement of the nucleus along the length of the zygote to promote recombination between sister chromatids (Chikashige et al., 1994; Robinow, 1977). This movement is driven by rapid rearrangements of microtubules, and requires the activity of microtubule-associating proteins, which include dynein, dynein light chains and the fission yeast homologue of p150 Glued, Ssm4 (Miki et al., 2002; Niccoli et al., 2004; Yamamoto et al., 1999). Upon completion of this event, chromosomes go through two rounds of chromosome segregation (Hagan and Yanagida, 1992) before the onset of sporulation, when spore walls form around each of the four haploid nuclei in an actin-dependent process (Petersen et al., 1998b).

Myosins are actin-associated motor proteins that have been shown to have important roles during this specialised cell cycle in both metazoan and fungal cells. In mammalian tissues, myosin II and myosin X localise to meiotic microtubule-organising centres (MTOCs), where they are required for the positioning and assembly of the meiotic spindle (Schuh and Ellenberg, 2008; Weber et al., 2004). In unicellular fungi, long-tailed type I myosins localise to the site of conjugation and to the leading edge of the forespore membrane, where they have been implicated in forespore assembly (Itadani et al., 2006; Toya et al., 2001). Although class V myosins are involved in transporting prespore membrane precursors to the site of spore formation in the budding yeast and conjugation tube formation in the plant pathogen *Ustilago maydis* (Taxis et al., 2006; Weber et al., 2003), as yet

no meiotic function has been assigned to either of the fission yeast class V myosins Myo51 or Myo52.

Here, we investigate the localisation and function of the fission yeast myosin V Myo51. Although this molecular motor has no discernible function during the vegetative cell cycle, we have discovered that Myo51 is involved in two distinct processes during the sexual life cycle of this yeast. We show that Myo51 and Myo52 both localise to the site of cell fusion during conjugation, where Myo51 facilitates cell wall breakdown at this locus. In addition, we show that Myo51 localises to the outer surface of the meiotic spindle pole body (SPB), where it undergoes turnover, regulated by the meiosis state. We show that Myo51 has an important role in maintaining the integrity of the SPB and thereby ensures that meiotic segregation is coordinated with spore wall synthesis.

Results

Fission yeast class V myosins recruit to the site of cell fusion

Inspection of the $myo51^+$ expression profile indicates that although its transcription does not vary during the vegetative life cycle, upon entry into meiosis and sporulation, the expression of $myo51^+$ increases dramatically (Mata et al., 2002; Rustici et al., 2004). We therefore examined whether the subsequent protein had any discernible localisation pattern during the meiotic lifecycle. To facilitate this, a series of strains expressing Myo51-fluorophore fusions were generated. The fluorophore or its position on Myo51 had no discernible effect upon cell morphology and each fusion protein localised to the cytokinetic actomyosin ring (CAR) during mitosis (Fig. 1A), as previously reported (Win et al., 2001). Live-cell imaging of nitrogen-starved homothallic $myo51$-gfp h^{90} cells undergoing pheromone-induced polarisation and cell fusion revealed that Myo51 transiently localised to the leading edge of polarised cell tips of agglutinating cells, immediately before cell fusion (Fig. 1B, 65 minutes). These foci coalesced into a single bright point (Fig. 1B, 75-120 minutes), which persisted until completion of cell wall degradation, when the Myo51 signal dispersed (Fig. 1B, 130-135 minutes).

The actin and microtubule cytoskeletons have key roles in the coordination and execution of cell fusion in fission yeast (Petersen et al., 1998a; Petersen et al., 1998b). We therefore explored the reliance of Myo51 upon the integrity of each cytoskeleton for recruitment to the fusing tip. Addition of carbendazim (CBZ) resulted in the rapid depolymerisation of microtubules, but had no effect upon the localisation of Myo51 to the site of cell fusion (Fig. 1C,D). By contrast, depolymerisation of the actin cytoskeleton by the addition of latrunculin A not only prevented cell fusion from taking place, but also abolished both actin and Myo51 localisation (Fig. 1E,F), indicating that the recruitment of this motor to the site of cell fusion requires an actin cytoskeleton.

We next determined whether Myo51 recruitment to the projection tip required Myo52, the other fission yeast class V myosin (Fig. 1G). Although $myo51$-gfp $myo52\Delta$ cells displayed the growth polarity defects associated with the absence of Myo52, Myo51 did not require Myo52 for its localisation during cell fusion. However, although there is no upregulation of Myo52 expression during meiosis (Mata et al., 2002), this protein was also seen to localise to the site of cell fusion (Fig. 1H) in an actin-dependent manner and independently of Myo51 (Fig. 1I). The localisation pattern for Myo51 and Myo52 during mating was reminiscent of that seen for the fission yeast formin Fus1 (Petersen et al., 1998c), a protein that is required for polymerisation of actin filaments at the projection tip of mating cells. Upon investigation, each myosin V protein failed
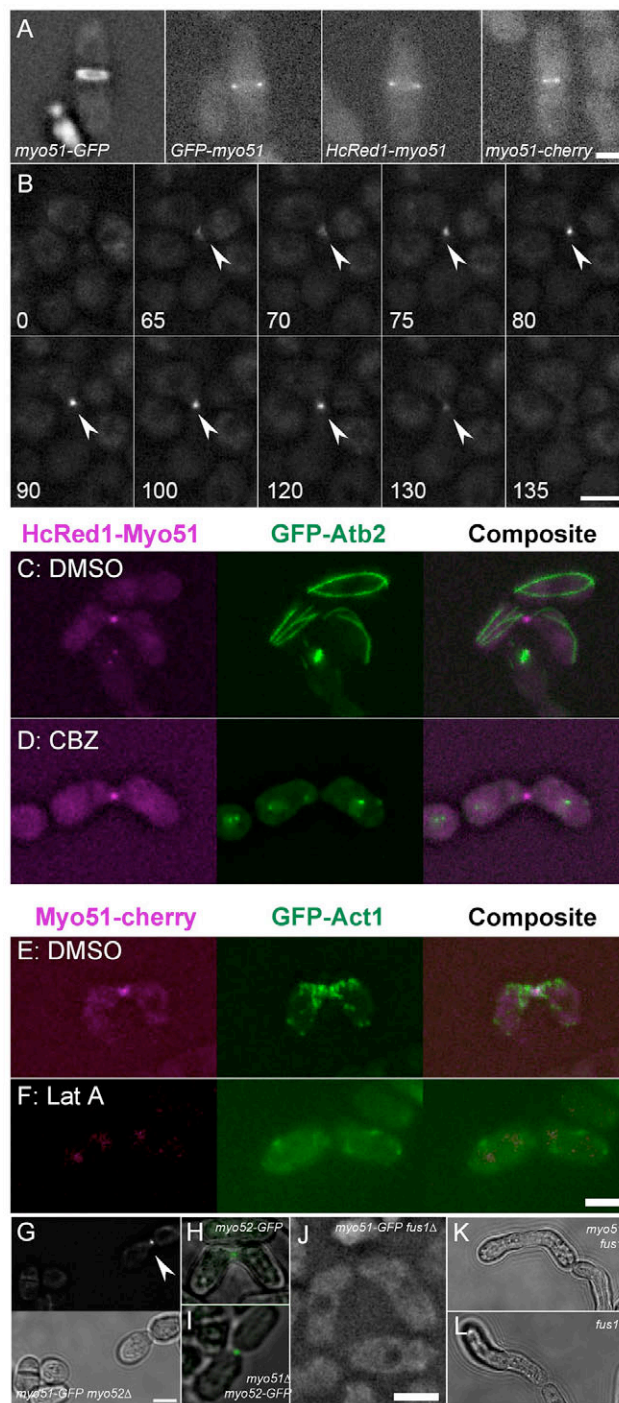
Fig. 1. Myo51 localises to the site of cell fusion. (A) Myo51 localises to the cell equator of mitotic cells when tagged at either its N-terminus (GFP-Myo51 and HcRed1-Myo51) or C-terminus (Myo51-GFP and Myo51-Cherry). (B) Time-lapse imaging of merged *z*-series reveals that Myo51 transiently localises to the site of cell fusion of mating cells. (C,D) Simultaneous visualisation of Myo51 (magenta) and microtubules (green) in live cells treated with either DMSO (C) or 25 μg/ml CBZ (D) show that its localisation is not microtubule dependent. In contrast to DMSO treatment (E), Myo51 (magenta) and actin (green) localisation is abolished in fusing *myo51-cherry gfp-act1* cells treated with 20 μM Lat A (F). (G) Myo51 localises to the fusing tip in the absence of Myo52. Myo52 localises to the site of conjugation in cells either possessing (H) or lacking (I) Myo51 (Myo52, green; phase-contrast, grey). (J) Myo51 fails to localise to the tip of *fus1Δ* cells, whereas phase-contrast images reveal that a lack of Myo51 (K, *myo51Δ fus1Δ*) does not alleviate the tip fusion phenotype of cells lacking this formin (L, *fus1Δ*). Scale bar: 5 μm.

to localise to the fusion site in agglutinating *fus1Δ* cells (Fig. 1J), indicating that they each require Fus1-activated actin filaments to localise to the site of conjugation. Interestingly the extended projection tip phenotype seen in *fus1Δ* cells (Petersen et al., 1995) (Fig. 1K) was still observed in *fus1Δ myo51Δ* cells (Fig. 1L).

Overexpression of the GTD of *S. pombe* myosin V disrupts cell fusion

Myosin V proteins bind their cargoes through their globular tail domain (GTD) (Reck-Peterson et al., 1999). We therefore generated strains in which GFP was fused to the N-terminus of Myo51-GTD and examined the effect its overexpression had upon cell fusion. Low-level expression of Myo51-GTD had no discernible effect upon the localisation of the endogenous full-length protein in vegetative (Fig. 2A) or conjugating cells (Fig. 2B). Overexpression of the Myo51-GTD had no detectable effect in vegetative cells, but had a discrete localisation as a single bright dot associated with the region of the cell membrane closest to the nucleus (Fig. 2C). When overexpressed in conjugating cells, the Myo51-GTD formed a randomly localised plaque-like structure, which displaced the full-length protein from the site of cell fusion (Fig. 2D), and prevented normal cell fusion between the two mating cells (Fig. 2E). Although this did not prevent these zygotes from undergoing nuclear fusion and meiosis, the lack of cell wall expansion at the site of fusion often prevented the movement of the large diploid nucleus between the two cell compartments. This resulted in meiotic segregation of chromosome and spore formation, which occurred in only one of the two mating cells, rather than the whole zygote (Fig. 2F-I). By contrast, when Myo51-GTD was overexpressed in conjugating cells on plates, they failed to fuse and instead developed elongated shmooing tips (Fig. 2J) that were similar to the structures observed upon failed conjugation in *fus1Δ* cells (Petersen et al., 1995).

Myo51 localises to the meiotic SPB

To examine whether Myo51 associated with actin structures during meiotic chromosome segregation, Myo51-GFP localisation was followed in conjugating cells expressing a labelled SPB component (Sid4) to allow the meiotic stage to be determined (Fig. 3). As noted earlier, Myo51 was seen localised to the shmoo tip (Fig. 3, 0:00), but this signal rapidly dispersed upon cell fusion. The protein had no discrete localisation during subsequent nuclear fusion and horsetail movement (Robinow, 1977) (supplementary material Fig. S1, 0:05-4:35). Once cells entered meiotic prophase, however, Myo51 recruited to the SPB, where it remained localised throughout both meiotic cycles (Fig. 3, 4:45-5:55). Upon entry into meiosis II, the intensity of the Myo51 signal at the SPB increased dramatically (Fig. 3, 5:25-5:35), indicating that significantly more protein was recruited to the SPB. The signal intensity of Myo51 at the SPB remained high throughout nuclear segregation of meiosis II, whereupon it rapidly disappeared. During anaphase of meiosis II, the Myo51 signal was also seen to associate to a discrete structure
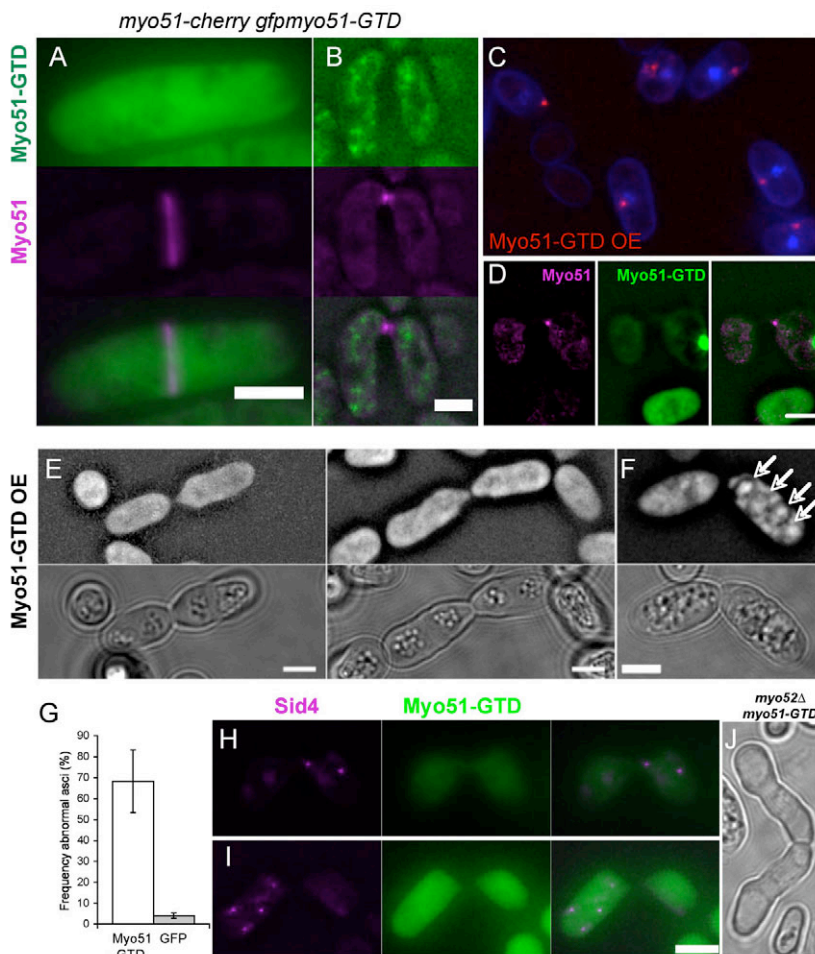
Fig. 2. Myosin V GTDs fail to localise correctly and inhibit cell fusion. (A,B) In contrast to the full-length protein (magenta), the Myo51-GTD (green) fails to localise in either mitotic (A) or conjugating (B) *myo51-cherry nmt41gfpmyo51-GTD* cells grown in the presence of thiamine. (C) When overexpressed in vegetative cells (no thiamine), Myo51-GTD (magenta) is recruited to a medial dot not associated with the nucleus (blue). (D) Overexpressed Myo51-GTD (green) localises to a cytoplasmic dot and causes mislocalisation of full-length Myo51 (magenta) in conjugating cells. (E,F) In the absence of discrete localisation (upper panels), cells overexpressing Myo51-GTD fail to fuse normally (E), with spores (arrows) forming in only one of the two mating cells (F). (G) The proportion of *leu1::nmt41myo51-GTD* cells with normal and abnormal asci when allowed to conjugate in the presence (grey bars) or absence (black bars) of 4 μM thiamine. (H,I) Sid4-tomato labelled SPBs (magenta) reveal that although nuclear fusion is unaffected in 90% of cells overexpressing the Myo51-GTD (green), nuclear distribution is disrupted in the subsequent meiosis. (J) Overexpression of Myo51-GTD can bring about the *fus1Δ* phenotype in cells on plates. Scale bars: 5 μm.

behind the nucleus (Fig. 3 arrowheads), reminiscent of the actin localisation at this time (Petersen et al., 1998b).

To confirm the SPB association of Myo51, its localisation was examined in a strain expressing HcRed1-Myo51 and Pcp1-GFP, which is an essential SPB component (Flory et al., 2002). Consistent with the C-terminal-tagged protein, HcRed1-Myo51 localised to the meiotic SPB from the onset of chromosome segregation of meiosis I (Fig. 4A). The signals continued to coalesce throughout meiosis I (Fig. 4B), until the end of telophase of meiosis II (Fig. 4C), when the signal from the two proteins did not overlap precisely, indicating that Myo51 is located on the periphery of the SPB. Consistent with this, a gap was often observed between the Myo51 signal and the growing meiotic spindle (Fig. 4D), the size of which was comparable with the distance between the Myo51 signal and that of the kinetochore component, Mal2 (Fig. 4E) (Jin et al., 2002), and indicate that Myo51 is located to the outer region of the SPB throughout meiotic chromosome segregation. This distribution was confirmed by examining the Myo51 signal in more than 100 cells from each stage of the meiotic cell cycle. As the distribution was consistent for all the cells we observed, we concluded that it reflected the normal cellular distribution for Myo51 during meiosis.

Myo52 concentrates near the SPB and nuclei during meiosis

We next examined whether the major mitotic cycle myosin V protein Myo52, colocalised with Myo51 to the meiotic SPB. During the first meiotic division, the Myo52 signal concentrated to a number of discrete foci (Fig. 4F,G) some of which were adjacent to, but never colocalised with, Myo51 at the SPB (Fig. 4J,K). During this period, other Myo52 foci appeared to be randomly distributed throughout the cytoplasm. Upon completion of meiosis I, Myo52 localised around the nuclear periphery (Fig. 4H), in a pattern that was similar to that seen for actin at this stage (Petersen et al., 1998b).

This Myo52 distribution rapidly dispersed after chromosome segregation, and Myo52 was subsequently only seen localised to cytoplasmic foci (Fig. 4I). Therefore, unlike Myo51, during meiosis Myo52 did not localise to the SPB, but had a distribution similar to that of actin.

Myo51 associates with the SPB independently of Myo52, Spo15, actin and the SIN network

We next examined whether Myo51 was dependent upon other cellular components for its localisation to the meiotic SPB. We first established that Myo51 recruited to the SPB in the absence of the second fission yeast class V myosin Myo52 (Fig. 4L). Similarly, Spo15, which is required for modification of the SPB during spore membrane formation (Ikemoto et al., 2000) was found to be dispensable for recruitment and localisation of Myo51 to the SPB (Fig. 4M). Core components of the fission yeast septation initiation network (SIN) are anchored to the outer plaque of the SPB by Cdc11 (Krapp et al., 2001), to regulate spore formation during meiosis (Krapp et al., 2006). Using a temperature-sensitive *cdc11* mutant, we assessed whether Myo51 was anchored to the SPB via Cdc11, or whether SIN activity is required for its localisation. When *cdc11-132 myo51-gfp* cells were cultured at 33°C (non-permissive temperature), Myo51 was seen to localise to the non-constricting CAR during mitosis, and also to the SPBs throughout meiosis (Fig. 4N), indicating that Myo51 localisation is independent of Cdc11 or SIN activity.

To explore the dependence of Myo51 upon actin for its localisation during meiosis, we created a strain containing the *myo51-mCherry* allele together with a *gfp-act1* allele under the control of the *nmt81* promoter, which had been integrated into the *leu1+* locus. mCherry-labelled Myo51 localised to the SPBs in the presence of DMSO (Fig. 4O). However, although fluorescence from GFP-actin foci was rapidly replaced with a non-discrete cytoplasmic background signal upon addition of the actin-depolymerising drug latrunculin A (Fig. 4P), Myo51 remained localised at the SPB, suggesting that this localisation was independent of its association with actin and therefore motor activity.

Myo51 turns over on the SPB during meiosis I

Having established that Myo51 does not require actin to localise to the SPB, we next examined whether this localisation was dynamic. Using fluorescence recovery after photobleaching (FRAP), we examined Myo51-GFP turnover at the SPB of cells in meiosis I (Fig. 5A) or meiosis II (Fig. 5C,E). During meiosis I, there was a rapid recovery of the GFP-Myo51 signal at the SPB after photobleaching, and full recovery took less than 5 minutes (Fig. 5A,B). These cells were then able to complete meiosis and spore formation normally (not shown), indicating that the laser had no detectable affect upon the integrity of the SPB. The recovered
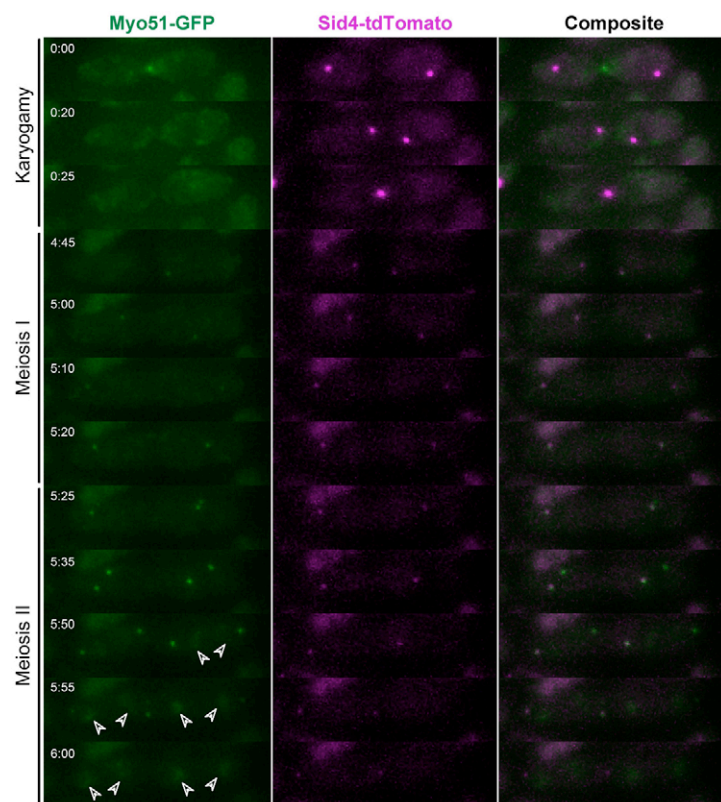
Fig. 3. Myo51 localises to the SPB during meiotic nuclear segregation. Maximum projections of 21 slice *z*-stacks selected from a time course of mating *myo51-gfp sid4-tomato* cells (Full time course is shown in supplementary material Fig. S1) reveal that Myo51 (green) not only localises to conjugating tips but also co-localises with the SPB component Sid4 (magenta) during meiotic chromosome segregation (4:35-6:00). At the end of meiosis II, Myo51 transiently localises to a dispersed structure behind each SPB (arrowheads in 5:50-6:00). Time is shown in hours:minutes.
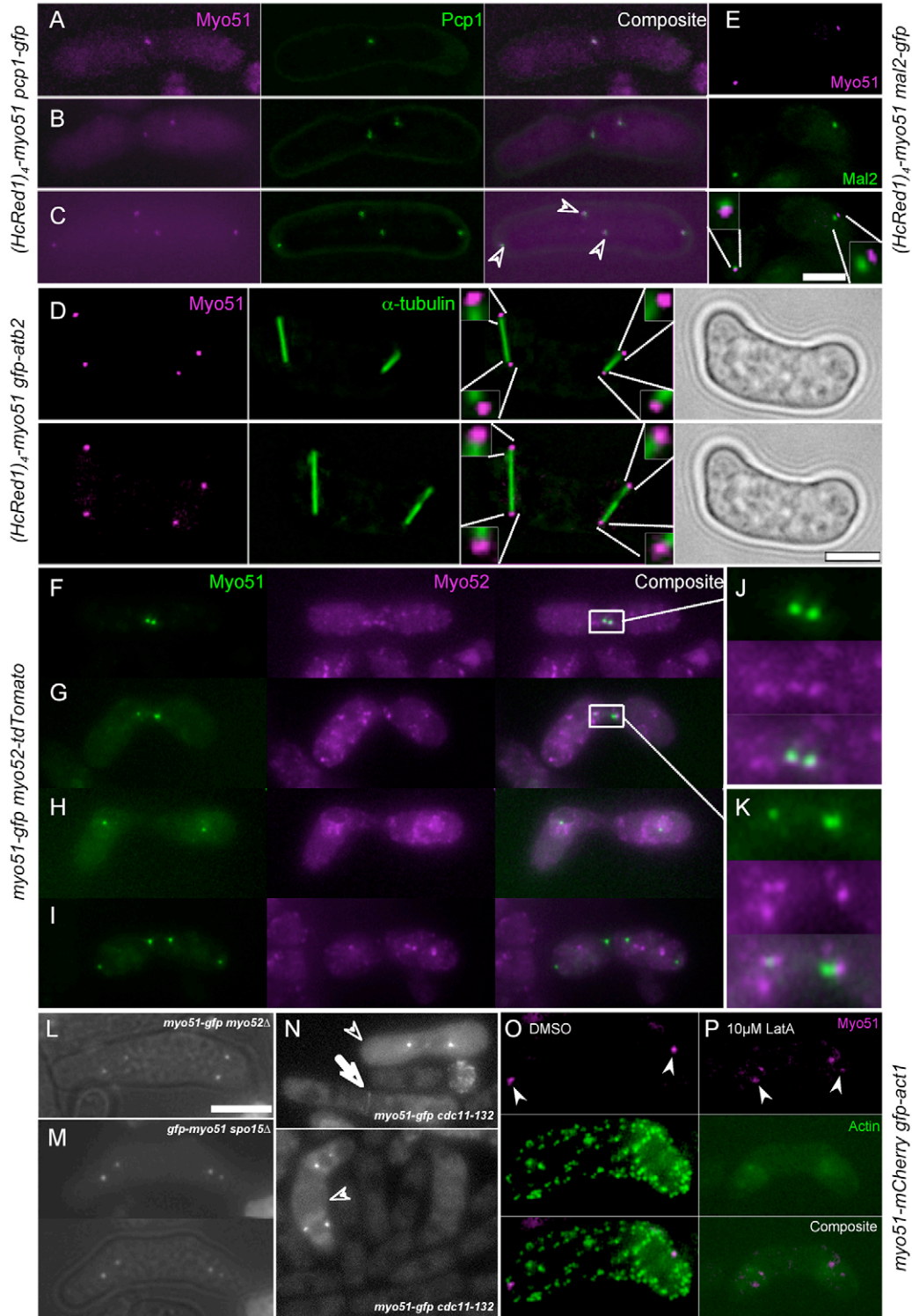
Fig. 4. Myo51 localises to the outside of meiotic SPBs. (A-C) Myo51 (magenta) colocalises with the essential SPB component Pcp1 (green) from the onset of meiotic nuclear segregation (A), throughout meiosis I (B), until the end of meiosis II (C) in *HcRed1-myo51 pcp1-gfp* cells. (D) A gap is seen between Myo51 (magenta) and the end of elongating GFP-Atb2 labelled meiotic spindles (green) (lower images taken 4 minutes after upper images) suggesting Myo51 localises to the outer side of the SPB. SPB regions are magnified in composite images. (E) Myo51 (magenta) failed to colocalise with the kinetochore component, Mal2 (green) in *HcRed1-myo51 mal2-gfp* cells. (F-K) Myo51 (green) and Myo52 (magenta) localisation in *myo51-gfp myo52tdTomato* cells during meiosis. Myo52 signal concentrates adjacent to Myo51-labelled SPBs during meiosis I (F,G), around the nucleus at the onset of meiosis II (H), and subsequently to punctate foci that are randomly distributed (I). (J,K) Magnified SPB regions highlighted in F and G, respectively. (L) Myo51 localises to the SPB of meiotic *myo52Δ* cells. (M) GFP (upper panel) and merged GFP phase-contrast image (lower panel) reveal that Myo51 localises to the SPB of *gfp-myo1 spo15Δ* cells during meiosis. (N) Myo51 localisation to the CAR (arrow) or meiotic SPB (arrowheads) is normal in *cdc11-132* cells grown at the non-permissive temperature. (O) DMSO has no effect on Myo51 (magenta) or actin (green) localisation during meiosis. (P) Treatment with 10 μM latrunculin A brings about rapid depolymerisation of actin, but has no effect on Myo51 localisation to the SPB (arrowheads). Scale bar: 5 μm.

GFP-Myo51 signal was comparable with that seen on the second unbleached SPB at the other end of the cell, indicating that SPB-associated Myo51 is very dynamic during meiosis I. By contrast, when the GFP-Myo51 signal was bleached from a single SPB of a cell in meiosis II (Fig. 5C,E), the GFP signal never recovered to normal levels (Fig. 5D), even after extended time-lapse analysis (Fig. 5F), by which time the GFP signal at the unaffected SPBs had started to disappear. These data indicate that Myo51 has distinct meiotic-cycle-dependent dynamic properties at the SPB.

MPF is required for Myo51 meiosis II SPB recruitment

To explore the precise timing of the increase in Myo51 signal at the SPB and allow us to correlate changes in its turnover, we examined Myo51 localisation in a strain lacking Mes1, which is a regulator of meiosis II onset. Cells lacking Mes1 arrest at the end of meiosis I because of the premature degradation of the pool of cyclin B required for onset of meiosis II (Izawa et al., 2005; Shimoda et al., 1985). As predicted, *mes1*^{+} cells underwent normal meiotic SPB segregation, with the expected increase in Myo51 signal during meiosis II (Fig. 6A). By contrast, a rapid reduction in Myo51 SPB signal was observed in *mes1Δ* cells upon their arrest at the end of meiosis I (Fig. 6B), and the motor was seen to associate with the nuclear membranes. This localisation pattern was observed in all *myo51*^{+} cells examined that were passing from prophase to the onset of anaphase of meiosis II (Fig. 6C). These data suggest that the increase in the Myo51 SPB signal correlates with the onset of meiosis II.

To confirm this, we generated a *mes1Δ* strain in which it was possible to regulate the induction of non-degradable cyclin B (Cdc13-des2) using an estradiol-regulatable hormone-binding domain (HBD) (Boe et al., 2008), and thereby increase Cdc13 levels at the *mes1Δ*-induced arrest point. In the absence of estradiol, the HBD-tagged non-degradable Cdc13 protein is targeted to the Hsp90 complex, rendering it inactive. However, the addition of estradiol brings about an almost instantaneous release of the HBD-Cdc13-des2 fusion protein (Boe et al., 2008). Although we were unable to detect Myo51 at the SPB of *myo51-gfp mes1Δ* pREP82 *cdc13des2-HBD*, control cells treated with ethanol (Fig. 6D, arrowheads), upon addition of estradiol, Myo51 was seen to localise to the SPB (Fig. 6D bottom panels). This finding is consistent with the increase in Myo51 signal at the SPB and the corresponding reduction observed in its turnover after the onset of meiosis II, indicating that Myo51 meiosis II SPB recruitment requires the presence of active MPF.

Modulating Myo51 protein levels disrupts meiotic chromosome segregation and spore formation

As Myo51 has a distinct localisation pattern at the SPB during meiosis, we examined whether Myo51 had a role during the latter stages of meiosis and subsequent spore formation. First, we examined the nuclear segregation within spores from a homozygous *myo51Δ* cell cross. Although each spore within asci from a homozygous cross between *myo51*^{+} cells contained nuclear material (Fig. 7A), a significant proportion (~10%) of asci from a similar cross between homozygous *myo51Δ* cells contained a spore lacking DNA (Fig. 7B; Table 1), indicative of a defect during meiosis II. This finding was confirmed using an allele expressing a GFP fusion of the nuclear membrane component, Dlc2 (Miki et al., 2002) and illustrated that nuclei often failed to segregate correctly in the absence of Myo51 (Fig. 7C). Introducing the *sid4-tdTomato* allele into this strain revealed that meiotic *myo51Δ* cells had an increased number of SPBs (Fig. 7D,E), which were often seen to associate with fragments of nuclear membrane (Fig. 7E, arrowheads). In addition, a proportion of the resultant asci also contained an increased number of spores, many of which contained no or reduced quantities of nuclear material, which was reflected in a reduction in spore viability (Table 1). These data indicate that Myo51 has a role in maintaining SPB integrity during meiosis.

This SPB integrity and spore formation phenotypes were more prominent in asci produced from mating cells overexpressing Myo51 (Fig. 7F,G; Table 1). Overexpression of Myo51 often resulted in the generation of asci containing an abnormal number of spores (Fig. 7F,G), which sometimes reached 36. However SPB and nuclear membrane localisation revealed that each spore did not always contain DNA, but instead contained an aberrant accumulation of SPB material (Fig. 7F), which was reflected in the dramatic reduction in
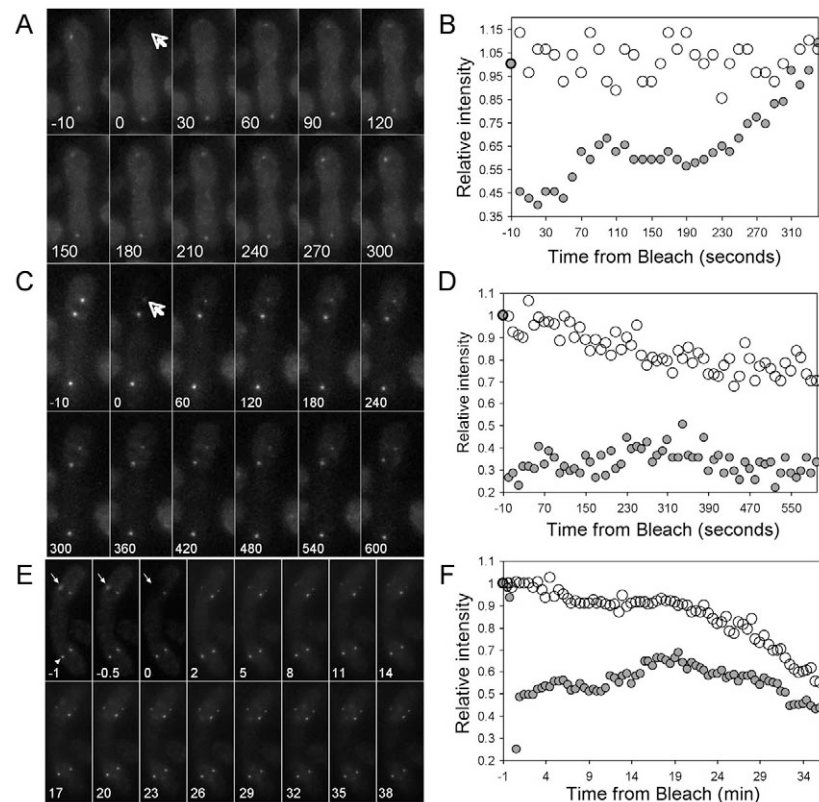
Fig. 5. The amount of Myo51 at the meiotic SPB is dynamic. (A-F) Time-lapse images and graphs of signal change of Myo51-GFP FRAP experiments. Myo51 on the SPB of cells in meiosis I (A) and meiosis II (C,E) was subjected to photobleaching (arrows), and its recovery was followed using 3D time-lapse imaging (left panels). Graphs (right) show change in relative intensity of Myo51 signal at the photobleached (grey filled circles) and an unbleached SPB (empty circles) throughout the experiment. Time is recorded as either seconds (A-D) or minutes (E,F).
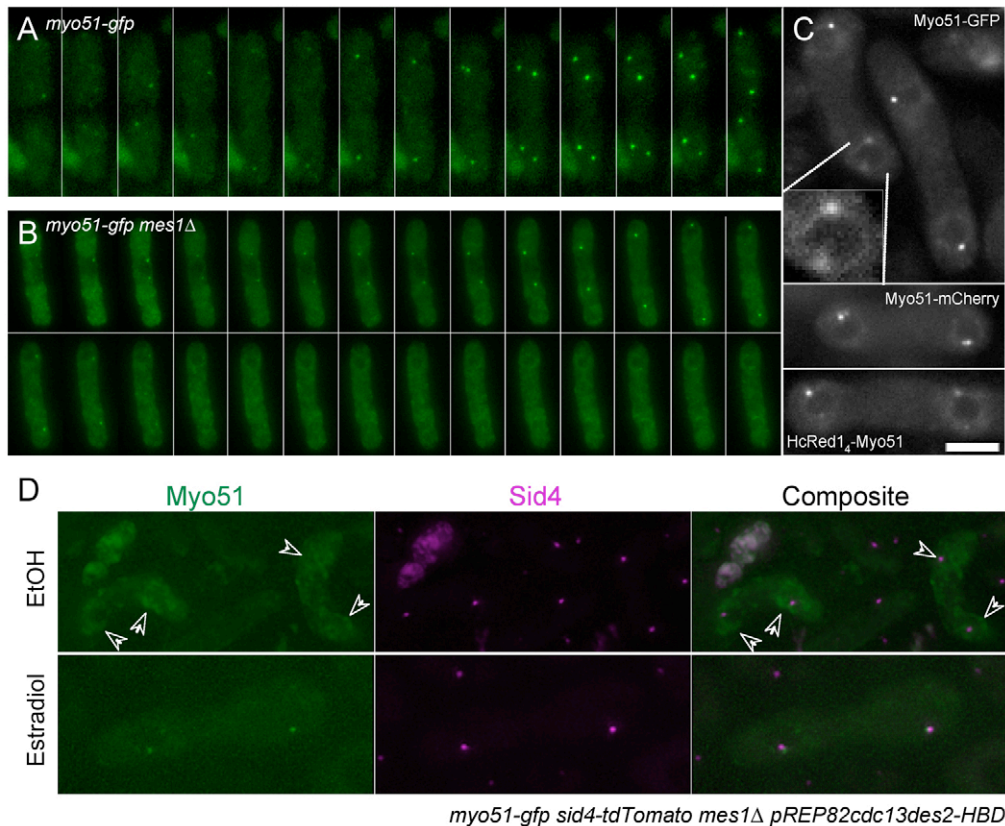
Fig. 6. Myo51 association with the SPB requires MPF. (A,B) Time-lapse Z-series maximum projections of *myo51-gfp mes1*[+] (A) *and myo51-gfp mes1Δ* (B) cells during meiosis. Images were captured either every 5 (A) or 2 (B) minutes. Although Myo51 remains localised to the SPB throughout meiosis II in *mes1*^{+} cells (A), Myo51 signal rapidly disappears from the SPB of *mes1Δ* cells arrested at the end of meiosis I and associates with the nuclear membrane (B). (C) Myo51-GFP (upper panel), Myo51-GFP (middle panel) and HcRed1-Myo51 (lower panel) localise to the SPBs and nuclear membrane of *mes1*^{+} cells before anaphase II. (D) *myo51-gfp sid4-tdTomato mes1Δ pREP82cdc13des2-HBD* cells were treated with either ethanol (upper panels) or estradiol (lower panels). Although ethanol has no effect upon the cellular distribution of Myo51 (green, arrowheads), estradiol-induced Cdc13 expression caused Myo51 to return to the SPB and colocalise with the SPB component Sid4 (magenta).

the viability of these cells (Table 1). These data suggest that Myo51 has a role in maintaining SPB integrity during meiosis and thereby coordinating spore formation with chromosome segregation during meiosis II.

Discussion

Myosin V function during conjugation

In this study, we investigated the function of the fission yeast myosin V, Myo51, and found that it has subtle roles during conjugation and in the regulation of spore formation. We discovered that both Myo51 and Myo52 colocalise with actin to the site of cell fusion (Fig. 8). The actin cytoskeleton has an essential role during conjugation, as illustrated by the fact that cells lacking the mating-cycle-specific formin Fus1, are sterile (Petersen et al., 1995). In contrast to wild-type cells, the cell wall at the site of conjugation of agglutinating *fus1Δ* cells remains intact, preventing the formation of a single zygote. It is appealing to conclude that this *fus1Δ* phenotype is brought about by the absence of Fus1-seeded actin filaments, which prevents Myo51 and Myo52 from delivering cargoes required for cell fusion to the site of conjugation. However, as cells lacking both myosin V proteins (*myo51Δ myo52Δ*) are not sterile (not shown), it would suggest these two motors are not the only proteins required for recruiting cell-wall-degrading enzymes to the cell tip. The fact that overexpression of the Myo51 GTD affects proper cell fusion

suggests that its cargoes are being sequestered by this cargo-binding tail domain, and preventing their recruitment to the site of conjugation, by an alternative mechanism that is independent of myosin V or Fus1 (Cartagena-Lirola et al., 2006).

The identity of potential cargoes remains unknown, however, it is intriguing to speculate on their identity. As *Schizosaccharomyces pombe* cells do not contain any obvious homologues of known exoglucanases or produce any detectable exoglucanase activity (Molero et al., 1999), candidates for conjugation-specific myosin V cargoes include the endoglucanases Eng1 (an endo-β-1,3-glucanase), Agn1 and Agn2 (endo-α-1,3-glucanases), which are required for the hydrolysis of cell wall sugars within the primary septum during cytokinesis (Dekker et al., 2004; Martin-Cuadrado et al., 2003). Although a role for Agn2 has been uncovered during the later stages of meiosis (Dekker et al., 2007), the fact that combinations of *agn1*, *eng1* and *agn2* mutants are not sterile suggests that they are not required for cell fusion to occur. It has been shown that the fission yeast exocyst complex is involved in targeting enzymes, including endoglucanases required for septum cleavage (Martin-Cuadrado et al., 2005; Wang et al., 2002). It is therefore possible that, as in budding yeast, a myosin V protein targets secretory vesicles to specific cellular locations (Pruyne et al., 1998), where they activate exocyst-dependent enzymes during cell fusion. Further investigations will hopefully uncover the
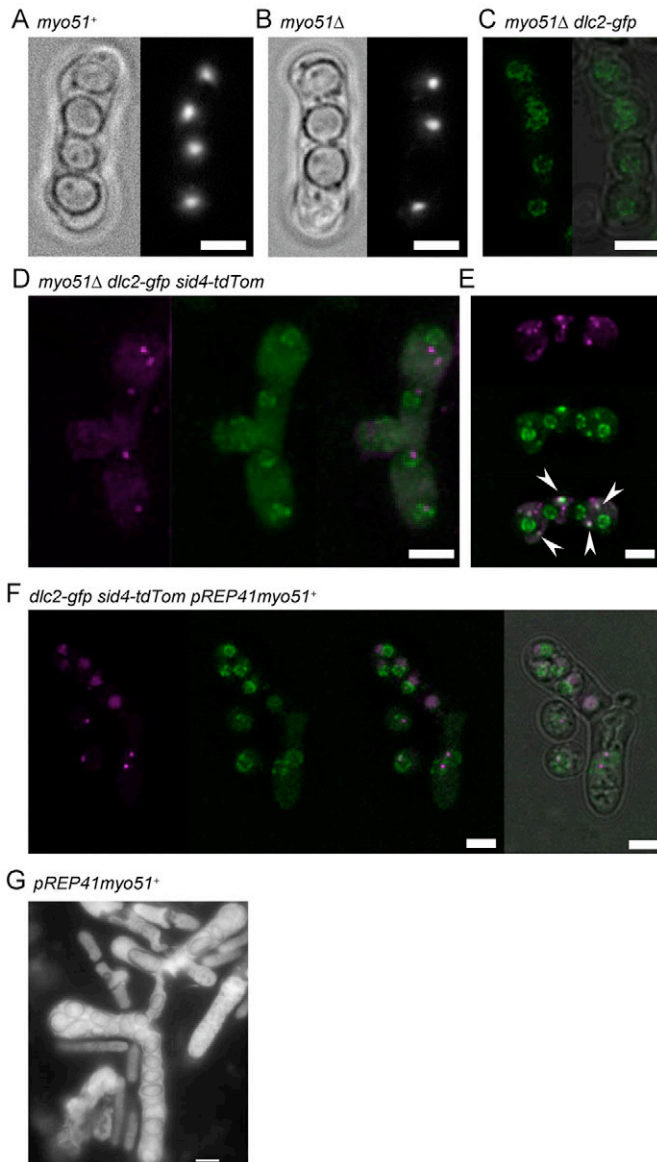
Fig. 7. Myo51 is required for normal meiotic chromosome segregation and spore formation. (A,B) Phase-contrast images (left panels) and DAPI staining (right panels) of asci produced from crosses between *myo51⁺* (A) or *myo51Δ* cells (B). (C) GFP signal (left panel) merged with phase-contrast image (right panel) from *dlc2-gfp myo51Δ* cells illustrate nuclear mis-segregation defects in these cells. (D,E) Sid4-tomato (magenta) and Dlc2 (green) signals reveal SPB defects in *myo51Δ* cells, which often lead to disruption of nuclear membrane integrity (arrowheads). (F,G) Overexpression of full-length Myo51 leads to the formation of asci containing an inappropriate number of spores. (F) These spores often contained aberrant SPB material (Sid4, magenta), rather than nuclei (Dlc2, green). Scale bars: 5 µm.

molecule(s) that Myo51 and Myo52 deliver to the fusion site, to allow normal conjugation to occur.

Myosin V at microtubule-organising centres

Similarly to class II and X myosins, myosin Vs have been reported to localise to the microtubule-organising centres in a number of vertebrate cell types (Espreafico et al., 1998; Tsakraklides et al., 1999), but although myosin II and X are known to be involved in the positioning and assembly of the meiotic spindle (Schuh and Ellenberg, 2008; Weber et al., 2004), the function of myosin Va at the MTOC is unknown. Similarly to Myo51, after recruitment to the MTOC the localisation of myosin Va is actin independent (Tsakraklides et al., 1999), suggesting that once recruited, their localisation to the MTOC occurs independently of its motor activity. Preliminary FRAP data suggest that Myo51 motor activity is required to recruit Myo51 to the SPB (not shown). This is consistent with our observation that the Myo51 tail alone is insufficient for SPB association, and in contrast to murine myosin Va, expression of Myo51-GTD alone is insufficient to displace the endogenous full-length protein from the MTOC in fission yeast (not shown). These data indicate that Myo51 motor activity is required for its recruitment to the SPB, where the tail then associates with a component of the meiotic SPB.

Myosin V and fungal meiosis

Although class V myosins have not been seen to localise to meiotic SPBs in other fungi, these motors have been implicated in transporting prespore membrane precursors to the site of spore formation and conjugation tube formation in budding yeast and the plant pathogen, *Ustilago maydis* (Taxis et al., 2006; Weber et al., 2003), suggesting a conserved meiotic function for this class of motor proteins in fungi. The fission yeast SPB undergoes structural changes during meiosis (Hirata and Shimoda, 1994; Tanaka and Hirata, 1982) when it develops into a site for forespore membrane organisation. We discovered that Myo51 remains associated with the nuclear membrane until onset of meiosis II, when most of these structural changes occur to the outer plaque of the SPB (Shimoda, 2004). This suggests the existence of a control mechanism to regulate Myo51 recruitment to the SPB and the timing of fore spore membrane assembly. This is not only consistent with the reduction in viability of spores produced from a cross between cells lacking Myo51, but is also supported by our unpublished finding that Myo51 interacts with the checkpoint protein Rad24. The function of this protein is essential during a diploid lifecycle (Tanaka et al., 2000), because it acts as a regulator of Byr2, a component of the signalling pathway that is essential for both conjugation and meiosis (Kjaerulff et al., 2005; Ozoe et al., 2002; Wang et al., 1991). However, the most dramatic and compelling evidence to support the existence of a Myo51-dependent mechanism to maintain SPB integrity during meiosis II at the onset of fore spore membrane assembly comes from the finding that overexpression of the full-length Myo51

Table 1. Percentage frequency of defects in spore formation and viability upon modulation of Myo51 expression*

	4 spores/4 nuclei	4 spores/3 nuclei	>4 spores	Spore viability
myo51⁺ × myo51⁺	95.70±2.75	3.17±1.44	1.17±1.6	93.2±5.2
myo51⁺ × myo51Δ	91.80±1.89	5.83±2.25	2.30±0.76	91.2±6.7
myo51Δ × myo51Δ	87.30±1.50	9.17±2.02	3.50±2.29	64.1±8.3
Myo51 overexpression	69.50±6.39	8.75±6.01	21.75±0.35	46.1±3.7

*Data averaged from three independent experiments.
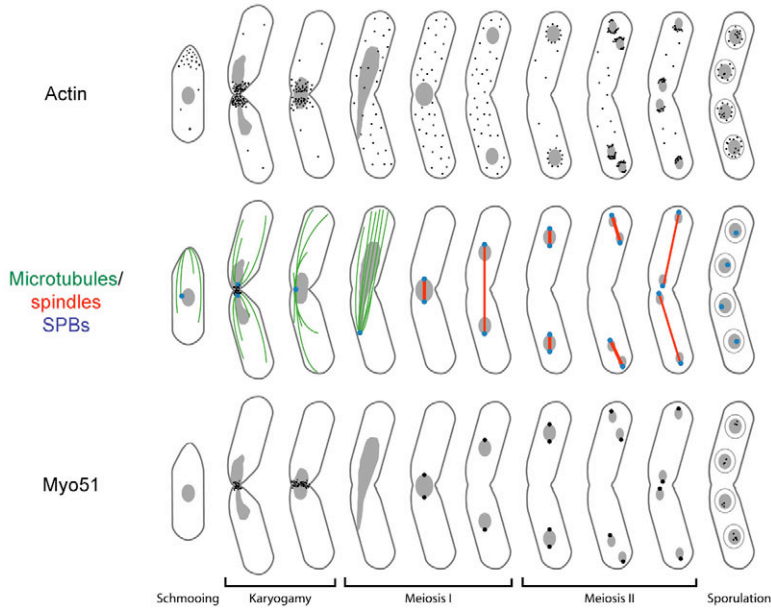
Fig. 8. Schematic representation of localisation of Myo51 and actin throughout the fission yeast meiotic lifecycle.

protein can bring about the formation of asci containing a huge number of spores, the majority of which are unviable.

MPF-dependent regulation of the association of Myo51 with the SPB

Although Myo51 might have a role maintaining the SPB during meiosis, it is unclear how its own recruitment is regulated. FRAP analysis of the Myo51 SPB signal suggests that Myo51 turnover at the SPB is normally reduced as meiosis progresses. Not only is this change in turnover a reflection of the reduction in cellular levels of Cdc13 (CyclinB), but also, when Mes1 is not present in the cell to prevent premature degradation of the pool of cyclin B required for meiosis II onset (Izawa et al., 2005), the Myo51 signal rapidly disappears from the SPB. The fact that it was possible to counteract this effect through the rapid induction of non-degradable Cdc13 indicates the Cdc2-cyclin-B complex is required for localisation of Myo51 at the meiotic MTOCs. In addition to this finding that MPF regulates Myo51 meiotic localisation, Cdc2 has been shown to have an important role in regulating meiosis II progression, but similarly to Myo51, it also coordinates this with subsequent spore formation (Dischinger et al., 2008; Hayles et al., 1986).

It is unclear why normal spindle formation did not occur upon induction of non-degradable Cdc13 in arrested *mes1Δ* cells. It could perhaps indicate that Mes1 prevents degradation of sub-populations of other as yet undetermined proteins required for meiosis II spindle formation, or that Mes1 is required for further unexplored functions during meiosis. However, expression of the non-degradable cyclin B was sufficient for enough activation during meiosis II to promote the Myo51-SPB association and provides an insight into the regulation and function of this actin-associated motor.

Does Myo51 always deliver the goods?

Class V myosins within vertebrate cells exist as dimeric processive motors to transport cellular cargoes throughout the cell (Mehta et al., 1999; Mermall et al., 1998). Although myosin V proteins from simpler organisms demonstrate non-processive in vitro motor activity (Krementsova et al., 2006; Reck-Peterson et al., 2001; Toth et al., 2005), it is clear that they still retain the ability to move rapidly

throughout the cell (Grallert et al., 2007; Schott et al., 2002). Although the processivity of mammalian myosin V and kinesins can be enhanced by associating with each other (Ali et al., 2008), the processivity of the budding yeast class V myosin Myo4, is regulated by binding partners that regulate its ability to dimerise, and thereby walk along actin (Hodges et al., 2008). The data presented here suggest that a similar mechanism might exist in fission yeast to regulate Myo51, the analogue of Myo4 in budding yeast.

Although it is clear that Myo51 is required for proper cell fusion during conjugation, and it is possible to observe Myo51 moving upon actin filaments to the site of cell fusion (not shown), it is not clear whether Myo51 is required to deliver cargoes to the meiotic SPB. We have, as yet, been unable to detect Myo51 foci moving to or from the meiotic SPB. These data illustrate that there is a large non-dynamic population of Myo51 at this organelle throughout meiosis II, and actin is not required for Myo51 to maintain its position there. Myo51 therefore probably interacts with the SPB via its tail domain during this period, which explains its ability to localise there in the absence of actin. This leaves its motor domain free to interact with actin, and thereby act as an anchor or tether for the growing actin polymers to facilitate forespore membrane formation (Petersen et al., 1998b; Taxis et al., 2006). Thus, Myo51 might not only have the ability to deliver cargoes to specific cellular locations, but could also act as a tether or tension sensor within the cell, in a cell-cycle- and indeed lifecycle-dependent manner.

Materials and Methods

Yeast cell culture and strains

S. pombe strains used in this study are listed in supplementary material Table S1. Cell culture and maintenance were carried out as described (Moreno et al., 1991). Cells were grown in supplemented minimal medium (EMM2) or sporulation medium (MSL) (Egel et al., 1994).

Molecular biology

Strains expressing fluorophore-tagged Myo51 at either its N-terminus (*myo51-nGFP* and *myo51-n4HcRed1*) or C-terminus (*myo51-mCherry*) were created using two distinct methods. The *myo51-mCherry* allele, in which the 3′ end of the endogenous chromosomal copy of the *myo51⁺* gene was fused to cDNA encoding the mCherry fluorophore (Shaner et al., 2004) was created using a method described previously

(Bahler et al., 1998) using pFA6a-cherry-kanMX6 as a template (Tanaka et al., 2005). Strains expressing N-terminal fusion proteins were created by isolating the *myo51⁺* gene as a *Sal*I-*Bam*HI fragment from pREP41*myo51⁺* (Win et al., 2001) and cloning this into the *Sal*I-*Bam*HI sites of the plasmids pINT41NGFP and pINT41N4HcRed1 to create pINT41NGFP*myo51⁺* and pINT41N4HcRed1*myo51⁺*. These plasmids were linearised and integrated into the *leu1⁺* locus to create the *myo51-nGFP* and *myo51-n4HcRed1* alleles, respectively. An *act1-nGFP* strain was created by isolating the *act1⁺* cDNA as a *Sal*I fragment by PCR, and cloning this into pINT81NGFP, which was subsequently sequenced, linearised and integrated into the *leu1⁺* locus. The *myo51-GTDnGFP* allele, encoding the Myo51 globular tail domain fused to GFP at its N-terminus, was created by isolating DNA encoding residues 996-1471 of *myo51⁺* as a *Sal*I-*Bam*HI fragment by PCR, which was sequenced and cloned into the *Sal*I-*Bam*HI sites of pINT41NGFP to create pINT41NGFPmyo51-GTD. This was subsequently linearised and integrated into the *leu1⁺* locus.

Fluorescence microscopy

Cells were mounted in a Bioptechs FCS2 (Bioptechs, Butler, PA) and the sample holder, objective lens and environmental chamber were maintained at the required temperature. Samples were visualised using one of two systems. The first was based upon an Olympus IX71 microscope with a PlanApo ×100 TIRFM-SP 1.45 NA lens mounted on a PIFOC Z-axis focus drive (Physik Instruments, Karlsruhe, Germany), and illuminated with an automated 300 W Xenon light source (Sutter, Novato, CA) using appropriate filters and visualised using either a Coolsnap HQ or QuantEM CCD camera (Photometrics, Tucson, AZ) controlled with Metamorph software (Molecular Devices, Downington, PA). The second system was a Deltavision Core (Applied Precision Instruments), mounted on an Olympus IX-71 microscope which utilises an Olympus Plan-apochromat ×100 1.45 NA objective lens and visualisation via a Cascade II 512B EMCCD camera (Photometrics). This system was used to visualise both eGFP and dTomato fluorophores using a 300 W Xenon API light source and ET-sedat filters (Chroma). This system has an additional 50 mW 488 nm laser for photobleaching investigations and the laser aperture was calibrated with a spot size of 0.4 μm.

References

Ali, M. Y., Lu, H., Bookwalter, C. S., Warshaw, D. M. and Trybus, K. M. (2008). Myosin V and Kinesin act as tethers to enhance each others' processivity. *Proc. Natl. Acad. Sci. USA* **105**, 4691-4696.

Bahler, J., Wu, J. Q., Longtine, M. S., Shah, N. G., McKenzie, A., 3rd, Steever, A. B., Wach, A., Philippsen, P. and Pringle, J. R. (1998). Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. *Yeast* **14**, 943-951.

Boe, C. A., Garcia, I., Pai, C. C., Sharom, J. R., Skjolberg, H. C., Boye, E., Kearsey, S., Macneill, S. A., Tyers, M. D. and Grallert, B. (2008). Rapid regulation of protein activity in fission yeast. *BMC Cell Biol.* **9**, 23.

Cartagena-Lirola, H., Duran, A. and Valdivieso, M. H. (2006). The Schizosaccharomyces pombe cfr1+ gene participates in mating through a new pathway that is independent of fus1+. *Yeast* **23**, 375-388.

Chikashige, Y., Ding, D. Q., Funabiki, H., Haraguchi, T., Mashiko, S., Yanagida, M. and Hiraoka, Y. (1994). Telomere-led premeiotic chromosome movement in fission yeast. *Science* **264**, 270-273.

Dekker, N., Speijer, D., Grun, C. H., van den Berg, M., de Haan, A. and Hochstenbach, F. (2004). Role of the alpha-glucanase Agn1p in fission-yeast cell separation. *Mol. Biol. Cell* **15**, 3903-3914.

Dekker, N., van Rijssel, J., Distel, B. and Hochstenbach, F. (2007). Role of the alpha-glucanase Agn2p in ascus-wall endolysis following sporulation in fission yeast. *Yeast* **24**, 279-288.

Dischinger, S., Krapp, A., Xie, L., Paulson, J. R. and Simanis, V. (2008). Chemical genetic analysis of the regulatory role of Cdc2p in the S. pombe septation initiation network. *J. Cell Sci.* **121**, 843-853.

Egel, R., Willer, M., Kjaerulff, S., Davey, J. and Nielsen, O. (1994). Assessment of pheromone production and response in fission yeast by a halo test of induced sporulation. *Yeast* **10**, 1347-1354.

Espreafico, E. M., Coling, D. E., Tsakraklides, V., Krogh, K., Wolenski, J. S., Kalinec, G. and Kachar, B. (1998). Localization of myosin-V in the centrosome. *Proc. Natl. Acad. Sci. USA* **95**, 8636-8641.

Flory, M. R., Morphew, M., Joseph, J. D., Means, A. R. and Davis, T. N. (2002). Pcp1p, an Spc110p-related calmodulin target at the centrosome of the fission yeast Schizosaccharomyces pombe. *Cell Growth Differ.* **13**, 47-58.

Grallert, A., Martin-Garcia, R., Bagley, S. and Mulvihill, D. P. (2007). In vivo movement of the type V myosin Myo52 requires dimerisation but is independent of the neck domain. *J. Cell Sci.* **120**, 4093-4098.

Hagan, I. and Yanagida, M. (1992). Kinesin-related cut7 protein associates with mitotic and meiotic spindles in fission yeast. *Nature* **356**, 74-76.

Hayles, J., Aves, S. and Nurse, P. (1986). suc1 is an essential gene involved in both the cell cycle and growth in fission yeast. *EMBO J.* **5**, 3373-3379.

Hirata, A. and Shimoda, C. (1994). Structural modification of spindle pole bodies during meiosis II is essential for the normal formation of ascospores in Schizosaccharomyces pombe: ultrastructural analysis of spo mutants. *Yeast* **10**, 173-183.

Hodges, A. R., Krementsova, E. B. and Trybus, K. M. (2008). She3p binds to the rod of yeast myosin V and prevents it from dimerizing, forming a single-headed motor complex. *J. Biol. Chem.* **283**, 6906-6914.

Ikemoto, S., Nakamura, T., Kubo, M. and Shimoda, C. (2000). S. pombe sporulation-specific coiled-coil protein Spo15p is localized to the spindle pole body and essential for its modification. *J. Cell Sci.* **113**, 545-554.

Itadani, A., Nakamura, T. and Shimoda, C. (2006). Localization of type I myosin and F-actin to the leading edge region of the forespore membrane in Schizosaccharomyces pombe. *Cell Struct. Funct.* **31**, 181-195.

Izawa, D., Goto, M., Yamashita, A., Yamano, H. and Yamamoto, M. (2005). Fission yeast Mes1p ensures the onset of meiosis II by blocking degradation of cyclin Cdc13p. *Nature* **434**, 529-533.

Jin, Q. W., Pidoux, A. L., Decker, C., Allshire, R. C. and Fleig, U. (2002). The mal2p protein is an essential component of the fission yeast centromere. *Mol. Cell. Biol.* **22**, 7168-7183.

Kjaerulff, S., Lautrup-Larsen, I., Truelsen, S., Pedersen, M. and Nielsen, O. (2005). Constitutive activation of the fission yeast pheromone-responsive pathway induces ectopic meiosis and reveals ste11 as a mitogen-activated protein kinase target. *Mol. Cell. Biol.* **25**, 2045-2059.

Krapp, A., Schmidt, S., Cano, E. and Simanis, V. (2001). S. pombe cdc11p, together with sid4p, provides an anchor for septation initiation network proteins on the spindle pole body. *Curr. Biol.* **11**, 1559-1568.

Krapp, A., Collin, P., Cokoja, A., Dischinger, S., Cano, E. and Simanis, V. (2006). The Schizosaccharomyces pombe septation initiation network (SIN) is required for spore formation in meiosis. *J. Cell Sci.* **119**, 2882-2891.

Krementsova, E. B., Hodges, A. R., Lu, H. and Trybus, K. M. (2006). Processivity of chimeric class V myosins. *J. Biol. Chem.* **281**, 6079-6086.

Kurahashi, H., Imai, Y. and Yamamoto, M. (2002). Tropomyosin is required for the cell fusion process during conjugation in fission yeast. *Genes Cells* **7**, 375-384.

Martin-Cuadrado, A. B., Duenas, E., Sipiczki, M., Vazquez de Aldana, C. R. and del Rey, F. (2003). The endo-beta-1,3-glucanase eng1p is required for dissolution of the primary septum during cell separation in Schizosaccharomyces pombe. *J. Cell Sci.* **116**, 1689-1698.

Martin-Cuadrado, A. B., Morrell, J. L., Konomi, M., An, H., Petit, C., Osumi, M., Balasubramanian, M., Gould, K. L., Del Rey, F. and de Aldana, C. R. (2005). Role of septins and the exocyst complex in the function of hydrolytic enzymes responsible for fission yeast cell separation. *Mol. Biol. Cell* **16**, 4867-4881.

Mata, J., Lyne, R., Burns, G. and Bahler, J. (2002). The transcriptional program of meiosis and sporulation in fission yeast. *Nat. Genet.* **32**, 143-147.

Mehta, A. D., Rock, R. S., Rief, M., Spudich, J. A., Mooseker, M. S. and Cheney, R. E. (1999). Myosin-V is a processive actin-based motor. *Nature* **400**, 590-593.

Mermall, V., Post, P. L. and Mooseker, M. S. (1998). Unconventional myosins in cell movement, membrane traffic, and signal transduction. *Science* **279**, 527-533.

Miki, F., Okazaki, K., Shimanuki, M., Yamamoto, A., Hiraoka, Y. and Niwa, O. (2002). The 14-kDa dynein light chain-family protein Dlc1 is required for regular oscillatory nuclear movement and efficient recombination during meiotic prophase in fission yeast. *Mol. Biol. Cell* **13**, 930-946.

Molero, G., Cid, V. J., Vivar, C., Nombela, C. and Sanchez-Perez, M. (1999). Candida albicans exoglucanase as a reporter gene in Schizosaccharomyces pombe. *FEMS Microbiol. Lett.* **175**, 143-148.

Moreno, S., Klar, A. and Nurse, P. (1991). Molecular genetic analysis of fission yeast Schizosaccharomyces pombe. *Methods Enzymol.* **194**, 795-823.

Niccoli, T., Yamashita, A., Nurse, P. and Yamamoto, M. (2004). The p150-Glued Ssm4p regulates microtubular dynamics and nuclear movement in fission yeast. *J. Cell Sci.* **117**, 5543-5556.

Ozoe, F., Kurokawa, R., Kobayashi, Y., Jeong, H. T., Tanaka, K., Sen, K., Nakagawa, T., Matsuda, H. and Kawamukai, M. (2002). The 14-3-3 proteins Rad24 and Rad25 negatively regulate Byr2 by affecting its localization in Schizosaccharomyces pombe. *Mol. Cell. Biol.* **22**, 7105-7119.

Petersen, J., Weilguny, D., Egel, R. and Nielsen, O. (1995). Characterization of fus1 of Schizosaccharomyces pombe: a developmentally controlled function needed for conjugation. *Mol. Cell. Biol.* **15**, 3697-3707.

Petersen, J., Heitz, M. J. and Hagan, I. M. (1998a). Conjugation in S. pombe: identification of a microtubule-organising centre, a requirement for microtubules and a role for Mad2. *Curr. Biol.* **8**, 963-966.

Petersen, J., Nielsen, O., Egel, R. and Hagan, I. M. (1998b). F-actin distribution and function during sexual differentiation in Schizosaccharomyces pombe. *J. Cell Sci.* **111**, 867-876.

Petersen, J., Nielsen, O., Egel, R. and Hagan, I. M. (1998c). FH3, a domain found in formins, targets the fission yeast formin Fus1 to the projection tip during conjugation. *J. Cell Biol.* **141**, 1217-1228.

Pruyne, D. W., Schott, D. H. and Bretscher, A. (1998). Tropomyosin-containing actin cables direct the Myo2p-dependent polarized delivery of secretory vesicles in budding yeast. *J. Cell Biol.* **143**, 1931-1945.

Reck-Peterson, S. L., Novick, P. J. and Mooseker, M. S. (1999). The tail of a yeast class V myosin, myo2p, functions as a localization domain. *Mol. Biol. Cell* **10**, 1001-1017.

Reck-Peterson, S. L., Tyska, M. J., Novick, P. J. and Mooseker, M. S. (2001). The yeast class V myosins, Myo2p and Myo4p, are nonprocessive actin-based motors. *J. Cell Biol.* **153**, 1121-1126.

Robinow, C. F. (1977). The number of chromosomes in SCHIZOSACCHAROMYCES POMBE: light microscopy of stained preparations. *Genetics* **87**, 491-497.

Rustici, G., Mata, J., Kivinen, K., Lio, P., Penkett, C. J., Burns, G., Hayles, J., Brazma, A., Nurse, P. and Bahler, J. (2004). Periodic gene expression program of the fission yeast cell cycle. *Nat. Genet.* **36**, 809-817.

Schott, D. H., Collins, R. N. and Bretscher, A. (2002). Secretory vesicle transport velocity in living cells depends on the myosin-V lever arm length. *J. Cell Biol.* **156**, 35-39.

Schuh, M. and Ellenberg, J. (2008). A new model for asymmetric spindle positioning in mouse oocytes. *Curr. Biol.* **18**, 1986-1992.

Shaner, N. C., Campbell, R. E., Steinbach, P. A., Giepmans, B. N., Palmer, A. E. and Tsien, R. Y. (2004). Improved monomeric red, orange and yellow fluorescent proteins derived from Discosoma sp. red fluorescent protein. *Nat. Biotechnol.* **22**, 1567-1572.

Shimoda, C. (2004). Forespore membrane assembly in yeast: coordinating SPBs and membrane trafficking. *J. Cell Sci.* **117**, 389-396.

Shimoda, C., Hirata, A., Kishida, M., Hashida, T. and Tanaka, K. (1985). Characterization of meiosis-deficient mutants by electron microscopy and mapping of four essential genes in the fission yeast Schizosaccharomyces pombe. *Mol. Gen. Genet.* **200**, 252-257.

Tanaka, K. and Hirata, A. (1982). Ascospore development in the fission yeasts Schizosaccharomyces pombe and S. japonicus. *J. Cell Sci.* **56**, 263-279.

Tanaka, K., Kohda, T., Yamashita, A., Nonaka, N. and Yamamoto, M. (2005). Hrs1p/Mcp6p on the meiotic SPB organizes astral microtubule arrays for oscillatory nuclear movement. *Curr. Biol.* **15**, 1479-1486.

Tanaka, Y., Okuzaki, D., Yabuta, N., Yoneki, T. and Nojima, H. (2000). Rad24 is essential for proliferation of diploid cells in fission yeast. *FEBS Lett.* **472**, 254-258.

Taxis, C., Maeder, C., Reber, S., Rathfelder, N., Miura, K., Greger, K., Stelzer, E. H. and Knop, M. (2006). Dynamic organization of the actin cytoskeleton during meiosis and spore formation in budding yeast. *Traffic* **7**, 1628-1642.

Toth, J., Kovacs, M., Wang, F., Nyitray, L. and Sellers, J. R. (2005). Myosin V from Drosophila reveals diversity of motor mechanisms within the myosin V family. *J. Biol. Chem.* **280**, 30594-30603.

Toya, M., Motegi, F., Nakano, K., Mabuchi, I. and Yamamoto, M. (2001). Identification and functional analysis of the gene for type I myosin in fission yeast. *Genes Cells* **6**, 187-199.

Tsakraklides, V., Krogh, K., Wang, L., Bizario, J. C., Larson, R. E., Espreafico, E. M. and Wolenski, J. S. (1999). Subcellular localization of GFP-myosin-V in live mouse melanocytes. *J. Cell Sci.* **112**, 2853-2865.

Wang, H., Tang, X., Liu, J., Trautmann, S., Balasundaram, D., McCollum, D. and Balasubramanian, M. K. (2002). The multiprotein exocyst complex is essential for cell separation in Schizosaccharomyces pombe. *Mol. Biol. Cell* **13**, 515-529.

Wang, Y., Xu, H. P., Riggs, M., Rodgers, L. and Wigler, M. (1991). byr2, a Schizosaccharomyces pombe gene encoding a protein kinase capable of partial suppression of the ras1 mutant phenotype. *Mol. Cell. Biol.* **11**, 3554-3563.

Weber, I., Gruber, C. and Steinberg, G. (2003). A class-V myosin required for mating, hyphal growth, and pathogenicity in the dimorphic plant pathogen Ustilago maydis. *Plant Cell* **15**, 2826-2842.

Weber, K. L., Sokac, A. M., Berg, J. S., Cheney, R. E. and Bement, W. M. (2004). A microtubule-binding myosin required for nuclear anchoring and spindle assembly. *Nature* **431**, 325-329.

Win, T. Z., Gachet, Y., Mulvihill, D. P., May, K. M. and Hyams, J. S. (2001). Two type V myosins with non-overlapping functions in the fission yeast Schizosaccharomyces pombe: Myo52 is concerned with growth polarity and cytokinesis, Myo51 is a component of the cytokinetic actin ring. *J. Cell Sci.* **114**, 69-79.

Yamamoto, A., West, R. R., McIntosh, J. R. and Hiraoka, Y. (1999). A cytoplasmic dynein heavy chain is required for oscillatory nuclear movement of meiotic prophase and efficient meiotic recombination in fission yeast. *J. Cell Biol.* **145**, 1233-1249.