The p150-Glued Ssm4p regulates microtubular dynamics and nuclear movement in fission yeast

Teresa Niccoli[1,*], Akira Yamashita[2,*], Paul Nurse[4,‡] and Masayuki Yamamoto[2,3,‡]

[1]Cancer Research UK, London Research Institute, Cell Cycle Laboratory, 44 Lincoln's Inn Fields, London, WC2A 3PX, UK
[2]Molecular Genetics Research Laboratory and [3]Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[4]The President's Office, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

*These authors contributed equally to this work
‡Authors for correspondence (e-mail: paul.nurse@mail.rockefeller.edu; myamamot@ims.u-tokyo.ac.jp)

Accepted 30 July 2004
Journal of Cell Science 117, 5543-5556 Published by The Company of Biologists 2004
doi:10.1242/jcs.01475

Summary

During vegetative growth of the fission yeast *Schizosaccharomyces pombe*, microtubules nucleate from multiple microtubule organising centres (MTOCs) close to the nucleus, polymerising until they reach the end of the cell and then shrinking back to the cell centre. In response to mating pheromone, *S. pombe* undergoes a morphological switch from a vegetative to a shmooing growth pattern. The switch in growth mode is paralleled by a switch in microtubular dynamics. Microtubules nucleate mostly from a single MTOC and pull on the ends of the cell to move the nucleus back and forth. This movement continues after cellular and nuclear fusion in the zygote and is important to ensure correct chromosome pairing, recombination and segregation during meiosis. Here we show that Ssm4p, a p150-Glued protein, is induced specifically in response to pheromone and is required for this nuclear movement. Ssm4p is associated with the cytoplasmic dynein complex and together with the CLIP-170 homologue Tip1p regulates dynein heavy chain localisation. We also show that Ssm4p collaborates with Tip1p in establishing the shmooing microtubular array.

Key words: Fission yeast, Microtubule dynamics, Mating, Meiosis, Dynactin

Introduction

The microtubular cytoskeleton and the regulation of its organisation are important for many cellular functions. Such regulation is executed mainly through microtubule-associated proteins (MAPs) and microtubule motors (Desai and Mitchison, 1997; Hunter and Wordeman, 2000). External signals inducing cell shape changes or differentiation can act via these factors to influence the dynamics of the microtubule cytoskeleton (Chausovsky et al., 2000; Hollenbeck, 2001; Spencer et al., 2000). The well-defined cell shape and microtubular organisation of the genetically amenable fission yeast makes this organism very suitable for the study of microtubular dynamics.

In fission yeast cells growing vegetatively, microtubules are arranged in three to five bundles that align along the long axis of the cell, extending from the centre to the ends of the cell (Drummond and Cross, 2000; Hagan and Hyams, 1988; Hagan, 1998). In response to the mating pheromone, fission yeast cells switch their growth mode from vegetative to shmooing (Fukui et al., 1986). Shmooing cells are often bent, with microtubules curving round the non-growing end and terminating at the shmooing end (Petersen et al., 1998). Cells then undergo cellular and nuclear fusion to form zygotes, which subsequently enter meiosis. During meiotic prophase there are thicker but fewer microtubule bundles, which often curl round one cell end and terminate at the opposite cell end (Ding et al., 1998; Petersen et al., 1998). The microtubules pull on the nuclear located spindle pole body (SPB), which is equivalent to the centrosome in higher eukaryotes, driving the nucleus back and forth, in an oscillatory 'horsetail movement' (Chikashige et al., 1994). A limited oscillatory nuclear movement has also been observed during shmooing growth (Chikashige et al., 1997) and the shmooing microtubular array is very similar to that of meiotic prophase (Niccoli and Nurse, 2002).

During the horsetail period, telomeres cluster close to the SPB, which is located at the leading edge of the migrating nucleus (Chikashige et al., 1994). The telomere clustering and the nuclear movement are thought to facilitate pairing of homologous chromosomes and recombination (Ding et al., 2004; Niwa et al., 2000; Yamamoto et al., 1999). The horsetail nuclear movement requires the cytoplasmic dynein heavy chain Dhc1p (encoded by the *dhc1* gene) (Yamamoto and Hiraoka, 2003; Yamamoto et al., 1999). In the absence of Dhc1p, the nucleus does not display oscillatory movement. The *dhc1* mutant fails in efficient pairing of homologous chromosomal regions and results in reduced frequencies of meiotic recombination (Yamamoto et al., 1999). During the horsetail period, Dhc1p localises to microtubules, the SPB and the point where microtubule plus ends contact the cell cortex (Yamamoto et al., 1999). Dhc1p attached to the cell cortex could haul the microtubules, generating a force pulling on the SPB to drive the nuclear oscillation (Yamamoto et al., 2001). Dhc1p might also contribute to the pulling force at the cortex by regulating the dynamics of microtubule disassembly (Yamamoto et al., 2001). Furthermore, Dhc1p is suggested to play some role in

karyogamy (Miki et al., 2002; Troxell et al., 2001; Yamamoto et al., 1999). We have observed that karyogamy is delayed significantly in the absence of Dhc1p, although it can eventually take place (A.Y. and M.Y., unpublished).

Cytoplasmic dynein, a conserved minus-ended directed motor required for the organisation of microtubular arrays in higher eukaryotes (Clark and Meyer, 1999; Ma et al., 1999), is a multisubunit complex essential for vesicular transport, spindle organisation and nuclear migration (Karki and Holzbaur, 1999; King, 2000). Cytoplasmic dynein is composed of heavy, intermediate, light-intermediate and light chains, and is associated with another protein complex called dynactin, which is required for most dynein-mediated activities. The dynactin complex includes Glued, a coiled-coil protein with a CAP-Gly microtubule-binding motif. Glued interacts with microtubules and cytoplasmic dynein (Gill et al., 1991; Holzbaur et al., 1991; Quintyne et al., 1999; Schafer et al., 1994; Waterman-Storer and Holzbaur, 1996) via a direct interaction with the dynein intermediate chain (Karki and Holzbaur, 1995; Vaughan and Vallee, 1995). CLIP-170, another protein containing a CAP-Gly motif, has also been implicated in the stabilisation of microtubules (Berlin et al., 1990; Brunner and Nurse, 2000) and in directing the localisation of the dynein-dynactin complex (Coquelle et al., 2002; Tai et al., 2002; Valetti et al., 1999; Vaughan et al., 1999).

We previously identified the *ssm4* gene, which encodes a coiled-coil protein carrying a CAP-Gly microtubule-binding domain (Yamashita et al., 1997). Ssm4p has a similar domain structure to Glued. Fission yeast has another coiled-coil protein carrying a CAP-Gly domain, named Tip1p (Brunner and Nurse, 2000; Jannatipour and Rokeach, 1998). Tip1p is a member of the CLIP-170 protein family and has been shown to regulate the stability of interphase microtubules (Brunner and Nurse, 2000). Tip1p has a carboxy-terminal metal binding motif, which is absent in Ssm4p. Based on their molecular design, we suspected that Ssm4p and Tip1p might belong to different subclasses, the former to the Glued family and the latter to the CLIP-170 family.

We aimed to prove that Ssm4p is a functional homologue of Glued and we also examined whether Ssm4p is involved in switching of the growth mode from vegetative to shmooing. Factors regulating vegetative microtubular dynamics are shut down and not required during shmooing growth (Niccoli and Nurse, 2002), suggesting that different factors regulate microtubular dynamics during shmooing. One candidate is the dynein-dynactin complex. Therefore, we analysed the role of Ssm4p in the regulation of microtubular dynamics during mating. Here we show that Ssm4p collaborates with the CLIP-170 homologue Tip1 in generating the shmooing microtubular array.

Materials and Methods

Fission yeast strains, genetic procedures and media

S. pombe strains used in this study are shown in Table 1. General genetic procedures for *S. pombe* were as published (Gutz et al., 1974). Complete medium YE, minimal medium SD, minimal medium MM (Moreno et al., 1991), synthetic sporulation medium SSA (Egel and Egel-Mitani, 1974) and sporulation medium SPA (Gutz et al., 1974) were used. Transformation of *S. pombe* was done as described (Okazaki et al., 1990). To monitor subcellular localisation of Ssm4p and Dhc1p, we constructed *S. pombe* strains JW290 and JW785. JW290 carried a fusion of the *ssm4* and green fluorescent protein (*GFP*) ORFs controlled by the authentic *ssm4* promoter, and JW785 carried a fusion of the *GFP* and *dhc1* ORFs controlled by the thiamine-repressible *nmt1* promoter (Maundrell, 1990). These fusion genes were integrated at the chromosomal *ssm4* and *dhc1* loci, respectively, according to standard protocols (Bahler et al., 1998). GFP-tagged Ssm4p and Dhc1p appeared to be fully functional, as judged by their ability to produce normal asci. For shmooing cells, PN3454, PN3785, PN3783, PN4431 and PN4445 were constructed similarly. Tagged Ssm4 and Dhc1 were created by fusing the C-terminus of the endogenous genes to GFP, MYC or HA according to published methods (Bahler, 1998).

Live analysis of nuclear dynamics

To visualise nuclei, JY450, JW652 and JW327 were transformed with a plasmid that expressed a GST-NLS-GFP fusion protein from the weak *nmt1* promoter (Basi et al., 1993). They were cultured in MM medium at 30°C up to mid-log phase, washed, shifted to MM-N medium and incubated for 3-5 hours. These cells were then mounted on a thin layer of 1% agarose containing MM-N medium, which was attached to a glass slide, to induce mating and meiosis. Live images were taken at room temperature (24-26°C), using a chilled CCD

Table 1. *Saccharomyces pombe* strains used

JW290	h^{90}	*ssm4GFP<<kan^r ade6-M216 leu1*
JW327	h^{90}	*dhc1::ura4$^+$ ade6-M216 leu1 ura4-D18*
JW612	h^+	*ssm4::ura4$^+$ ade6-M210 his2*
JW652	h^{90}	*ssm4:: kan^r ade6-M216 leu1*
JW695	h^{90}	*ssm4:: kan^r dhc1::ura4$^+$ ade6-M216 leu1 ura4-D18*
JW785	h^{90}	*kan^r>>nmt1-GFP-dhc1 ade6-M216 leu1*
JX528	h^-	*ssm4::ura4$^+$ ade6-M216 leu1 ura4-D18*
JX648	h^{90}	*tip1::ura4$^+$ ade6-M216 leu1 ura4-D18*
JX650	h^{90}	*ssm4::ura4$^+$ tip1::ura4$^+$ ade6-M216 leu1 ura4-D18*
JY245	h^+	*ade6-M210 his2*
JY333	h^-	*ade6-M216 leu1*
JY450	h^{90}	*ade6-M216 leu1*
PN3454	h^-	*cyr1::ura4$^+$ sxa2::ura$^+$ dhc1GFP<<LEU2 ura4-D18 leu1*
PN3783	h^-	*cyr1::ura4$^+$ sxa2::ura4$^+$ ssm4HA<<kan^r ura4-D18 leu1*
PN3785	h^-	*cyr1::ura4$^+$ sxa2::ura4$^+$ ssm4GFP<<kan^r ura4-D18 leu1*
PN3855	h^-	*cyr1::ura4$^+$ sxa2::ura4$^+$ tip1::kan^r ssm4::kan^r ura4-D18 leu1*
PN3864	h^-	*cyr1::ura4$^+$ sxa2::ura4$^+$ tip1::kan^r ura4-D18 leu1*
PN4429	h^-	*cyr1::ura4$^+$ sxa2::ura4$^+$ nmt1-GFP-atb2 ura4-D18 leu1*
PN4430	h^-	*cyr1::ura4$^+$ sxa2::ura4$^+$ nmt1-GFP-atb2 ssm4::kan^r ura4-D18leu1*
PN4431	h^-	*cyr1::ura4$^+$ sxa2::ura4$^+$ ssm4HA<<kan$^+$ dhc1MYC<<kan^{r}ura4-D18 leu1*
PN4445	h^-	*cyr1::ura4$^+$ sxa2::ura4$^+$ dhc1GFP<< LEU2 tip1YFP<<kan^{r}ura4-D18 leu1*

camera (Photometrics, Quantix) attached to a fluorescence microscope (Carl Zeiss, Axioplan 2) and the MetaMorph software (Universal Imaging Corporation).

Observation of Ssm4p-GFP and GFP-Dhc1p in living cells

JW290 carrying pREP81-*CFP-atb2* and JW785 were grown in MM medium at 30°C to the mid-log phase, washed and spotted onto SPA medium. After incubation for 6-8 hours at 30°C, cells were observed as described above. For shmooing cells, PN3785 and PN3454 were induced with 3 μg/l P factor for 5 hours. The cells were then placed on a slide and monitored with a fluorescence microscope mounted with a CCD camera. YFP-GFP double-labelled strain PN4445 was monitored (Niccoli and Nurse, 2002). The brightness of Ssm4p-GFP dots was measured with NIH Image, subtracting the background for every time point.

Live analysis of microtubular dynamics

Live microtubules were visualised with *nmt1-GFP-atb2* integrated into the genome (Ding et al., 1998) in the wild type and *ssm4Δ* cells, PN4429 and PN4430, respectively. In *tip1Δ* cells and *tip1Δ ssm4Δ* cells, PN3864 and PN3855, microtubules were visualised by transforming the pDQ105 plasmid carrying *nmt1-GFP-atb2* (Ding et al., 1998). Cells were grown in MM medium with low thiamine (0.06 μg/ml) to partially induce the *nmt1* promoter, mounted on a soybean lectin (25 μg/ml; Calbiochem)-coated glass bottom dish and imaged with a Zeiss LSM150 confocal microscope. *tip1Δ* microtubules, which were not bright enough to be monitored with a confocal microscope, were monitored with a fluorescence microscope.

The lengths of live microtubules were measured in LSM 5 Image Browser. The length of a microtubule bundle at each time point was compared to the previous time point, if the length was increasing the dynamics was marked as polymerising (+), if decreasing, as depolymerising (–), and if staying the same, as stalling (0). The dynamics of the microtubules were calculated for each time point and then the dynamics of all microtubule bundles at the same end were compared. In 80% of cases, the majority of microtubules at a single end displayed the same dynamics, which we called consensus dynamics. We then compared the consensus dynamics of both ends for each time point to verify whether they were the same or different. In the comparison between opposite ends, time points that presented stalling consensus dynamics, which occurred 9% of the time, were not taken into account.

Bleaching of microtubules

Cells were prepared as above and filmed on an inverted confocal ZEISS LSM 150 microscope. A small area along a microtubule was selected each time and bleached with 600 nm laser on 75% power and using as many iterations as needed; these had to be determined every time because of laser variability, and then the cells were monitored for recovery of fluorescence. To depolymerise microtubules, 1 ml pre-conditioned medium with MBC (Carbendazim) (25 μg/ml from a 5 mg/ml stock in DMSO) was added to the dish with the cells after bleaching, rapid adjustments to the focus were sometimes needed. Cells were continuously filmed throughout the experiment.

Immunofluorescence

Cells were fixed in –80°C methanol and processed according to published methods (Mata and Nurse, 1997). Tubulin was visualised using TAT1 monoclonal antibody (a gift from Prof. K. Gull, University of Manchester, UK) (Woods et al., 1989) and Sad1p with anti-Sad1 (Hagan and Yanagida, 1995). The secondary antibodies were goat anti-mouse Alexa546 and goat anti-rabbit Cy5. Images were taken with a Zeiss LSM 510 laser scanning confocal microscope.

Immunoprecipitation

Native cell extracts were made as described (Yamaguchi et al., 2000) in HB buffer (25 mM MOPS pH 7.2, 15 mM $MgCl_2$, 15 mM EGTA, 1% Triton X100) with the protease inhibitor set (Roche) and phosphatase inhibitors. Anti-HA (1:200) antibody or anti-MYC antibody (1:200) was pre-bound to protein G beads for 1 hour. Extracts were incubated with the beads for 2 hours. The beads were washed three times and then boiled in the presence of loading buffer to release the bound complex. Boiled samples were then separated by 7% low-bis SDS-PAGE. Western blot analysis and detection were carried out as previously described (Yamaguchi et al., 2000). For detection, both anti-HA (12CA5) and anti-MYC (9E10) were used at 1:1000.

Two-hybrid system

Two-hybrid screening was carried out using a commercially supplied *S. pombe* cDNA library (Clontech) and a meiotic cDNA library constructed from *S. pombe pat1* diploid cells undergoing synchronised meiosis (Y. Akiyoshi and M.Y., unpublished). Bait plasmids that expressed a full-length Ssm4p, fused to either the Gal4 DNA-binding domain on pGBT9 or the LexA DNA-binding domain on pBTM116, were used for each screening. Dlc1p was isolated as a positive clone from both libraries. For β-galactosidase assay, *S. cerevisiae* L40 (*Mata ade2 his3 leu2 trp1 LYS2::lexA-HIS3 URA3::lexA-lacZ*) was used.

Results

Ssm4p is essential for the oscillatory nuclear movement during meiotic prophase

Ssm4p is a microtubule-binding protein produced under nutrient starvation (Yamashita et al., 1997) and has a similar domain arrangement to Glued-type proteins (Fig. 1), with a CAP-Gly domain at its N terminus followed by a coiled-coil stretch (Brunner and Nurse, 2000) (Fig. 1). CLIP-170-like proteins, including Tip1p, have a similar domain structure to Glued-type proteins, but they have in addition a metal binding motif at their C-terminus (Fig. 1). To test whether Ssm4p is a Glued-type protein, acting as part of the putative dynein-dynactin complex, we analysed the role of Ssm4p during the meiotic horsetail movement. We investigated whether it played a similar function to the dynein heavy chain Dhc1p, which is required for the nuclear movement and the regulation of microtubular dynamics (Yamamoto et al., 2001).

To visualise the nucleus in living cells, GFP was fused to a nuclear localisation signal and expressed in the wild type and *ssm4Δ* strain. In wild-type cells, the nucleus started oscillating between the cell ends immediately after karyogamy (Chikashige et al., 1994; Ding et al., 1998; Yamamoto et al., 1999) (Fig. 2A). In contrast, in *ssm4Δ* cells the nucleus remained in the middle of the cell, similar to *dhc1Δ* cells (Fig. 2A). This indicates that both Ssm4p and Dhc1p are required for horsetail nuclear movement. Comparison of meiotic divisions in the wild type and *ssm4Δ* strain showed that *ssm4Δ* cells proceeded through the meiotic divisions with the same timing as wild-type cells (Fig. 2B,C), suggesting that Ssm4p, like Dhc1p, is not required for meiotic nuclear divisions.

During meiotic prophase, telomeres cluster close to the SPB, which is located at the leading edge of the oscillating nucleus (Chikashige et al., 1994). To examine telomere clustering in the *ssm4Δ* cells, we induced meiosis in those cells expressing Taz1-GFP, which serves as a marker for telomeres (Cooper et

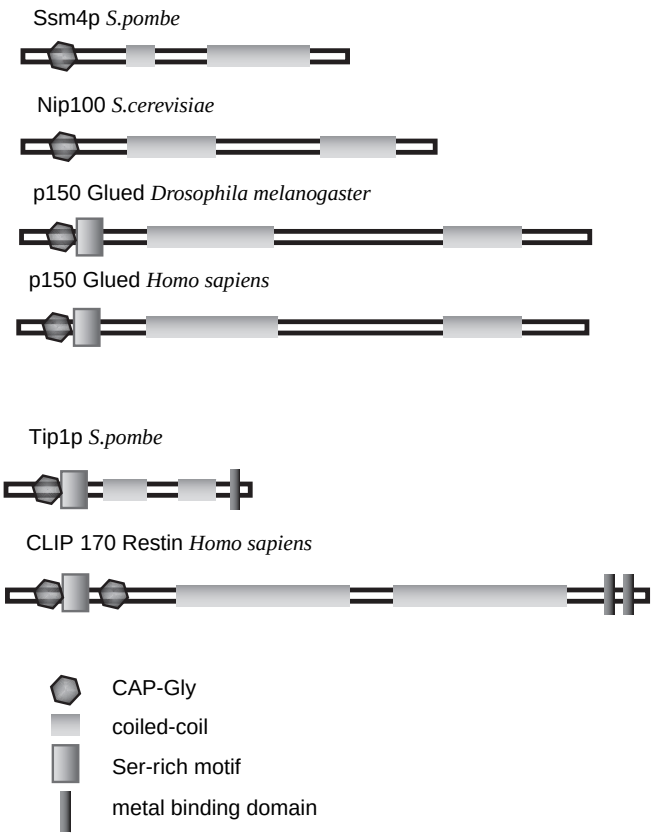

Fig. 1. Domain arrangement of Ssm4p in *Saccharomyces pombe* and related proteins. Ssm4p domain structure and related proteins were identified in the Pfam database. The protein diagrams are not exactly to scale.

al., 1997; Jin et al., 2002). Cells were fixed and stained with anti-GFP monoclonal antibody and anti-Sad1 antibody (Hagan and Yanagida, 1995). Clustering of telomeres at the SPB was observed to occur normally in the *ssm4* mutant (data not shown).

Chromosome pairing is suppressed and meiotic recombination is reduced in the *ssm4* mutant

Meiotic recombination is significantly reduced in the *dhc1* mutant (Yamamoto et al., 1999). We examined meiotic recombination in *ssm4Δ* cells by tetrad analysis, measuring genetic linkage between the *leu1* and *his2* loci on chromosome II. The results showed that genetic recombination between these two loci was reduced almost 12-fold in the *ssm4Δ* strain (Table 2).

Pairing of chromosomes at homologous loci is an apparent prerequisite for efficient meiotic recombination. It has been shown that homologous chromosome regions do not colocalise efficiently in meiotic prophase in the *dhc1* mutant (Yamamoto

Table 2. Tetrad analysis of genetic linkage between *his2* and *leu1* in the *ssm4* fission yeast mutant

Crossed strains	Number of tetrads				Calculated genetic distance (cM)
	Tetratype	Non-parental ditype	Parental	Total	
wt	20	1	96	117	11.1
ssm4Δ	2	0	109	111	0.9

JY245 was crossed with JY333 and JW612 was crossed with JX528 on SSA medium at 30°C. Produced asci were dissected and the genotype of each spore was tested. The genetic distance was calculated according to the equation, D=50×(T+6NPD)/Total.

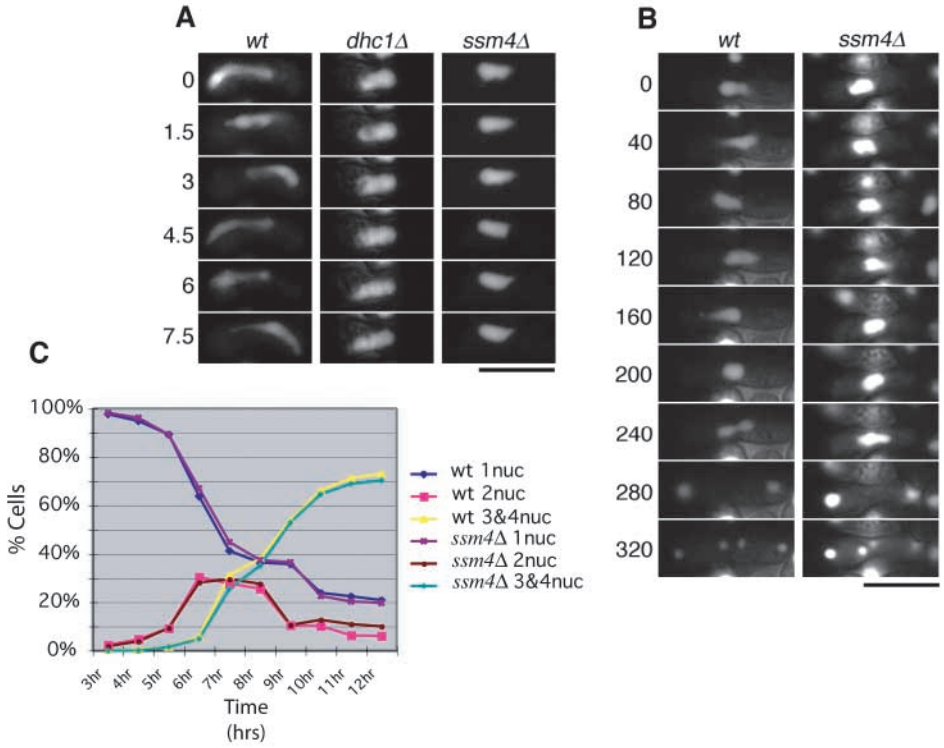

Fig. 2. Ssm4p is essential for the horsetail movement in fission yeast. Nuclei of conjugated cells were monitored and the fluorescence of the artificial nuclear marker GST-NLS-GFP was followed. (A) Nuclear behaviour in living wild type, *dhc1Δ* and *ssm4Δ* strains. Serial images were obtained from a single cell at meiotic prophase. The numbers on the left indicate time span in minutes. (B) Time-lapse recording of a nucleus in a wild-type cell and a *ssm4Δ* cell undergoing meiosis. The numbers on the left indicate the time from the nuclear fusion in minutes. Bars, 10 μm. (C) Diploid wild-type and *ssm4Δ* cells were cultured to late log phase, synchronously induced to meiosis at 30°C by depleting nitrogen from the medium. The number of nuclei was counted at the times indicated. More than 200 cells were examined at each time point. Open circles, open squares, open triangles indicate percentage of cells with one nucleus, two nuclei and three or four nuclei, respectively.

et al., 1999). Therefore, we investigated pairing of homologous chromosomal regions in the *ssm4* mutant. To visualise homologous regions by GFP fluorescence, a tandem repeat of the *Escherichia coli lac* operator sequence was integrated at the *his2* locus of the wild type and *ssm4Δ* strain, which resides near the centromere of chromosome II. GFP-tagged LacI, which binds to the *lac* operator sequence, was expressed in these strains. After mating, 88% of wild-type cells at meiotic prophase showed a single GFP dot, indicating the loci were paired. In contrast, only 42% of *ssm4Δ* cells showed a single GFP dot at meiotic prophase. These observations indicate that, as in *dhc1Δ* cells, homologous chromosomes fail to pair efficiently in the *ssm4* mutant. A failure in chromosome pairing is a probable cause of missegregation at meiosis I and generation of asci with less than four spores (see below). Staining of chromosomes sometimes revealed a missegregated chromosome mass during meiosis I in *ssm4Δ* cells (data not shown), as has been reported for the *dhc1* mutant (Yamamoto et al., 1999).

The *ssm4Δ* strain frequently generated aberrant asci carrying less than four spores (Yamashita et al., 1997) (Table 3). Disruption of the dynein heavy chain gene *dhc1* caused similar abnormal sporulation (Yamamoto et al., 1999) (Table 3). We constructed an *ssm4 dhc1* double mutant strain and found that this strain gave aberrant asci at nearly the same frequency as each single mutant (Table 3). These observations strongly suggest that Ssm4p and Dhc1p are responsible for similar subcellular function and are part of the same pathway.

Ssm4p interacts with the dynein complex

Various reports have shown that Glued-type proteins co-immunoprecipitate with the dynein complex (Karki and Holzbaur, 1995). We investigated if this was also the case in fission yeast by constructing a strain in which Ssm4p and Dhc1p were tagged with different peptides in a *cyr1Δ sxa2Δ* background, which can synchronously switch from vegetative to shmooing growth in response to exogenously added pheromone (Niccoli and Nurse, 2002). Ssm4p-HA and Dhc1p-myc were not detectable during vegetative growth but appeared after 6 hours in the presence of pheromone (Fig. 3A), indicating that the proteins are induced during shmooing growth. Immunoprecipitation of Ssm4p-HA in shmooing cells brought down Dhc1p-myc and vice versa (Fig. 3B), indicating that Ssm4p and Dhc1p are part of the same complex.

Table 3. Number of spores per ascus in the *ssm4, dhc1* and *tip1* fission yeast mutants*

	Number of asci carrying the following number of spores (%)			
Strain	1	2	3	4
wt	<0.1	<0.1	1.2	98.8
ssm4Δ	0.5	8.6	7.6	83.3
dhc1Δ	1.2	10.1	9.1	79.6
ssm4Δ dhc1Δ	1.0	10.5	10.3	78.2
tip1Δ	<0.1	1.4	8.9	89.7
ssm4Δ tip1Δ	1.7	14.1	22.9	61.3

*Sporulation was induced in each strain on SSA medium at 30°C. Spores were scored after 3 days.

More than 500 asci were examined for each strain.

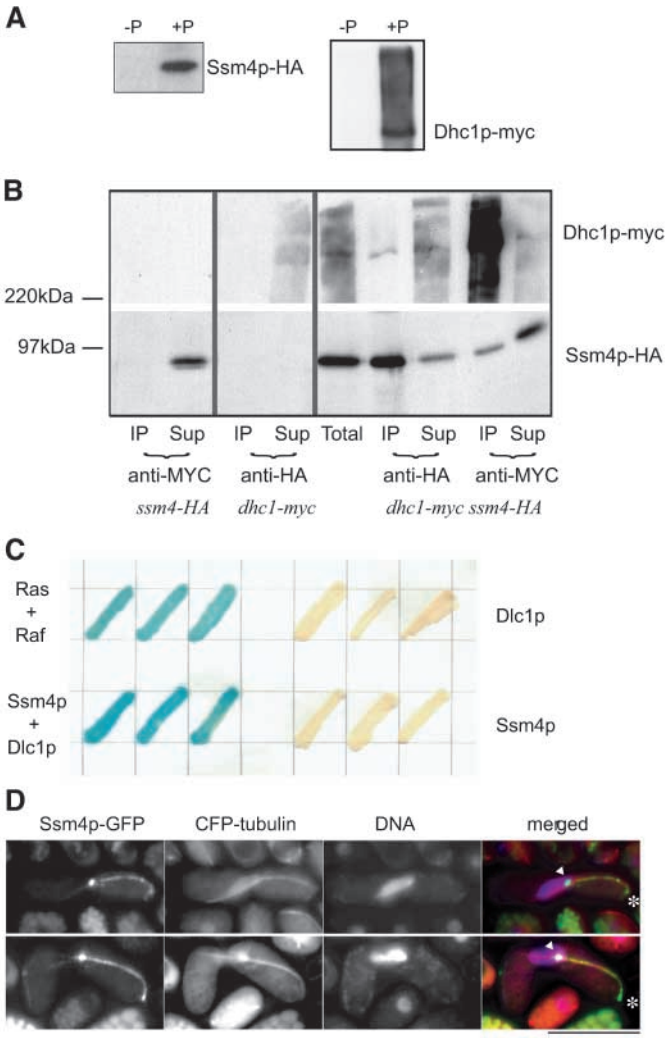

Fig. 3. Ssm4p and the dynein complex interact. (A) *cyr1Δ sxa2Δ ssm4-HA* and *cyr1Δ sxa2Δ dhc1-myc* fission yeast cells were grown in the absence (–P) and in the presence (+P) of pheromone for 6 hours. Boiled cell extracts were prepared as described and western blots were probed with anti-HA or anti-MYC antibodies. (B) *cyr1Δ sxa2Δ dhc1-myc ssm4-HA* cells were induced with pheromone for 6 hours. Native cell extracts were immunoprecipitated with anti-HA and anti-MYC antibodies. Western blots of immunoprecipitates (IPs), supernatants and total cell extracts were carried out as described and probed with HA and MYC antibodies (right panel). Control IPs with anti-HA of *cyr1Δ sxa2Δ dhc1-myc* did not precipitate any Dhc1p-myc (middle panel) and IPs with anti-MYC of *cyr1Δ sxa2Δ ssm4-HA* did not precipitate any Ssm4p-HA (left panel). (C) Two-hybrid interaction of Ssm4p with Dlc1p. β-galactosidase activity was assayed in *Saccharomyces cerevisiae* cells expressing proteins as indicated. The combination of Ras and Raf was used as a positive control. (D) Localisation of Ssm4p-GFP in meiotic cells. Homothallic haploid cells that carried the *ssm4-GFP* fusion gene and the *CFP-atb2* fusion gene encoding CFP-tubulin were subjected to nitrogen starvation to induce mating and subsequent meiosis in zygotes. They were monitored by fluorescence microscopy for localisation of Ssm4p and microtubules. Nuclear DNA was stained with Hoechst 33342. Merged images are shown in the right panels. Green, GFP; red, CFP; blue, DNA. The arrowheads and the asterisks indicate intense GFP fluorescence at the leading edge of the nucleus and at the microtubular tip contacting the cell cortex, respectively. Bar, 10 μm.

To identify novel factors that might interact directly with Ssm4p, we carried out a yeast two-hybrid screen using the full-length Ssm4p as bait. We isolated Dlc1p, a cDNA clone encoding the dynein light chain, in this screen (Fig. 3C). Dlc1p is suggested to play Dhc1p-independent roles in karyogamy, meiotic recombination and sporulation (Miki et al., 2002). However, Dlc1p is required to anchor Dhc1p at the cell cortex during the horsetail period and a defect in *dlc1* affects the oscillatory nuclear movement (Miki et al., 2002). Thus, Dlc1p is likely to be a component of the cytoplasmic dynein complex, at least temporarily. The positive two-hybrid interaction between Ssm4p and Dlc1p suggests that Ssm4p may mediate the binding between the dynein and putative dynactin complexes in fission yeast.

It has been reported that Glued can directly bind to the dynein intermediate chain in rat (Karki and Holzbaur, 1995; Vaughan and Vallee, 1995). The fission yeast genome sequencing project has predicted a putative gene encoding a possible homologue of the dynein intermediate chain (ORF SPBC855.01c; GenBank accession number AL391016). We cloned this gene and designated it *dic1*. Disruption of *dic1* resulted in the same phenotype as the *ssm4* and *dhc1* mutants, i.e., lack of horsetail nuclear movement and production of aberrant asci. No positive interaction of Ssm4p and Dic1p, however, has been observed in two-hybrid assays (data not shown).

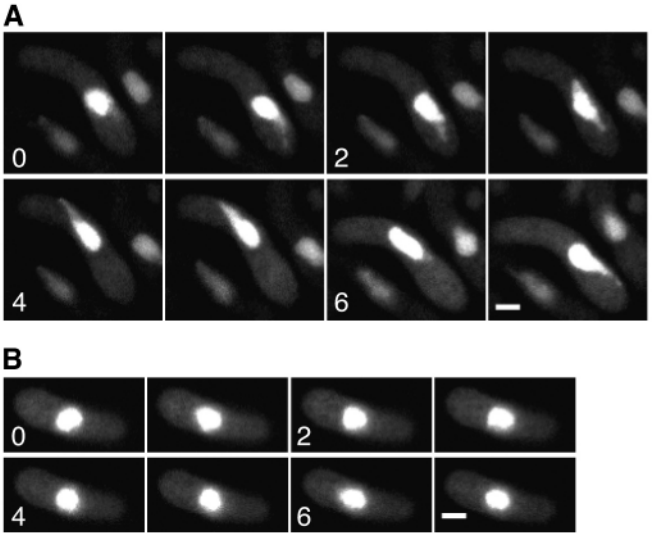

Fig. 4. Ssm4p is essential for the oscillatory nuclear movement in shmooing fission yeast cells. (A,B) *cyr1Δ sxa2Δ* cells (A) and *cyr1Δ sxa2Δ ssm4Δ* cells (B) carrying a nuclear GFP marker were treated with pheromone for 6 hours, placed on a slide, and filmed under a fluorescent microscope. Images were taken every minute. Bar, 3 μm.

Ssm4p is localised to the SPB, microtubules and the point where microtubule plus ends contact the cell cortex in meiotic cells

We previously reported that Ssm4p colocalised with microtubules in both mitotic and meiotic cells when expressed ectopically (Yamashita et al., 1997). To examine localisation of Ssm4p under natural conditions, we tagged endogenously expressed Ssm4p with GFP. Ssm4p-GFP fluorescence was not detectable during vegetative growth (data not shown). Green fluorescence could be observed during meiotic prophase, which indicated colocalisation of Ssm4p-GFP with microtubules (Fig. 3D). Localisation of Ssm4p-GFP to the leading edge of the horsetail nucleus, where the SPB resides, was also evident. In addition, Ssm4p-GFP was detected at the site where microtubule plus ends contacted the cell cortex at the cell tips. These localisations of Ssm4p-GFP coincide with those of Dhc1p (Yamamoto et al., 1999). Altogether, Ssm4p appears to act in the same pathway as Dhc1p, to interact with Dhc1p and Dlc1p, and localises in a similar manner to Dhc1p, indicating that Ssm4p is a functional homologue of Glued-type proteins.

The nucleus oscillates in shmooing cells in an Ssm4p-dependent manner

A limited nuclear movement has been observed in cells under shmooing growth (Chikashige et al., 1997). We investigated this movement in cells of a *cyr1Δ sxa2Δ* background, in which shmooing growth could be induced synchronously in response to exogenously added pheromone. Similar to zygotes, the nucleus in shmooing cells oscillated back and forth (Fig. 4A). However, the oscillatory movement did not occur in the absence of Ssm4p (Fig. 4B). This suggested that similar mechanisms, if not completely the same, were likely to be responsible for nuclear oscillation during the shmooing and horsetail periods.

We then investigated localisation of Ssm4p-GFP in shmooing cells. Ssm4p-GFP became visible in response to pheromone, arranged in linear arrays that colocalised with microtubules, and dispersed when the cells were treated with carbendazim or MBC (a microtubule-depolymerising drug) (Fig. 5A,B). This suggests that the protein is localised along microtubules. Ssm4p-GFP also appeared as bright dots (Fig. 5A,C). One dot coincided with the SPB marker Sad1p (Hagan and Yanagida, 1995), whereas the others localised to a point where the microtubule plus ends touched the cortex (Fig. 5A, arrows and arrowheads, respectively). To verify if Ssm4p moved within the cell, we analysed the behaviour of the protein by time-lapse fluorescence microscopy of Ssm4p-GFP in shmooing cells. Ssm4p-GFP fluorescence along microtubules had a dotted pattern but none of these dots displayed a clear pattern of movement (Fig. 5C). The bright dot at the SPB moved in the cell as expected (Fig. 5C, yellow arrowhead). The fluorescence at the tips of the microtubules increased when microtubules stalled or depolymerised (Fig. 5C, white arrow and 5D), suggesting that Ssm4p can bind to depolymerising or stalling ends.

Ssm4p and Tip1p are required to localise Dhc1p in shmooing and meiotic cells

We analysed if the absence of Ssm4p had any effect on Dhc1p localisation and vice versa. In the absence of Dhc1p, Ssm4p could no longer accumulate at sites where the microtubule plus ends contacted the cortex in meiotic cells (Fig. 6A compare with Fig. 3D). Similarly, Dhc1p required Ssm4p to accumulate at the site where the microtubule plus ends contacted the cortex in both meiotic and shmooing cells (Fig. 6B,C). This could

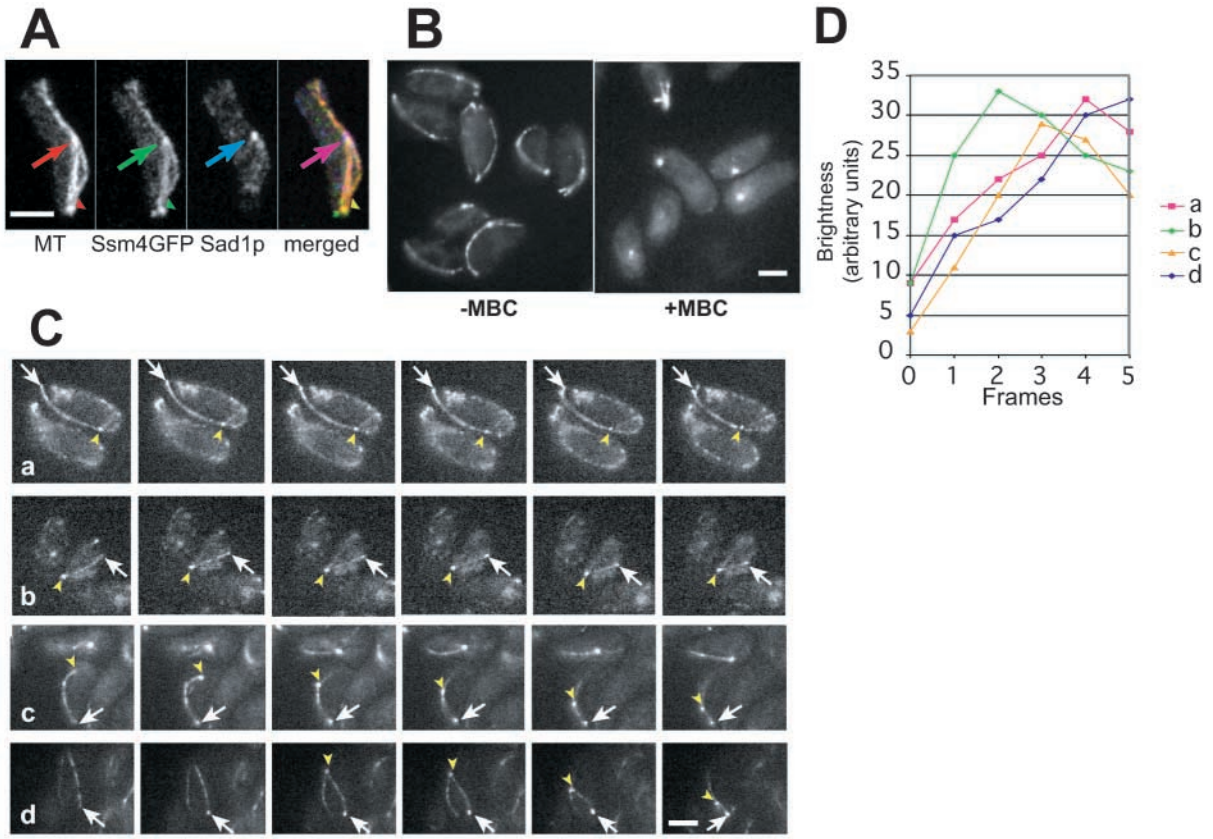

Fig. 5. Ssm4p is expressed during fission yeast mating and localises to microtubules. (A) *cyr1Δ sxa2Δ ssm4GFP* cells were induced for 6 hours with pheromone, cells were then fixed in methanol and immunostained with anti-tubulin (MT) and anti-Sad1 antibodies. Arrows indicate the SPB and arrowheads the point where microtubule plus ends contact the cell surface. (B) *cyr1Δ sxa2Δ ssm4GFP* cells were induced for 6 hours with pheromone, treated with MBC and imaged on a fluorescent microscope before and after the treatment. (C) *cyr1Δ sxa2Δ ssm4GFP* cells were induced with pheromone for 6 hours, placed on a slide and filmed with a fluorescence microscope. Frames are 8-10 seconds apart. White arrows indicate the microtubule tips and yellow arrowheads the fluorescent dot at the SPB, which can be identified as a point where more than one microtubule originates. The microtubular tip is anchored in a fixed position in a, c and d and the SPB is moving towards it as the microtubule depolymerises. In b, the SPB is not moving and the microtubule is depolymerising back towards it. Bars, 3 μm. (D) Fluorescence at the tip of microtubules in C (marked by the white arrow) was measured with NIH image for each frame and plotted.

explain why no pulling force is generated in the absence of Ssm4p. However, both Ssm4p and Dhc1p could independently localise to microtubules and the SPB in the absence of each other (Fig. 6A-E).

CLIP-170-like proteins have been found to interact with the dynactin complex (Coquelle et al., 2002). We next investigated if Tip1p also played a role in localising Dhc1p and Ssm4p. Dhc1p-GFP and Ssm4p-GFP localisation was similar to that of wild-type cells in the absence of Tip1p (Fig. 6A-C), but in the double mutant *ssm4Δ tip1Δ* Dhc1p-GFP totally lost its microtubular localisation, although it retained its SPB localisation (Fig. 6B-E). Dhc1p-GFP colocalised with Tip1p-YFP at the tips of microtubules in response to pheromone (Fig. 6F), suggesting that they might interact.

We investigated if Tip1p was required for the formation of wild-type asci. The *tip1* mutant sporulated at a slightly lower frequency than the wild type (71% versus 82%), and produced aberrant asci like *dhc1* or *ssm4* mutants, although the deficiency was less severe (Table 3). However, the defect in the *ssm4Δ tip1Δ* mutant was more severe than the single mutants *ssm4Δ* or *tip1Δ* (Table 3). These results suggest that both Tip1p and Ssm4p contribute to the formation of spores but probably act through independent pathways.

Ssm4p is required for oscillatory microtubular movement during shmooing

Microtubules have been implicated in nuclear migration during meiotic prophase, and we therefore tested whether the nuclear oscillation defect seen in haploid *ssm4Δ* cells was due to a microtubular cytoskeleton defect by analysing the microtubules in *ssm4Δ* responding to pheromone. To generate oscillatory movement, microtubular dynamics at opposite ends of the cell might be coordinated, with microtubules polymerising at one end while depolymerising at the opposite end. To verify if wild-type microtubules exhibited such coordinated behaviour, we monitored live microtubular dynamics during shmooing. Each cell end had one to five microtubule bundles at any given time, and we measured the length of each of these bundles (Fig. 7A,B) and calculated whether they were polymerising or depolymerising. We compared the dynamics of microtubules at the same end for

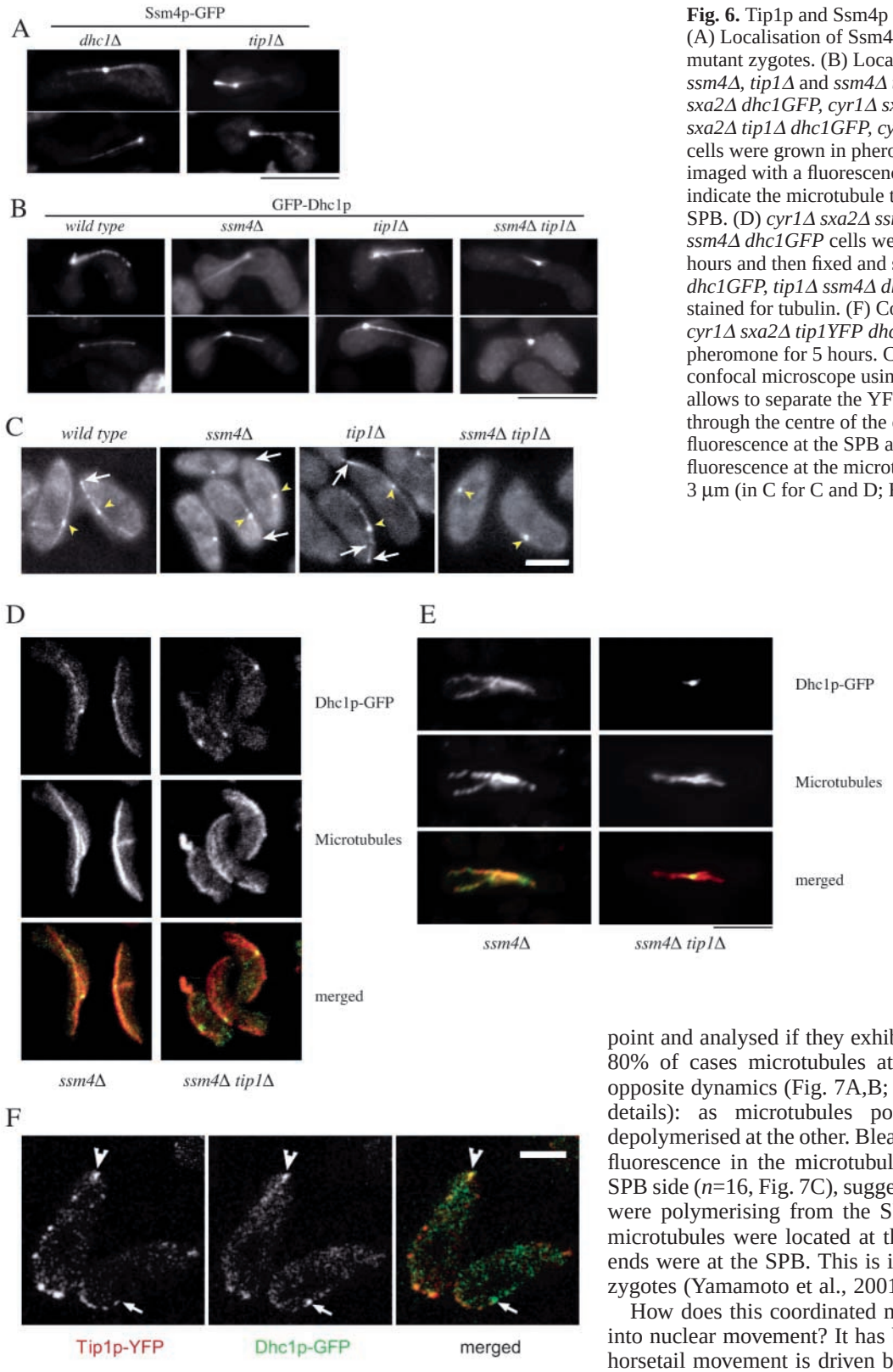

Fig. 6. Tip1p and Ssm4p collaborate to localise Dhc1p. (A) Localisation of Ssm4p-GFP in the *dhc1* and *tip1* mutant zygotes. (B) Localisation of GFP-Dhc1p in the *ssm4Δ*, *tip1Δ* and *ssm4Δ tip1Δ* mutant zygotes. (C) *cyr1Δ sxa2Δ dhc1GFP, cyr1Δ sxa2Δ ssm4Δ dhc1GFP, cyr1Δ sxa2Δ tip1Δ dhc1GFP, cyr1Δ sxa2Δ tip1Δ ssm4Δ dhc1GFP* cells were grown in pheromone for 6 hours and then imaged with a fluorescence microscope. The white arrows indicate the microtubule tips and the yellow arrowheads the SPB. (D) *cyr1Δ sxa2Δ ssm4Δ dhc1GFP, cyr1Δ sxa2Δ tip1Δ ssm4Δ dhc1GFP* cells were grown in pheromone for 6 hours and then fixed and stained for tubulin. (E) *ssm4Δ dhc1GFP, tip1Δ ssm4Δ dhc1GFP* zygotes were fixed and stained for tubulin. (F) Colocalisation of Tip1p and Dhc1p. *cyr1Δ sxa2Δ tip1YFP dhc1GFP* cells were treated with pheromone for 5 hours. Cells were visualised with a confocal microscope using a CFP-YFP filter set which allows to separate the YFP and GFP signals. A single plane through the centre of the cells was taken. Arrows indicate fluorescence at the SPB and arrowheads indicate fluorescence at the microtubular tip. Bars, 10 μm (A,B); 3 μm (in C for C and D; F); 5 μm (E).

each time point. When a majority of microtubules at a single end displayed the same dynamics, we called this ‘consensus dynamics’; this occurred in 80% of cases. We then compared the consensus dynamics at opposite cell ends for each time point and analysed if they exhibited coordinated behaviour. In 80% of cases microtubules at opposite cell ends displayed opposite dynamics (Fig. 7A,B; see Materials and Methods for details): as microtubules polymerised at one end they depolymerised at the other. Bleaching experiments showed that fluorescence in the microtubules always recovered from the SPB side (n=16, Fig. 7C), suggesting that nascent microtubules were polymerising from the SPB, and that the plus ends of microtubules were located at the cell tips whereas the minus ends were at the SPB. This is in accordance with results with zygotes (Yamamoto et al., 2001).

How does this coordinated microtubule behaviour translate into nuclear movement? It has been proposed that the meiotic horsetail movement is driven by a pulling force generated on microtubules at the cell ends (Yamamoto et al., 2001). The same could be true in shmooing cells and as microtubules do not curl around the body of the cell, they must depolymerise as they are pulled. To verify this possibility, we bleached a small area on a microtubule and monitored its dynamics after MBC addition, as it depolymerised. After addition of MBC the

bleached area moved towards one end of the cell. As microtubules were all depolymerising after the addition of the drug, the movement must be due to a pulling force exerted on the shrinking microtubule at the cortex as it depolymerised (Fig. 7D).

In contrast, microtubules of *ssm4Δ* shmooing cells did not appear to oscillate. Microtubule bundles appeared much thicker than wild-type microtubules, and their length did not change significantly over a filming period (Fig. 8A,B). However, the fluorescence intensity along the bundle did change, suggesting that individual microtubules might be growing and shrinking without coordination of the bundle as a whole (Fig. 8C). Microtubule fluorescence after bleaching recovered with a similar timing to the wild type (Fig. 8D), and the fluorescence always recovered from the SPB side (n=17), suggesting that microtubule polarity is similar to the wild type. There was also no coordination of the dynamics at the opposite ends of the cell, as only in 35% of cases did microtubules display coordinated behaviour (Fig. 8B). We bleached a small area on a microtubule bundle in *ssm4Δ* cells, and then depolymerised microtubules with MBC. The bleached area did not move as microtubules depolymerised and shrank back to a stud in the middle of the cell (Fig. 8E). This indicates that a pulling force is not generated on shrinking microtubules in *ssm4Δ* cells, and that microtubules are not anchored at the cortex.

We conclude that in the absence of Ssm4p, microtubule bundles are thicker, presumably because of more microtubules per bundle, that the coordination within the bundle and between opposite cell ends is lost, and that microtubules are no longer anchored or able to generate a pulling force at the cell cortex.

Tip1p and Ssm4p collaborate to allow the switch to shmooing microtubular dynamics

Tip1p is required to stabilise microtubules in vegetative cells (Brunner and Nurse, 2000), whereas Ssm4p appears to be required to coordinate the dynamics but not to stabilise microtubules in shmooing cells. Tip1p is still present during shmooing growth, and so may be

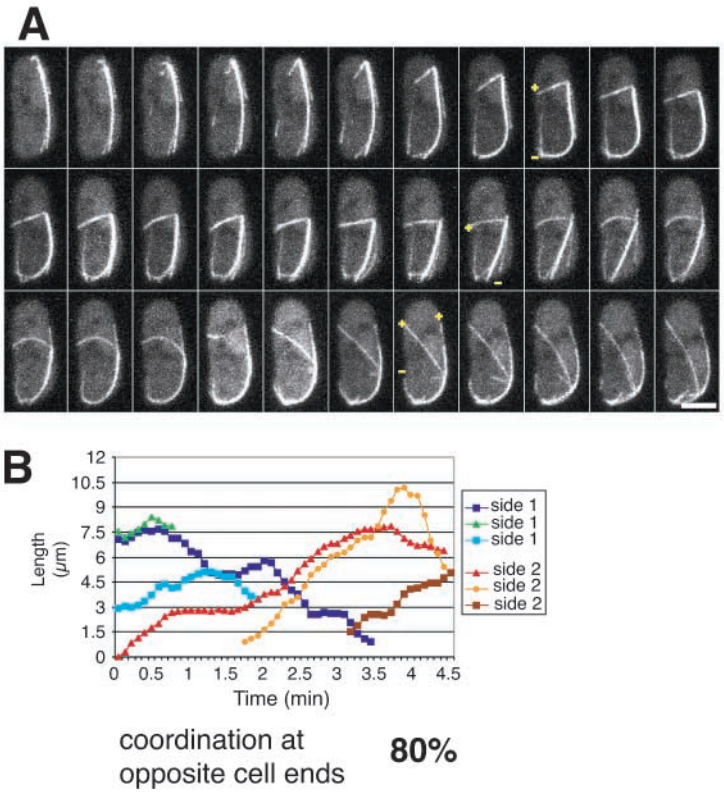

Fig. 7. Microtubular dynamics in wild-type fission yeast cells are coordinated. (A) *cyr1Δ sxa2Δ nmt1atb2GFP* cells were treated with pheromone for 5 hours. Cells were then imaged every 6 seconds on a confocal microscope. The panels show the first 3.3 minutes of a typical time course. Images shown are projections of sections through the whole cell. + and – indicate growing and shrinking microtubules respectively. (B) Microtubule lengths for single cells were measured distinguishing the side of the cell in which they lie. The diagram describes the dynamics of the microtubules of the cell shown in A over the total filming period of 4.5 minutes. The concordance of dynamics at each cell end and between opposite cell ends was then calculated as a percentage of total time points, where eight cells were scored for a total imaging time of 45 minutes. (C) *cyr1Δ sxa2Δ nmt1atb2GFP* were induced with pheromone for 5-6 hours, placed on a lectin-coated glass bottom dish, bleached with a localised laser beam and then filmed every 6 seconds to monitor the pattern of recovery. Images show a single plane through the middle of the cell. The yellow arrows indicate the position of the SPB, from which more than one microtubule originates. The grey lines mark the initial bleached area. The bleached area moves in the same direction as the SPB, with the distance between the SPB and the further boundary of the bleached area remaining the same, as indicated by a green bar in two panels, and the fluorescence recovers from the boundary closer to the SPB. (D) *cyr1Δ sxa2Δ nmt1atb2GFP* cells were induced with pheromone for 6 hours. Microtubules were bleached with a pulse of laser light; MBC was then added to depolymerise the microtubules, which were filmed with a confocal microscope (see Materials and Methods for details). Images shown are single planes through a cell. The red arrow marks the initial bleaching position. Bar, 3 μm.

able to partially substitute for the absence of Ssm4p. To test this possibility we monitored microtubular dynamics in shmooing *tip1Δ* and *ssm4Δ tip1Δ* cells. Microtubular dynamics in a *tip1Δ* strain varied from cell to cell. Some microtubules did not behave in a coordinated manner in certain cases (Fig. 9A,B, light blue and orange microtubules), whereas in other cases microtubules appeared similar to the wild type (Fig. 9C). The SPB oscillated in the former cases, suggesting that a pulling force was still generated (Fig. 9A, yellow arrow). Overall, the coordination between opposite ends was 73%, similar to that in the wild type. The microtubule growth rate was not significantly different from the wild-type growth rate (Tables 4, 5), although the shrinkage rate was a little slower. This confirms that Tip1p does not play a major role in the regulation of microtubular dynamics during shmooing.

In contrast, the double mutant *ssm4Δ tip1Δ* shmooing cells displayed a dramatically different microtubular pattern to that in wild-type shmooing cells. Microtubule bundles spanned the whole cell length, terminating at the cell end and they no longer displayed coordinated dynamics at opposite cell ends (Fig. 9D,E). This is similar to that of wild-type vegetative cells. Both growth and shrinkage rates were slower than in the wild type (Tables 4, 5). The phenotype of the double mutant was also different from that of the *ssm4Δ* single mutant, in which microtubule bundles were thick and did not show much apparent oscillation, as stated above. Microtubule dynamics in *ssm4Δ* were difficult to determine because of this trait.

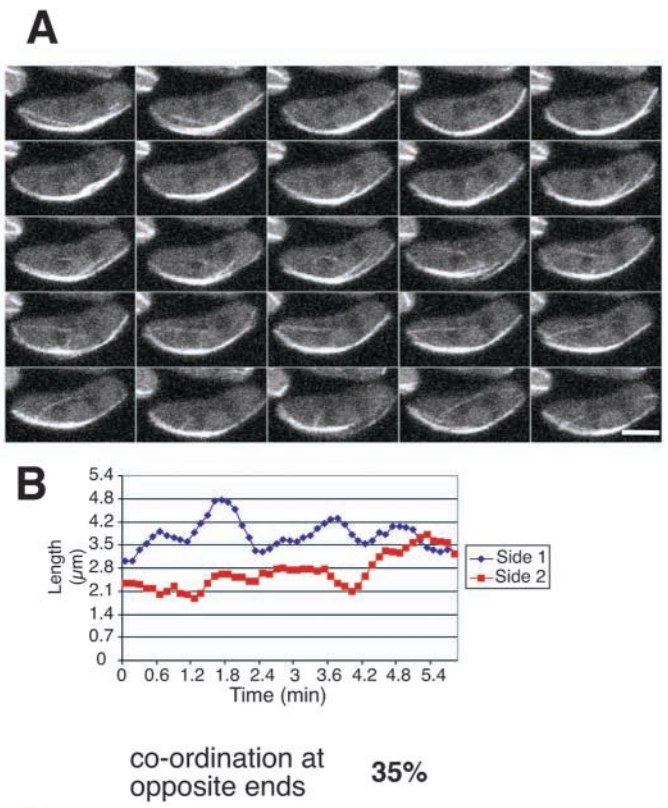

Fig. 8. Microtubules in *ssm4Δ* fission yeast mutants are less dynamic and do not oscillate. (A) *cyr1Δ sxa2Δ ssm4Δ nmt1atb2GFP* cells were treated with pheromone for 5 hours. Cells were then imaged every 12 seconds on a confocal microscope. Images shown are projections of sections through the whole cell. (B) The lengths of microtubules for single cells were measured distinguishing on which side of the cell they lie. The diagram describes the dynamics of the microtubules of the cell in A. The coordination of dynamics between opposite cell ends was then calculated as a percentage of total time points. Four cells were scored for a total imaging time of 20 minutes. At least 10 cells were observed for a total imaging time of 50 minutes and they all exhibited a similar behaviour. (C) *cyr1Δ sxa2Δ ssm4Δ nmt1atb2GFP* cells were induced with pheromone for 6 hours. Cells were imaged every 7 seconds on a confocal microscope. Images shown are projections of sections through the cell. The yellow arrow indicates where there is a change of fluorescence along a bundle. (D) *cyr1Δ sxa2Δ ssm4Δ nmt1atb2GFP* cells were induced with pheromone for 5-6 hours, placed on a lectin-coated glass bottom dish, bleached with a localised laser beam and then filmed every 6 seconds to monitor the pattern of recovery. Images show a single plane through the middle of the cell. The yellow arrows indicate the position of the SPB. The grey lines mark the initial bleached area. The bleached area recovers from the SPB. The time taken by fluorescence to recover over a 2.3 μm bleached area was 43.3±11.1 seconds (n=16) for the wild type (from Fig. 7C) and 40.3±11.3 seconds (n=17) for *ssm4Δ* cells. (E) *cyr1Δ sxa2Δ ssm4Δ* cells were induced with pheromone for 6 hours. Microtubules were bleached with a pulse of laser light, MBC was then added to depolymerise the microtubules and microtubules were filmed with a confocal microscope. Images shown are single planes through a cell. The yellow arrowheads mark the stud remaining after microtubules had depolymerised. Bar in A,C, 3.5 μm; D,E, 3 μm.

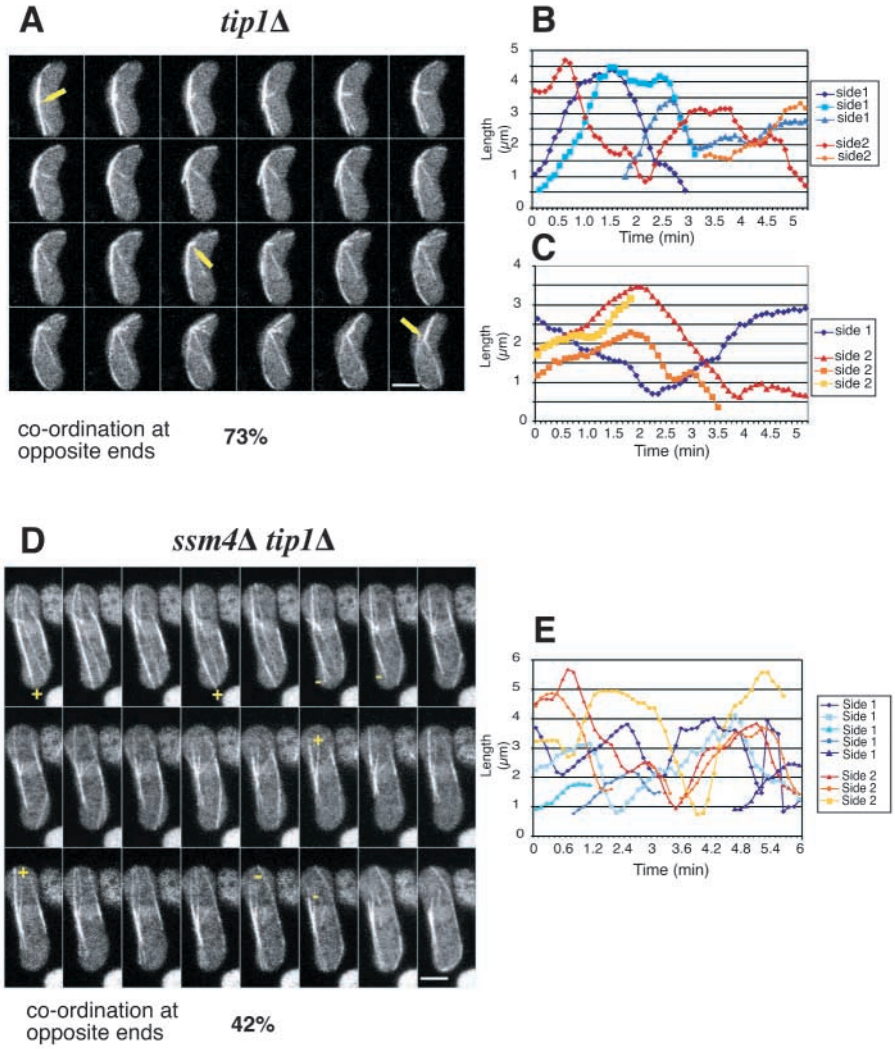

Fig. 9. Microtubular dynamics have lost coordination in *ssm4Δ tip1Δ* cells. (A,B,C) *cyr1Δ sxa2Δ tip1Δ nmt1atb2GFP* and (D, E) *cyr1Δ sxa2Δ tip1Δ ssm4Δ nmt1atb2GFP* cells were treated with pheromone for 5 hours. (A,D) Cells were then imaged every 6 seconds on a confocal microscope. Images shown are projections of sections through the whole cell. Yellow arrows mark the position of the SPB. + and – indicate growing and shrinking microtubules respectively. (B,C,E) Microtubule lengths for single cells were measured distinguishing the side of the cell in which they lie. B describes the dynamics of the microtubules of the cell in A, diagram E corresponds to D, and diagram in C describes a different *tip1Δ* cell. The coordination of dynamics between opposite cell ends was then calculated as a percentage of total time points; eight cells were scored for a total imaging time of 45 minutes. Bar, 2 μm.

Altogether, we conclude that Ssm4p and Tip1p collaborate in the switch of microtubular behaviour from a vegetative to a shmooing array.

Discussion

In this paper we show that Ssm4p behaves as a homologue of the Glued family of proteins and that it collaborates with Tip1p both to allow the switch to shmooing microtubular dynamics and to localise the dynein heavy chain Dhc1p. Cells lacking Ssm4p have an almost identical phenotype to cells lacking Dhc1p and do not show any horsetail nuclear movement. This nuclear movement is required for efficient pairing of homologous chromosomes and efficient recombination, and both processes are impaired in *ssm4Δ* cells. This study has demonstrated that Ssm4p is involved in the same cellular activities as dynein and that it interacts with the dynein complex in fission yeast, indicating that Ssm4p is functionally equivalent to p150-Glued.

During the oscillatory nuclear movement in shmooing cells, microtubular dynamics at opposite ends of the cell are coordinated, with microtubules polymerising at one end as they depolymerise at the other, similar to that observed in zygotes (Yamamoto et al., 2001). A pulling force is exerted at the cortex on depolymerising microtubules, which drives the nucleus and SPB to one end of the cell (Yamamoto et al., 2001) (this study). Once the nucleus reaches the cell end, the pulling force at that end stops and the microtubules at the opposite end start to depolymerise and pull on the nucleus in the opposite direction.

Table 4. Parameters of microtubular dynamics

Strain	Growth (μm/minute)*	Shrinkage (μm/minute)*
wt	2.97±1.23 (*n*=30)	4.47±2.58 (*n*=28)
tip1Δ	2.45±1.25 (*n*=38)	2.75±1.11 (*n*=21)
tip1Δ ssm4Δ	2.23±1.41 (*n*=39)	3.02±1.41 (*n*=37)

*Growth and shrinkage rates were calculated for *wt*, *tip1Δ* and *tip1Δssm4Δ* cells. Microtubular dynamics could not be calculated in *ssm4Δ* because the bundles were thicker and did not display a dissectible dynamic behaviour in our films. The number of microtubules observed is indicated by *n*.

Table 5. Probability values for comparisons of microtubular dynamics using a two-tailed Student's *t*-test

Strain	Growth	Shrinkage
wt vs *tip1Δ*	0.10	0.003
wt vs *tip1Δ ssm4Δ*	0.025	0.010
tip1Δ vs *tip1Δ ssm4*	0.45	0.43

For this mechanism to work four conditions have to be met: (1) The whole bundle has to depolymerise at the same time; (2) A pulling force has to be generated on the microtubules; (3) The microtubules have to be anchored to the cortex as they depolymerise; (4) The coordination of microtubule dynamics at opposite ends ensures that the movement occurs continuously and smoothly. Notably, all these conditions are lost in *ssm4Δ* cells and microtubule bundles appear brighter, suggesting that they might be thicker. Dhc1p is thought to generate the pulling force at the site where microtubule plus ends contact the cell cortex (Yamamoto et al., 2001). In *ssm4Δ* cells Dhc1p can no longer accumulate at that point, and therefore might not be able to pull on microtubules. The dynactin complex is also known to regulate dynein motor activity by directly influencing its ATPase activity (Kumar et al., 2000) and increasing its processivity (King and Schroer, 2000). Ssm4p might therefore regulate dynein both by activating its motor activity and by localising it to the microtubule tips, where it can exert its pulling force.

Tip1p is a protein with similar domain structure to Ssm4p and is a CLIP-170-like protein. Proteins of this type have also been implicated in dynein function; CLIP-170 colocalises with dynein and dynactin (Dujardin et al., 1998; Smith et al., 2000) and co-immunoprecipitates with the dynein intermediate chain (Coquelle et al., 2002). Tip1p appears to have no role in localising Dhc1p or Ssm4p in fission yeast, and has only a minor role in the regulation of microtubular dynamics during shmooing. Surprisingly the double mutant *ssm4Δ tip1Δ* was unable to generate a shmooing microtubular array, could not localise Dhc1p-GFP along microtubules in meiotic cells and displayed severe aberrations in spore formation. This suggests that Ssm4p and Tip1p collaborate in the coordination of microtubular dynamics during shmooing and after shmooing to generate the characteristic oscillatory nuclear movement. The two deletions display a synergistic interaction, as the phenotype of the double mutant is more severe than the sum of the two individual phenotypes. Ssm4p and Tip1p could act independently of each other, regulating two independent aspects of shmooing microtubular dynamics, both of which have to be absent for the microtubule array to completely disassemble. For example, Tip1p might be required to bundle microtubules, as in its absence, microtubules appear less bright (data not shown), and to regulate microtubule growth and shrinkage rates, as the double mutant displays similar dynamics to *tip1Δ* cells. Ssm4p, on the other hand, might coordinate microtubular dynamics within a bundle and between opposite ends of the cell. In the absence of both, microtubules are less bundled and uncoordinated, and consequently take on the appearance of a vegetative microtubular array. Alternatively, Tip1p might partially substitute for Ssm4p function in *ssm4Δ* cells and only when both proteins are missing is the real function of Ssm4p unveiled.

The dynein-dynactin complex is involved in nuclear orientation or movement in many organisms such as *S. cerevisiae*, *Aspergillus*, *Neurospora*, *Drosophila*, *Caenorhabditis elegans* and also in mammalian cells. In many of these organisms disruption of dynein function leads to microtubular defects, suggesting that, as in *S. pombe*, dynein plays a role in regulating microtubular dynamics (Gaglio et al., 1997; Han et al., 2001; Merdes et al., 1996; Shaw et al., 1997). The role of dynein-dynactin in nuclear migration is probably best understood in *S. cerevisiae*. During mitosis, the nuclear spindle has to align with the mother-daughter neck and then elongate through the neck. The latter requires microtubule sliding along the cortex and dynein is required for this movement (Adames and Cooper, 2000). The dynactin Nip100 and CLIP-170 Bik1 have been implicated in this process (Kahana et al., 1998; Sheeman et al., 2003). In contrast to *S. pombe*, it has been recently shown that dynein heavy chain Dyn1 accumulates at the tips of microtubules in a dynactin mutant (Lee et al., 2003; Sheeman et al., 2003) and that Bik1 is required for Dyn1 localisation at the tips of microtubules (Lee et al., 2003; Sheeman et al., 2003), suggesting that although the same components are involved in nuclear migration they might be regulated differently.

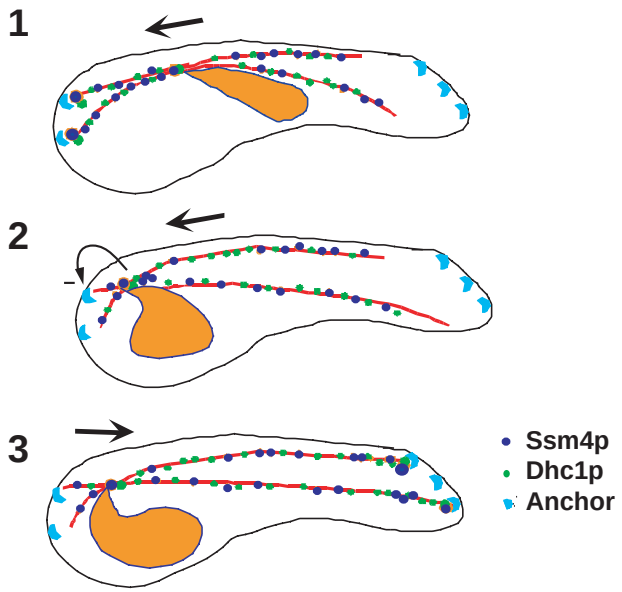

Fig. 10. Model of microtubular oscillations. (1) The microtubules ahead of the nucleus depolymerise as they are pulled by Dhc1p, as proposed by Yamamoto et al. (Yamamoto et al., 2001). Ssm4p activates Dhc1p motor activity and ensures that the microtubules can interact with the cortical anchor, whose presence was proposed by Yamamoto et al. The microtubules behind the nucleus polymerise more slowly than the ones ahead are depolymerising and so they cannot reach the cell end and interact with the cortical anchor. (2) When the overlap region reaches the end of the cell, microtubules can no longer depolymerise and they dissociate from their cortical anchor, the putative dynein-dynactin complex, which is moving in the minus direction, is no longer anchored at the cortex and so could move towards the SPB. (3) When microtubules lose their cortical anchor they will no longer be pulled, the microtubules behind will be able to grow until they reach the cortical anchors in the opposite end. The anchors, or another factor at the cell ends, might induce microtubules to pause. Ssm4p will then accumulate at the microtubule tips, generating a stable interaction with the cortex and triggering microtubule depolymerisation. Dhc1p will also accumulate at the tips of microtubules, stabilised by Ssm4, which will also activate the motor activity of Dhc1p, which will start pulling the nucleus in the opposite direction. Alternatively, when the SPB reaches the cell end, it sends a signal to the opposite microtubule tip, activating it for an interaction with the cortical anchor.

In the *ssm4Δ* and the *dhc1Δ* strains, the process of karyogamy was somewhat delayed (Yamamoto et al., 1999) (our unpublished results). However, nuclear fusion eventually occurred in these strains at a comparable frequency to the wild type. Therefore, it is likely that some other factor(s) is responsible for regulating microtubular dynamics to allow nuclear fusion. One possible candidate is a kinesin-like protein Klp2p, as a double disruptant of *klp2* and *dhc1* was severely impaired in karyogamy (Troxell et al., 2001).

The present study suggests that a new set of regulators, which rely on the putative dynein-dynactin complex, are induced to establish microtubular arrays for shmooing and meiosis. Ssm4p is among such regulators, which appears to play a key role in coordinating microtubular dynamics between the opposite ends and driving the nucleus back and forth in a shmooing cell. Our model, based on that proposed by Yamamoto and colleagues (Yamamoto et al., 2001), is presented in Fig. 10. Our model differs as we attribute to Ssm4p some of the activities previously attributed to dynein, such as the regulation of microtubule dynamics. Moreover, we do not postulate the presence of an anchor-inactivating factor at the SPB but we propose that the dynamics of the microtubules themselves could be sufficient to explain the nuclear oscillations. Ssm4p may contribute to this in three ways: it ensures that microtubules within a bundle display similar dynamics; it guarantees that microtubules at opposite cell ends display opposite dynamics; and it supports the anchoring and pulling on depolymerising microtubules at the cell cortex, maybe via Dhc1p. It is probable that Ssm4p regulates microtubule dynamics similarly during the meiotic horsetail period, though this remains to be proven critically

We thank Mercedes Pardo, Rafael Carazo-Salas, Stefania Castagnetti and Takashi Toda for their comments on the manuscript, and the Cell Cycle Lab, in particular Damian Brunner for helpful discussions. We also thank Da-Qiao Ding for the tubulin-GFP plasmid and strain, Ayumu Yamamoto and Yasushi Hiraoka for the *dhc1* strains and Minoru Yoshida for the nuclear marker. T.N. was supported by a Cancer Research UK graduate studentship. Part of this study was supported by a Grant-in-Aid for Scientific Research (A.Y.) and a Grant-in-Aid for Specially Promoted Research (M.Y.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. We would like to dedicate this paper to the memory of Heidi Browning, who provided invaluable support and discussions.

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