

# The role of *Schizosaccharomyces pombe dma1* in spore formation during meiosis

Andrea Krapp, Elena Cano del Rosario and Viesturs Simanis\*

EPFL SV ISREC UPSIM, SV2.1830, Station 19, CH-1015 Lausanne, Switzerland

\*Author for correspondence ([viesturs.simanis@epfl.ch](mailto:viesturs.simanis@epfl.ch))

Accepted 30 June 2010

Journal of Cell Science 123, 3284-3293

© 2010. Published by The Company of Biologists Ltd

doi:10.1242/jcs.069112

## Summary

Meiosis is a specialised form of the cell cycle that gives rise to haploid gametes. In *Schizosaccharomyces pombe*, the products of meiosis are four spores, which are formed by encapsulation of the four meiosis II nuclei within the cytoplasm of the zygote produced by fusion of the mating cells. The *S. pombe* spindle pole body is remodelled during meiosis II and membrane vesicles are then recruited there to form the forespore membrane, which encapsulates the haploid nucleus to form a prespore. Spore wall material is then deposited, giving rise to the mature spore. The septation initiation network is required to coordinate cytokinesis and mitosis in the vegetative cycle and for spore formation in the meiotic cycle. We have investigated the role of the SIN regulator *dma1p* in meiosis; we find that although both meiotic divisions occur in the absence of *dma1p*, asci frequently contain fewer than four spores, which are larger than in wild-type meiosis. Our data indicate that *dma1p* acts in parallel to the leading-edge proteins and septins to assure proper formation for the forespore membrane. *Dma1p* also contributes to the temporal regulation of the abundance of the meiosis-specific SIN component *mug27p*.

**Key words:** Meiosis, Spore formation, *Dma1*, Fission yeast

## Introduction

Meiosis is a form of the cell cycle that gives rise to haploid gametes after recombination and reassortment of the genome. In *Schizosaccharomyces pombe*, the products of meiosis are four spores that are highly resistant to environmental insults. Under starvation conditions, cells of opposite mating types undergo a pheromone-dependent arrest of their cell cycles in G1, followed by cytoplasmic and nuclear fusion. Cells then commit to the meiotic cell cycle and undergo premeiotic DNA synthesis, recombination, two meiotic divisions and then form four haploid spores (for reviews, see Shimoda, 2004; Shimoda and Nakamura, 2004; Yamamoto, 2004). Filming of spore formation in living cells (Nakamura et al., 2008), coupled with genetic and cytological analysis, has given rise to a model for spore formation. During meiosis II, the spindle pole body (SPB) changes its appearance from a dot to a crescent shape (Hagan and Yanagida, 1995). This requires the products of the *spo2*, *spo13* and *spo15* genes (Ikemoto et al., 2000; Nakase et al., 2008). Membrane vesicles are recruited to the modified SPB and fuse to give rise to the forespore membrane (FSM), which encapsulates the haploid nucleus to form the prespore. Components such as *meu14p*, found at the leading edge of the spore, are involved in its closure (Okuzaki et al., 2003). The leading edge proteins (LEPs) function in parallel with the meiotic septin complex to orient FSM extension (Onishi et al., 2010). Spore wall material is then deposited between the membranes to form the spore (Tanaka and Hirata, 1982; Yoo et al., 1973).

## The SIN and its regulators

During vegetative growth, cells of the fission yeast *S. pombe* divide by medial fission. A contractile ring (CAR) is assembled at the centre of the cell during mitosis; at the end of anaphase, CAR contraction guides synthesis of the septum that bisects the

cell. A group of protein kinases called the ‘septation initiation network’ (SIN) is essential for cytokinesis. Loss of SIN signalling produces multinucleate cells, whereas constant activation of the SIN results in multiseptated cells (Krapp and Simanis, 2008). The SIN also collaborates with the anillin-related protein *mid1p* to promote CAR assembly early in mitosis (Hachet and Simanis, 2008; Huang et al., 2008). The SIN and *mid1p* are both regulated by *plp1p* (Bahler et al., 1998a; Tanaka et al., 2001), which might provide global coordination of cytokinesis and mitosis (for a review, see Roberts-Galbraith and Gould, 2008). The SIN also plays an essential role during the meiotic cell cycle (Krapp et al., 2006; Yan et al., 2008); SIN mutants complete apparently normal meiotic nuclear divisions but fail to form spores, suggesting a common function for the SIN in coordinating septation and nuclear division in the mitotic cycle, and spore formation with nuclear division in the meiotic cycle.

SIN signalling originates from the spindle pole body (SPB) and is modulated by the nucleotide status of the GTPase *spg1p* (Schmidt et al., 1997; Sohrmann et al., 1998). This is determined by the balance of spontaneous nucleotide exchange, a putative GEF, *etd1p* (Garcia-Cortes and McCollum, 2009), and a GTPase-activating protein (GAP), *cdc16p* (Fankhauser et al., 1993; Minet et al., 1979), with which *spg1p* interacts through a scaffold, *byr4p* (Furge et al., 1999; Furge et al., 1998; Song et al., 1996). Signal transmission requires the activity of three protein kinases, each of which has a regulatory subunit: *cdc7p-spg1p* (Fankhauser and Simanis, 1994; Mehta and Gould, 2006), *sid1p-cdc14p* (Fankhauser and Simanis, 1993; Guertin et al., 2000; Guertin and McCollum, 2001) and *sid2p-mob1p* (Hou et al., 2000; Salimova et al., 2000; Sparks et al., 1999). These proteins associate with the SPB via a tripartite scaffold comprising *ppc89p*, *sid4p* and *cdc11p* (Chang and Gould, 2000; Krapp et al., 2001; Morrell et al., 2004; Rosenberg et al., 2006; Tomlin et al., 2002).

During meiosis, the SIN scaffold proteins, spg1p and sid2p-mob1p are located at the SPB during the horsetail stage, meiosis I and meiosis II (Krapp et al., 2006). Byr4p and cdc16p are on the SPB during the horsetail stage and on both SPBs during meiosis I, whereas cdc7p and sid1p-cdc14p are absent. During meiosis II, byr4p and cdc16p disappear from the SPBs, to be replaced by sid1p-cdc14p and cdc7p. Furthermore, although these proteins all show some asymmetric behaviour during the mitotic cycle (for a review, see Lattmann et al., 2009), they are observed on all the SPBs during the meiotic cycle. This is consistent with the fact that each meiosis II nucleus will be engulfed by a spore that develops from the SPB.

A sid2p-related kinase called *mug27* (also known as *ppk35* or *slk1*; hereafter called *mug27*) is expressed during meiosis (Ohtaka et al., 2008; Perez-Hidalgo et al., 2008; Yan et al., 2008). A *sid2-250 mug27-D* double mutant makes no spores at all, indicating that the two kinases cooperate. The *mug27-D*-null mutant makes smaller spores and the FSM fails to engulf the nucleus, which results in decreased spore viability. Sid2p and mug27p are both observed at the SPB; in addition, mug27p is also present at the SPB and then associates with the FSM during spore formation. Mug27p does not require the SIN scaffold proteins for SPB-association (Ohtaka et al., 2008). Increased expression of the syntaxin psy1p rescues the sporulation defect of *mug27-D sid2-250* (Yan et al., 2008), suggesting that mug27p and sid2p facilitate recruitment of components of the secretory apparatus to allow FSM expansion.

### The SIN inhibitor *dma1*

Dma1p, which is related to the mammalian CHFR or RNF8 family of ubiquitin ligases, is an inhibitor of the SIN (Murone and Simanis, 1996), which might function by regulating plo1p at the SPB (Guertin et al., 2002). Dma1p has two functional domains: an N-terminal forkhead-associated (FHA)-domain, which promotes phosphorylation-dependent interaction with other proteins and is required for proper localisation of dma1p (Guertin et al., 2002), and a C-terminal RING-finger, which is a motif found in ubiquitin-protein ligases. Proteins related to dma1p, such as CHFR, have been shown to function as protein-ubiquitin ligases in vitro (e.g. see Bothos et al., 2003; Kang et al., 2004); both domains are essential for its mitotic function in preventing septum formation during mitosis. Strong overexpression of *dma1* inhibits septum formation, producing multinucleate cells. Increased expression of *dma1* does not significantly affect the steady-state level of SIN proteins such as sid2p and mob1p during the mitotic cycle (Guertin et al., 2002) (A.K. and V.S., unpublished data). Genome-wide analysis of meiotic gene expression (Mata et al., 2002) indicates that *dma1* expression increases strongly as cells enter meiosis II.

In this study, we have examined the role of dma1p in meiosis. Mating between cells lacking dma1p produces a majority of asci with less than four spores, despite the fact that the two meiotic divisions are completed normally. Analysis of spore formation indicates that the spores are larger than those of wild type, and reveal problems in FSM formation. Our data indicate that the timing of the degradation of the sid2p-family protein kinase mug27p might be regulated by dma1p.

## Results

### Analysis of *dma1* expression and dma1p during meiosis

Because dma1p is an important regulator of the SIN in the mitotic cycle (see Introduction), we examined whether dma1p plays any role in meiosis. Northern blotting of RNA extracted from diploid

cells undergoing a *pat1-114*-induced meiosis revealed a marked increase in the steady-state level of *dma1* mRNA when cells were undergoing the transition from meiosis I to meiosis II (Fig. 1A), consistent with the data from a genome-wide meiotic expression analysis (Mata et al., 2002). Protein extracts prepared under denaturing conditions revealed that dma1p-GFP levels increased significantly in parallel with RNA levels and remained elevated through to the end of meiosis II (Fig. 1B).

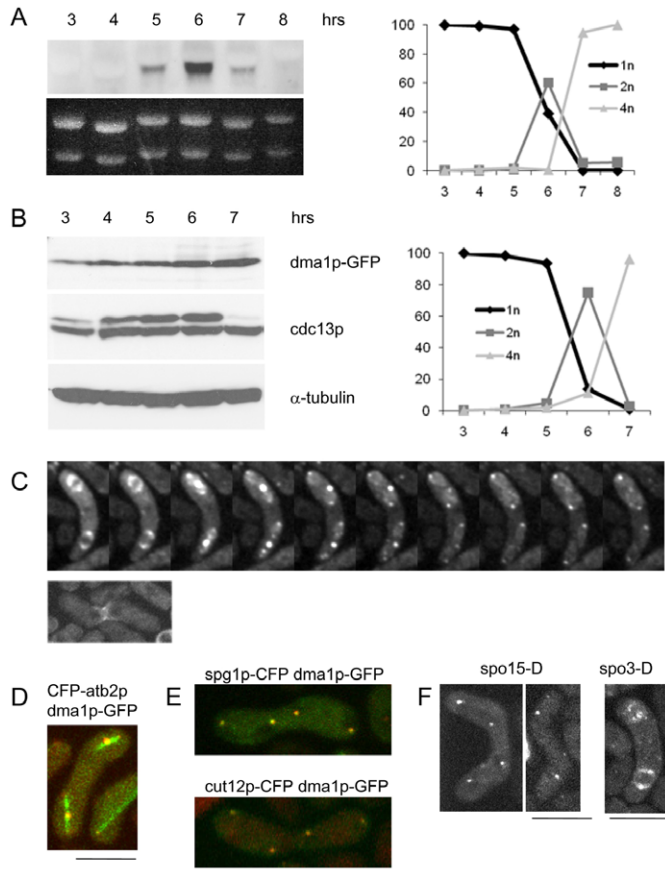
Localisation of dma1p-GFP in mating cells revealed a broad signal at the point of fusion between the two cells (Fig. 1C, lower panel). No signal was observed associated with any discrete structure during horsetail movement or meiosis I, although western blotting indicated that dma1p-GFP is present (Fig. 1B). In meiosis II, dma1p-GFP localised to the SPB and the FSM (Fig. 1C, upper panel). It was then observed as an intense dot between the separating nuclei, overlying the spindle midzone in anaphase II (Fig. 1D). At the end of meiosis II, the dma1p-GFP signal was observed as a dot, which colocalised with two different SPB markers, spg1p and cut12p (Fig. 1E). Formation of the crescent-shaped meiotic spindle pole plaque, and hence FSM formation, requires spo15p (Ikemoto et al., 2000); in a *spo15-D*-null mutant, dma1p-GFP was still observed as a dot at the meiosis II SPBs and also between the separating nuclei, although the signal was less intense than in wild-type cells (Fig. 1F). This indicates that SPB-association of dma1p-GFP in meiosis II is independent of SPB remodelling. In a *spo3-D*-null mutant in which FSM expansion is compromised (Nakamura et al., 2008), dma1p-GFP was observed at the SPB and on the FSM (Fig. 1F).

### Meiosis in the absence of *dma1* function produces asci with less than four spores

Previous studies have shown that dma1p regulates the SIN during the mitotic cell cycle (Guertin et al., 2002; Murone and Simanis, 1996). Because the SIN is essential for spore formation, we examined whether *dma1::ura4<sup>+</sup>* cells (*dma1-D*) were affected during meiosis. A *dma1-D h<sup>90</sup>* strain was allowed to mate and undergo meiosis; examination of asci revealed that only 40% of them contained four spores, whereas the remainder contained three (35%), two (17%), one (8%) or no (1%) spores (Fig. 2A,C; figures for a typical experiment are given). Staining of asci revealed that 97% of asci that contained at least one spore also contained four condensed nuclei, consistent with completion of the two meiotic divisions (Fig. 2B). The control mating of *h<sup>90</sup> dma1<sup>+</sup>* cells gave rise to >99% four-spored asci. Similar results were obtained when a *h<sup>+</sup>/h<sup>-</sup> dma1-D/dma1-D* diploid was starved and sporulated (4 spores, 48%; 3 spores, 38%; 2 spores, 5%; 1 spore, 1.5%; no spores, 7%). Measurement of the diameter of the spores indicated that those produced following a *dma1-D* meiosis had a diameter of  $3.45 \mu\text{m} \pm 0.47 \mu\text{m}$  ( $n=292$ ) compared with wild-type spores, which had a diameter of  $3.0 \mu\text{m} \pm 0.38 \mu\text{m}$  ( $n=280$ ;  $P<0.001$ ).

Dma1p comprises two functional domains (see Introduction); *h<sup>90</sup>* RF and FHA point mutants of *dma1* were allowed to mate and undergo meiosis. Analysis of asci revealed that inactivation of either domain produced a result similar to the *dma1-D* mutant (Fig. 2C), indicating that both the RF and FHA domains of dma1p are essential for its meiotic role in spore formation. The increased number of asci with no spores suggests that the presence of a mutant dma1p might be more deleterious than its absence.

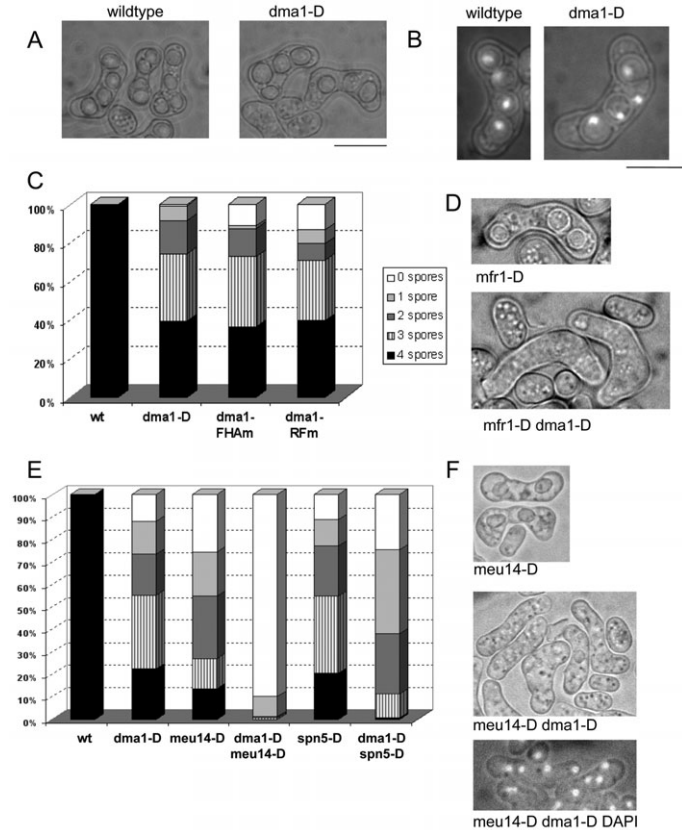
The *dma1-D* meiotic phenotype is reminiscent of that of the null mutant of the APC/C regulator *mfr1*, where asci frequently contain fewer than four spores (Blanco et al., 2001). This prompted us to



**Fig. 1. Analysis of *dma1* expression and localisation during meiosis.** (A) A culture of *pat1-114* diploid cells was induced to enter meiosis; samples were withdrawn at the indicated times after shift and RNA was extracted. A northern blot was probed as described in the Materials and Methods. The upper panel shows the *dma1* signal, the lower panel shows the Ethidium-Bromide-stained gel prior to transfer. The graph shows progression through meiosis in the experiment; 1n, 2n and 4n indicate one, two and four nuclei, respectively. (B) A culture of diploid *pat1-114 dma1-GFP* cells was induced to enter meiosis; samples were withdrawn and protein was extracted at the indicated time (hours) after shift. A western blot was probed with antiserum to detect GFP, *cdc13p* or  $\alpha$ -tubulin. The kinetics of meiotic progression are shown in the panel on the right. 1n, 2n and 4n indicate one, two and four nuclei, respectively. (C) *dma1-GFP h<sup>+</sup>* and *h<sup>-</sup>* cells were mated; cells were mounted for imaging as described in the Materials and Methods. The upper panels show a cell pictured at 4-minute intervals during meiosis II. The lower panel shows a pair of cells fusing during conjugation. (D) A cell expressing *CFP-atb2p* and *dma1-GFP* was photographed during meiotic anaphase II. Note the presence of a bright GFP signal between the dividing nuclei, overlying the spindle midzone. (E) Cells expressing *dma1-GFP* and either *spg1-CFP* or *cut12-CFP* were photographed at the end of meiosis II. The image presented is a merge of the CFP (red) and GFP (green) channels; note that the SPB signal is yellow. (F) *dma1-GFP spo15-D* and *dma1-GFP spo3-D* cells were examined during meiosis II. Scale bars: 10  $\mu$ m.

examine the meiotic phenotype of *mfr1-D dma1-D* cells; we found a synergistic effect in the double-null mutant in that few or no spores were formed after meiosis (Fig. 2D), indicating that they do not function in a single linear pathway.

Microdissection of three- and four-spored asci from a *dma1-D h<sup>90</sup>* mating revealed that the overall spore viability was 89% and 94%, respectively; the wild-type control gave 99% viable spores



**Fig. 2. Meiosis in the absence of *dma1p* frequently gives rise to asci with fewer than four spores.** (A) The indicated *h<sup>90</sup>* strains were mated as described in the Materials and Methods. After three days at 25°C, asci were suspended in EMM-N and photographed. Scale bar: 10  $\mu$ m. (B) Cells from the same mating as A were fixed, stained with DAPI and photographed with combined fluorescence and transmission illumination. (C) Asci from the indicated *h<sup>90</sup>* matings were scored according to the number of spores in the asci produced. *Dma1-RFm* and *Dma1-FHAm* indicate mutants in the RING-finger and Forkhead-associated domains, respectively. The number of spores in the asci was determined after 60 hours at 29°C. At least 100 asci were scored for each strain. (D) *mfr1-D* and *mfr1-D dma1-D h<sup>90</sup>* cells were allowed to self-mate. After three days, asci were suspended in EMM-N and photographed. Note the absence of spores in the double-null mutant. (E) Matings between *h<sup>+</sup>* and *h<sup>-</sup>* strains of the indicated genotypes were performed. In the case of *meu14-D*, an *h<sup>90</sup>* strain was used. The number of spores in the asci was determined after 60 hours at 29°C. At least 100 asci were scored for each strain. (F) Samples of the indicated matings analysed in E were photographed. Note that although spores are almost completely absent in the *dma1-D meu14-D* mutant, DAPI staining reveals the presence of four nuclei in the asci.

(Table 1A). One spore gave rise to a microcolony of approximately 20 cells; this phenotype might be produced by a spore inheriting an unbalanced number of chromosomes (Niwa and Yanagida, 1985). All the viable progeny examined appeared haploid (irrespective of the number of spores in the ascus), as judged by the size of cells and colony colour on medium containing Phloxin B. To examine this further, three- and four-spored asci from the cross *dma1::ura4<sup>+</sup> ade6-M210 leu1-32 h<sup>-</sup>*  $\times$  *dma1::ura4<sup>+</sup> ade6-M216 leu1-32 h<sup>+</sup>* were dissected and replica-plated onto media without adenine and containing limiting adenine (Moreno et al., 1991) to reveal the colour difference between the two *ade6* alleles. These two *ade6* alleles complement in trans and are frequently



**Table 1**

	Total	Number of viable spores				
		Four	Three	Two	One	None
<i>dma1-D</i> four-spored asci	63	49 (78%)	12 (19%)	2 (3%)	0	0
<i>dma1-D</i> three-spored asci	91	NA	72 (79%)	12 (13%)	3 (3%)	4 (4%)
Wild type	52	50 (96%)	2 (4%)	0	0	0

NA, not applicable. Spores from asci containing either three or four visible spores were separated by microdissection and colonies were allowed to form at 29°C. Colonies were counted after 3 or 4 days, and the fate of 'missing' spores was determined by microscopy. Only asci in which spores were liberated in 24 hours or less were counted in this analysis. For the wild-type control, 52 four-spored asci were dissected from an *ade6-M210 leu1-32 h<sup>-</sup>* × *ade6-M216 leu1-32 h<sup>+</sup>* cross.

	Number of asci	Spores released in 24 hours		Spores released in 72 hours	
		Number	Percentage	Number	Percentage
<i>dma1-D</i> four-spored asci	59	38	64%	51	86%
<i>dma1-D</i> three-spored asci	72	40	56%	56	78%
Wild type	70	69	99%	69	99%

Asci were placed on YE (yeast extract) plates at 25°C and examined at intervals thereafter for the liberation and germination of spores from the ascus.

used to select and maintain diploids. If diploid progeny are generated in a *dma1-D* meiosis, then some cells should inherit both alleles and be adenine prototrophs. After dissection of 37 four-spored asci, we observed that 32 gave rise to four colonies and five gave rise to only three colonies (overall spore viability of 96%). In 32 three-spored asci, 25 gave rise to three colonies, whereas five produced only two; in both instances, the remaining spores failed to germinate (overall viability 93%). After replica plating, all the progeny were adenine auxotrophs and growth on limiting adenine medium produced dark red (*ade6-M210*) to light red (*ade6-M216*) colony colours, consistent with 2:2 segregation of the *ade6* alleles in the four-spored asci and either one or two of each kind in the three-spored asci where three colonies arose. This is consistent with all the progeny being haploid. In addition, we noted that significant numbers of asci from *dma1-D* homozygous meiosis failed to release spores in the first 24 hours after placement on yeast extract (YE) plates (Table 1B). The reason for the delayed germination of *dma1-D* spores is unclear, but might reflect alterations in the composition of the spore wall or alterations in the expression of *agn2*, which is required for the release of spores from the ascus (Dekker et al., 2007).

To examine chromosome segregation, we used the lacI-GFP-lys1::lacO detection system (Nabeshima et al., 1998) to examine the segregation of the chromosome I centromere in *dma1-D h<sup>90</sup>* mating. In crosses where both parent cells carried the lacI-GFP-lys1::lacO detection system, we observed in *dma1-D* cells that 90.9% (*n*=88) of asci had four detectable cen1 signals after meiosis II; a wild-type control showed only 98.3% (*n*=120) of nuclei with a cen1 signal. Because >90% of spores are viable in four-spored asci, the absence of strong cen1 signal at the end of meiosis II in might not reflect loss or mis-segregation of chromosomes because *S. pombe* cells are intolerant of aneuploidy (Niwa and Yanagida, 1985).

A recent study revealed that the fission yeast meiotic septins and the proteins at the leading edge of the FSM (leading-edge proteins, LEPs) function in parallel pathways to orient growth of the FSM. Double mutants between the meiotic septins and a LEP, *meu14-D*, revealed an additive effect upon spore formation (Onishi et al., 2010). To examine the genetic relationship of *dma1* to the LEPs and meiotic septins, we examined the effects of constructing double mutants between them. We found that a *dma1-D* produced a synergistic effect with both the meiotic septin mutant *spn5-D* and the LEP mutant *meu14-D* (Fig. 2E). In the *spn5-D dma1-D* double

mutant, less than 1% of asci contained four spores, whereas almost 20% contained none. The effect observed in the *dma1-D meu14-D* double mutant was even more pronounced, with 90% of asci containing no visible spores (Fig. 2E,F). DAPI staining of asci revealed four nuclei in the *dma1-D meu14-D* asci, suggesting that the meiotic divisions had been completed (Fig. 2F). The observation that *dma1-D* displayed additive effects with both the *spn5-D* and *meu14-D* suggests that *dma1p* does not act in a single linear pathway with either of these two protein complexes and is consistent with *dma1p* having multiple roles in meiosis.

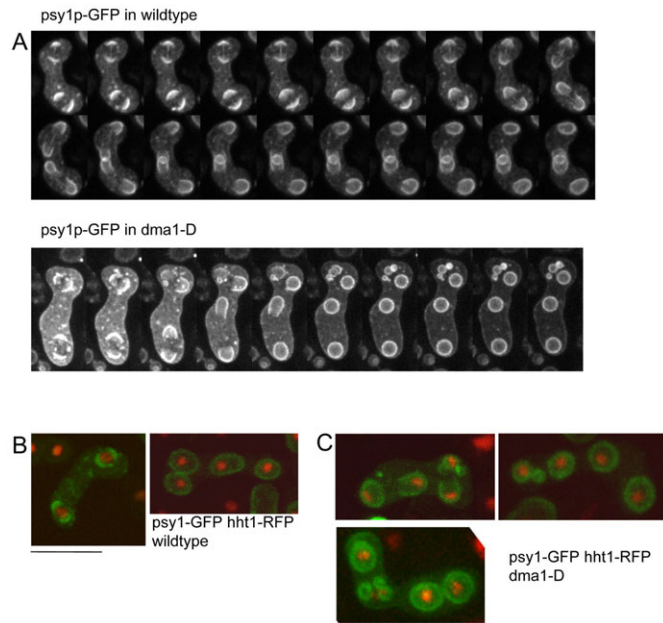
Taken together, the data presented above lead us to conclude that meiosis in a *dma1-D* background produces spores that are larger than those of wild type and of reduced viability. A majority of asci contain fewer than four spores, although the two meiotic divisions appear to be completed normally. Analysis of *dma1* point mutants indicates that both of its functional domains are required during meiosis. Thus, whereas *dma1p* is not essential for meiosis or spore formation, it is important for the formation of normal, four-spored asci.

**Forespore development is abnormal in *dma1-D* cells**

The observation that the size and number of spores produced following meiosis in the absence of *dma1p* are aberrant suggests a role for *dma1p* in regulating synthesis of the FSM. Therefore, we analysed formation of the FSM using a GFP-tagged syntaxin *psy1p*, which is an FSM-resident protein (Nakamura et al., 2008). As shown in Fig. 3A,B, in wild-type meiosis, the FSM expands from the plaque-like SPB to engulf the four nuclei, forming the prespore (Nakamura et al., 2008). By contrast, in *dma1-D* meiosis, although FSM expansion began normally, FSM development became aberrant on one of the nuclei, producing bleb-like structures that eventually coalesced to give small multi-lobed structures (Fig. 3A); these usually did not contain any DNA (Fig. 3C). Similar data were obtained using *spo3p-GFP* to follow FSM formation (data not shown).

We also examined the localisation of *meu14p*, which is localised at the leading edge of the FSM (Fig. 4A,B) and might also affect meiotic SPB function (Okuzaki et al., 2003). We observed extra *meu14p-GFP* rings forming during FSM expansion (Fig. 4A,C), consistent with the data obtained using *psy1p-GFP* and *spo3p-GFP* as markers.

FSM development requires *spo15p*-dependent remodelling of the SPB in meiosis II (Ikemoto et al., 2000). Examination of

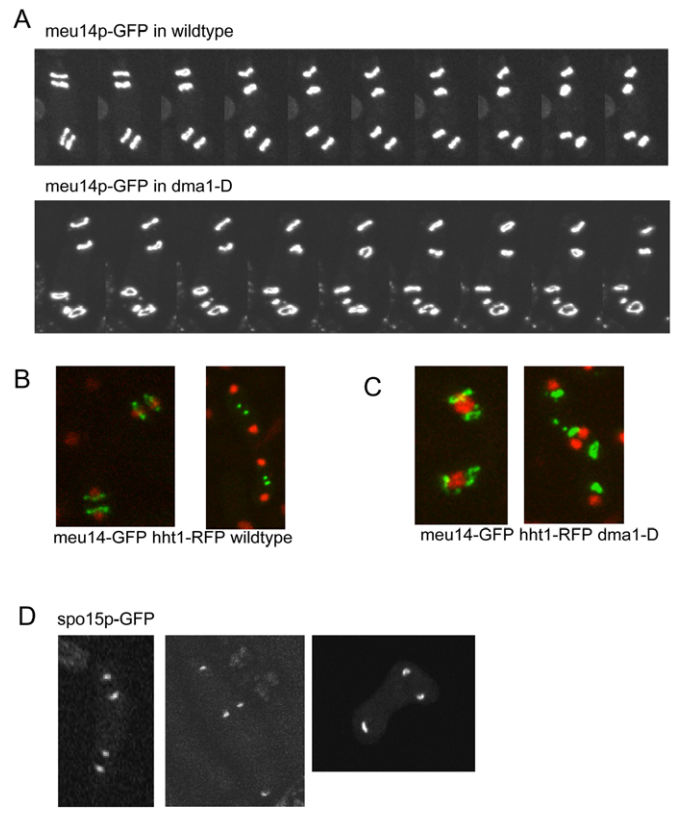


**Fig. 3. Forespore membrane formation is aberrant in *dma1-D* meiosis.** (A) Matings between  $h^-$  and  $h^+$  wild-type or  $h^-$  and  $h^+$  *dma1-D* cells expressing psy1p-GFP were filmed during forespore membrane (FSM) development in meiosis II. The time interval between frames is 2 minutes in the wild-type and 4 minutes in the *dma1-D* cells. (B,C) Matings between  $h^-$  and  $h^+$  wild-type or  $h^-$  and  $h^+$  *dma1-D* cells expressing spo3p-GFP (green) and hht1-RFP (red) were photographed. Scale bars: 10  $\mu$ m.

spo15p-GFP revealed the presence of elongated and crescent-shaped SPB structures in meiotic cells (Fig. 4D); whether the crescent structure of the meiotic SPB is normal in *dma1-D* will require further analysis by electron microscopy. However, the observation that FSM deposition, which absolutely requires SPB remodelling and spo15p function (Ikemoto et al., 2000), occurs in *dma1-D* cells suggests that the SPB is modified at the onset of meiosis II. Taken together, these data indicate that FSM development during meiosis II is aberrant in *dma1-D* cells, which leads to the formation of asci containing fewer than four spores.

#### Localisation of SIN proteins and SIN regulators in *dma1-D* meiosis

Because the SIN is required for spore formation (Krapp et al., 2006; Yan et al., 2008) and dma1p regulates the SIN in the mitotic cycle (Guertin et al., 2002; Murone and Simanis, 1996), we examined the localisation of SIN proteins in meiosis in the *dma1-D* background. We found that the localisation of all SIN-GFP proteins examined (Fig. 5; data not shown) was qualitatively similar to that previously described in *dma1^+* cells (Krapp et al., 2006), in that the signals were associated with all SPBs depending on the stage of the meiotic cycle, with sid1p and cdc7p associated with the SPB only in meiosis II, as expected (Fig. 5A). The localisation of both sid2p and mob1p also resembled that seen in a wild-type meiosis (see Fig. 5B,C); both GFP-tagged proteins were observed on all four SPBs in meiosis II. Western blotting showed that the steady-state levels of both mob1p (Fig. 5B) and sid2p (Fig. 5C) did not change significantly during meiosis. However, we observed that sid2p displayed a slower migrating form at 7 hours after induction of meiosis in *dma1-D* cells (Fig. 5C); the wild-type cells

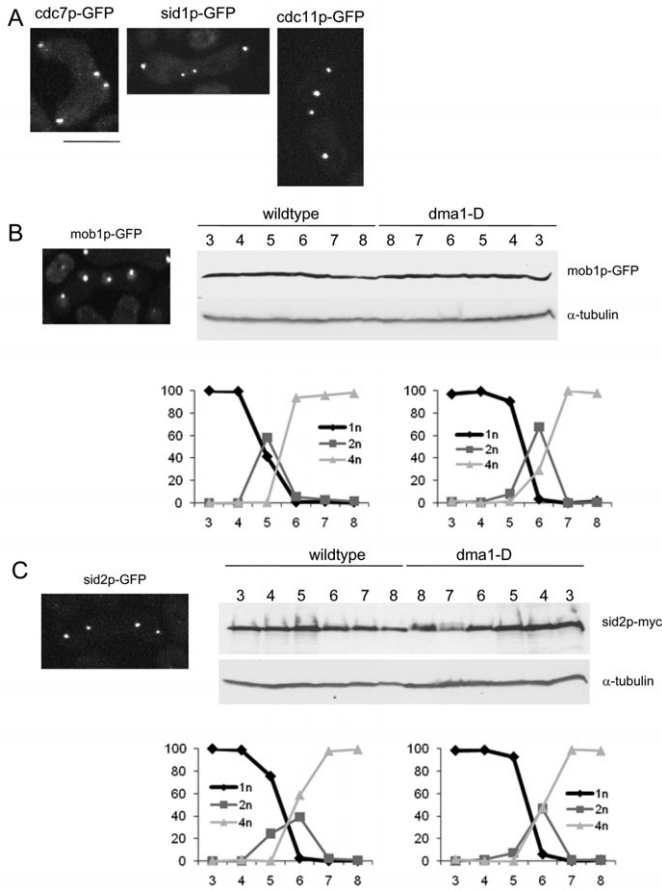


**Fig. 4. The distribution of meul4p is aberrant in *dma1-D* meiosis.** (A) Matings between  $h^-$  and  $h^+$  wild-type or  $h^-$  and  $h^+$  *dma1-D* cells expressing meul4p-GFP were filmed during FSM development in meiosis II. The time interval between frames is 2 minutes. (B,C) Matings between  $h^-$  and  $h^+$  wild-type or  $h^-$  and  $h^+$  *dma1-D* cells expressing meul4p-GFP (green) and hht1-RFP (red) were photographed. (D) Meiotic *dma1-D* cells expressing spo15p-GFP were examined. Meiosis II cells are shown in each case. Note that in the left-most panel the two other nuclei overlap. Scale bars: 10  $\mu$ m.

did not accumulate detectable amounts of this form of sid2p. The nature of the modification is unknown (see Discussion), but it was observed in three separate experiments (data not shown).

As dma1p might regulate the association of plo1p with the SPB (Guertin et al., 2002), we examined whether the absence of dma1p influenced the meiotic localisation of plo1p-GFP (Bahler et al., 1998a). We found that, in *dma1^+* meiosis, three spots were observed during the horsetail stage (Fig. 6A, cell 1), which colocalise with the kinetochore marker cnp1p (Fig. 6B). During meiosis I, plo1p-GFP was observed first as a number of dots along the spindle and on the SPB, then on the SPBs and faintly on the elongating spindle (Fig. 6A, cells 2 and 3; Fig. 6B), becoming fainter at the end of anaphase I (Fig. 6A, cell 4). During meiosis II, plo1p-GFP was observed at all four SPBs (Fig. 6A) and faintly on the spindle. The intensity of the SPB signal decreased during meiosis II (Fig. 6A, cells 5–9;  $n=5$ ), eventually becoming too faint to detect during anaphase; this resembles the mitotic localisation of plo1p (Mulvihill et al., 1999). Thus, in addition to its mitotic association with the SPB and spindle, during meiosis I, plo1p-GFP also associates with kinetochores.

In *dma1-D* meiosis, the localisation was similar up to meiosis II (Fig. 7A, cells 1–3); during meiosis II, a signal was observed initially at all four SPBs, then, during anaphase II, the signal



**Fig. 5. Localisation of SPB markers and proteins involved in SIN signalling during wild-type and *dma1-D* meiosis.** (A) Matings between *h<sup>+</sup>* and *h<sup>-</sup>* *dma1-D* cells expressing the indicated GFP-tagged SIN proteins were examined. Meiosis II cells are shown in each case. Scale bar: 10  $\mu$ m. (B,C) *pat1-114* and *pat1-114 dma1-D* cells expressing either *mob1p-GFP* or *sid2p-myc* or *sid2p-GFP* (for imaging) were induced to undergo meiosis. Protein samples were prepared at the indicated times (hours) after shift and western blots were probed with sera against the indicated proteins. The kinetics of meiotic progression are shown below the western blot (wild type on the left, *dma1-D* on the right). The images show a meiosis II cell in each case. B, data for *mob1p-GFP*; C, data for *sid2-GFP* and *sid2-myc*.

became asymmetric. Nine cells were filmed; in seven cells, *plo1p-GFP* remained associated with two of the four SPBs, one from each spindle (Fig. 7A, cells 4–8), whereas in two cells, *plo1p-GFP* remained associated with only one of the four SPBs (data not shown). Analysis of the *plo1p-GFP* by western blotting revealed that the level of *plo1p-GFP* decreased as cells progressed through meiosis II and that this was preceded by the appearance of a modified, slower-migrating form of the protein at the transition from meiosis I to meiosis II in both *dma1<sup>+</sup>* and *dma1-D* meiosis (Fig. 7B). In summary, we conclude that although *plo1p-GFP* localises symmetrically in meiotic *dma1<sup>+</sup>* cells, in the absence of *dma1p*, the meiosis II SPBs behave asymmetrically with regard to *plo1p-GFP* localisation.

**Dma1p is required for timely elimination of *mug27p***

*Mug27p* is the meiosis-specific orthologue of *sid2p* (see Introduction). Because *mug27-D* cells produce small spores, whereas

*dma1-D* cells produce large spores, we tested whether the steady-state levels of *mug27p* were affected in *dma1-D*. We observed that *mug27p* levels peaked at the transition from meiosis I to meiosis II and then declined at the time of spore formation (Fig. 8A), consistent with previous studies (Ohtaka et al., 2008; Perez-Hidalgo et al., 2008; Yan et al., 2008). By contrast, in *dma1-D* meiosis, *mug27p* persisted beyond the onset of meiosis II (Fig. 6A). Significantly, whereas in wild-type *mug27p* levels decline before *cdc13p*, in *dma1-D* they remain elevated after *cdc13p* levels have decreased (Fig. 8A). However, localisation of *mug27p-GFP* to the SPB was similar in wild-type and *dma1-D* meiosis (Fig. 8B). We conclude that *dma1p* plays a role in the timely elimination of *mug27p*. To study the extent to which the effects of the absence of *dma1p* upon spore formation were due to *mug27p*, we constructed a *dma1-D mug27-D* double-null mutant; we found that although meiosis produced four nuclei, less than 1% of asci contained any spores (Fig. 8C). Filming of *psyl-GFP* in this mutant showed strongly aberrant FSM extension, which rapidly collapsed to form small multi-lobed structures (Fig. 8D). This synergy between the null mutants demonstrates that the meiotic effects caused by the absence of *dma1p* are not mediated solely via *mug27p*. We attempted to examine the effects of increased *dma1* expression on spore formation and *mug27p* levels; however, increased expression of *dma1<sup>+</sup>* blocks cytokinesis and interferes with mitotic progression (Murone and Simanis, 1996), and we found that expression of *dma1<sup>+</sup>* from the full-strength *nmt1* promoter interferes with chromosome segregation during meiosis and, hence, no spores were observed (data not shown). Whether intermediate levels of overexpression of *dma1* affects spore formation will be the subject of future studies.

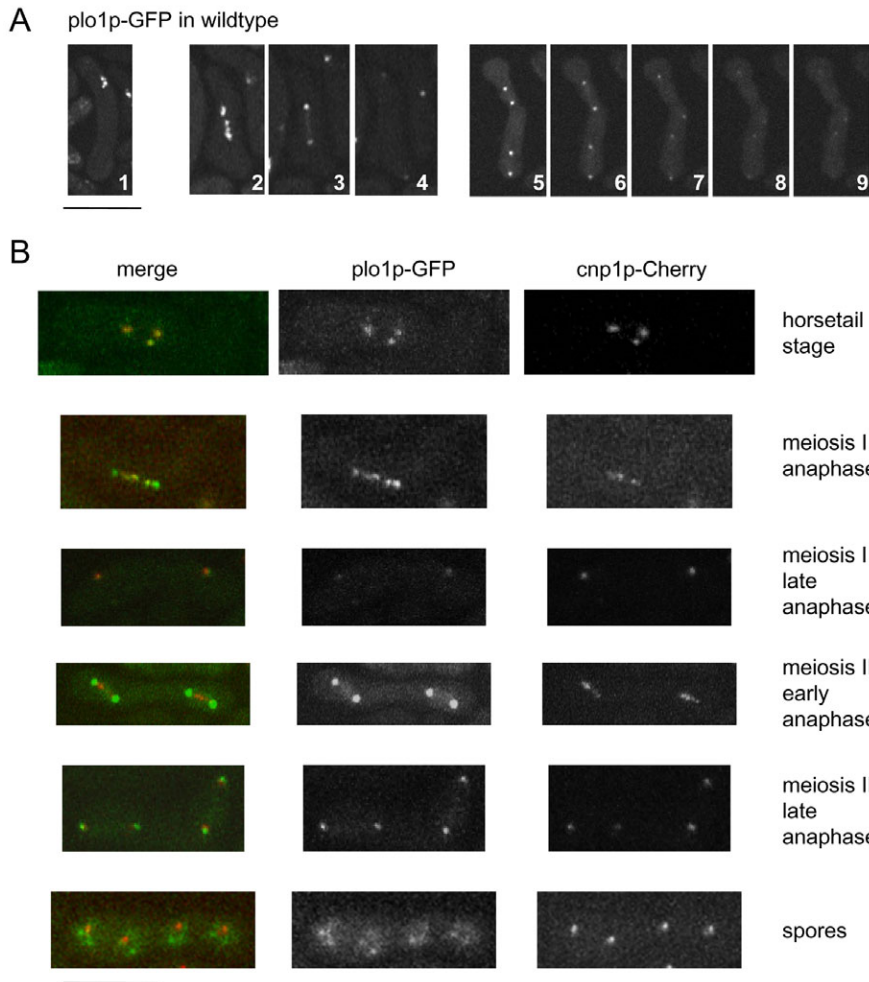
**Discussion**

In the present study, we have examined whether the SIN regulator *dma1p* plays a role in meiosis. We have found that if cells undergo the meiotic cycle without *dma1p*, more than 60% of asci contain fewer than four spores. However, in the vast majority of cases, the asci contain four nuclei, indicating that the two meiotic divisions have been completed successfully.

Analysis of three-spored asci indicates that the viability of the spores is high and the progeny are haploid. This argues against extensive mis-segregation of chromosomes, as *S. pombe* is intolerant of aneuploidy (Niwa and Yanagida, 1985). The presence of four DAPI staining bodies also argues against encapsulation of more than one nucleus by a growing FSM as being responsible for the absence of one or more spores.

*Dma1p* localises to the SPB in meiosis II; however, its localisation there does not require this remodelling, as it is still observed at the dot-like SPB in a *spo15* mutant, which does not reorganise the SPB. The nature of the meiotic SPB anchor for *dma1p* is unknown, although by analogy with the mitotic cycle (Guertin et al., 2002), *sid4p* might play this role. *Dma1p* also appears to associate with the expanding FSM early in meiosis II. In mid-anaphase, *dma1p-GFP* is observed as very bright dot between the two dividing nuclei; the only protein we are aware of that shows a similar localisation is *pad1p-GFP*, which is a subunit of the proteasome (Wilkinson et al., 1998). To date, we have been unable to find conditions that allow both *dma1p* and the *pad1p* to be visualised simultaneously; nonetheless, this raises the possibility that *dma1p* might regulate the proteasome at some point(s) in meiosis. The role for *dma1p* at the point of fusion between mating cells is at present unclear. F-actin is present around, but excluded from, the region of cell fusion (Petersen et al., 1998). It is possible





**Fig. 6. Localisation of plo1p-GFP in *dmal1*<sup>+</sup> meiosis.** (A) *Dmal1-D* cells expressing plo1-GFP were mated and filmed at intervals during meiosis; see text for details. Cells 2–4 are images of the same cell taken 15 minutes apart. Cells 5–9 are images of a single cell taken 5 minutes apart. (B) The strains *plo1-GFP cnp1-Cherry* and *plo1-GFP cnp1*<sup>+</sup> were mated. Cells were mounted 24 hours later and photographed. The panel shows cells at various stages of meiosis, with a merge and the separated red and green channels. Scale bars: 10  $\mu$ m.

that the presence of *dma1p* prevents any attempt to construct a CAR or division septum at this site.

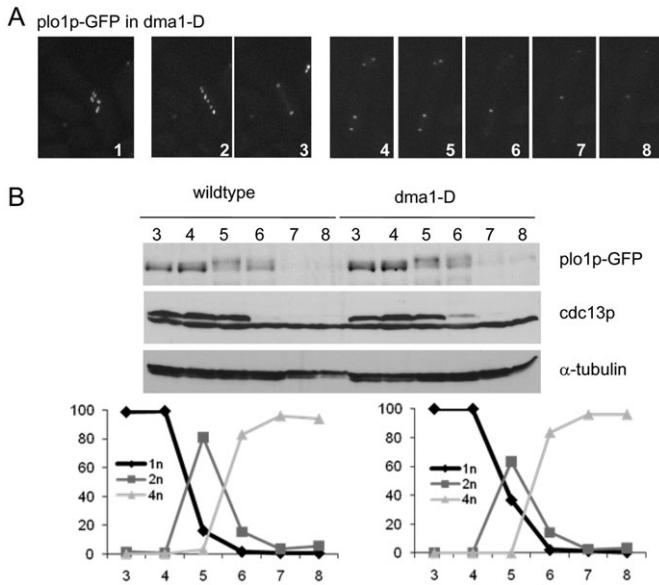
The data presented here demonstrate that *dmal1-D* meiosis frequently produces asci containing fewer than four spores. Analysis of null mutants for meiosis-specific genes has identified a number of mutants that produce the same phenotype (Martin-Castellanos et al., 2005). The one whose mutant phenotype most strongly resembles that of *dmal1-D* is *mfr1-D* (Blanco et al., 2001). This raised the possibility that *dma1p* might regulate *mfr1p* and thus control the activity of the meiotic APC/C. However, the strong additive effects of these two null mutants mean that this simple hypothesis is unlikely to be correct and imply that if *dma1p* does act upon *mfr1p* (or vice versa) to regulate the APC/C during meiosis then *dma1p* and/or *mfr1p* must have additional targets.

This screen also identified *mug27p*. The absence of *mug27p* produces smaller spores and it has been proposed that *mug27p* regulates FSM expansion; we have found that *mug27p* persists for longer than usual in *dmal1-D* meiosis. It is possible that this accounts for the production of larger spores. Preliminary data indicate that increased expression of the *mug27* gene from the *nmt1* promoter produces spores that are approximately 10% larger than those in wild type but most asci contain four spores (data not shown). Because *mug27p* levels do eventually decrease in *dmal1-D*, this task is either performed by a redundant mechanism that does not depend upon *dma1p*, or the absence of *dma1p* reduces the

efficiency of the elimination mechanism for *mug27p*. At present, it is not understood how *mug27p* levels are reduced at the end of meiosis; our data indicate clearly that *dma1p* contributes to this, although it is unclear whether it does so directly. To date, we have failed to detect any two-hybrid interaction between *dma1p* and *mug27p* (data not shown) and analysis of proteins associated with *dma1p* in meiotic cells by mass spectrometry did not reveal any peptides derived from *mug27p* (our unpublished data).

The data presented here indicate that growth of the FSM is aberrant in *dmal1-D*. Although FSM expansion begins normally, we observe asynchrony in membrane closure and what appear to be additional FSM initiation events, producing multi-lobed structures. FSM organisation and shape are determined by the cooperative action of LEPs and the meiotic septins (Onishi et al., 2010). Our data reveal significant negative genetic interactions of *dmal1-D* both with an LEP-null mutant and a septin-null mutant. This indicates that *dma1p* does not act in a single linear pathway with either of these two protein complexes in the process of spore formation and is consistent with *dma1p* having multiple roles in meiosis.

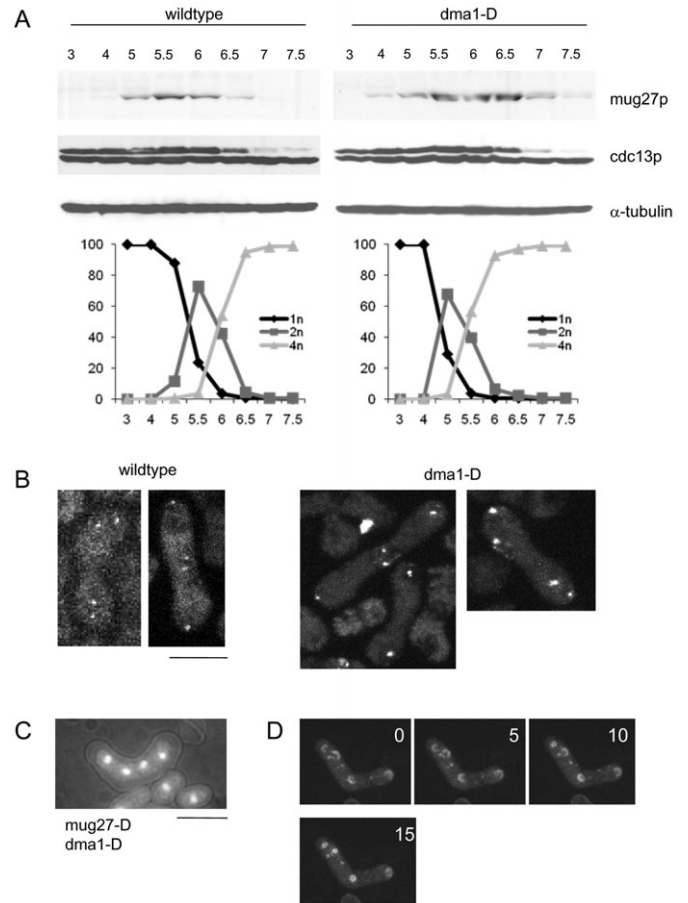
The study of Yan et al. (Yan et al., 2008) demonstrated that *sid4p* is required for spore formation by using a *sid4* shut-off strain; this frequently gave rise to asci with two, rather than four, spores even though the two meiotic divisions had been completed. *Dma1p* is known to localise at the SPB via *sid4p* (Guertin et al., 2002); however, the phenotypes are different, so it is not clear at



**Fig. 7. Protein levels of plo1-GFP during meiosis and localisation of plo1p-GFP in *dma1-D* meiosis.** (A) Cells expressing plo1-GFP were mated and filmed at intervals during meiosis; see text for details. Cells 2 and 3 are images of the same cell taken 15 minutes apart. Cells 4–8 are images of a single cell taken 5 minutes apart. (B) *pat1-114* and *pat1-114 dma1-D* cells expressing plo1-GFP were induced to undergo meiosis. Protein samples were prepared at the indicated times (hours) after shift and western blots were probed with sera against GFP, cdc13p or α-tubulin. The kinetics of meiotic progression are shown below the western blot (wild type on the left, *dma1-D* on the right).

present whether the effects of the absence of *dma1p* are mediated via regulation of the SIN. In this context, it is noteworthy that the failure to form four-spored asci in *dma1-D* is not rescued by increasing SIN activity through overexpression of *spg1* (data not shown).

Localisation of SIN proteins and regulators of the SIN show that all the proteins examined segregate as previously described in a wild-type meiosis. Thus, mislocalisation of the ‘core’ mitotic SIN proteins is unlikely to be the primary cause of the failure to form spores. A notable exception to this is the SIN regulator plo1p. Although the role of plo1p in *S. pombe* meiosis has not been analysed extensively to date, its horsetail–meiosis I localisation at the kinetochores is consistent with the role described for its *Saccharomyces cerevisiae* orthologue cdc5p in assuring kinetochore orientation during meiosis I (Lee and Amon, 2003). The localisation of plo1p during the ‘mitotic-like’ meiosis II division resembles its localisation in mitosis (Mulvihill et al., 1999). The plo1p-GFP signal is first detected strongly on all four meiosis II SPBs; as anaphase progresses, the signal grows fainter and plo1p-GFP is also detected on the spindle. In meiotic *dma1-D* cells, the behaviour of SPBs in meiosis II differs from that in wild type; although plo1p-GFP initially associates with all four SPBs, it subsequently becomes asymmetric during meiosis II. Although the SIN proteins *sid1p*, *cdc14p*, *cdc16p*, *byr4p* and *cdc7p* display such behaviour in the mitotic cycle, they segregate symmetrically during meiosis, even in the absence of *dma1p*. The majority of meioses examined showed 2:2 segregation of meiosis II SPBs that retained plo1p to those that did not, whereas the most frequent class of aberrant asci had three spores. Thus, there is not an obvious correlation between the aberrant segregation of plo1p-GFP and a failure to form spores.



**Fig. 8. Dma1p affects the timing of the elimination of mug27p during meiosis.** (A) *pat1-114* and *pat1-114 dma1-D* cells expressing *mug27-GFP* were induced to undergo meiosis. Protein samples were prepared at the indicated times (hours) after shift and western blots were probed with sera against the indicated proteins. The kinetics of meiotic progression are shown below the western blot (wild type on the left, *dma1-D* on the right). (B) Localisation of mug27p-GFP. Wild-type or *dma1-D h<sup>+</sup>* and *h<sup>-</sup>* cells expressing mug27p-GFP were mated and imaged as described in the Materials and Methods. The images show meiosis II cells for each strain. Scale bar: 10 μm. (C) *Mug27-D dma1-D h<sup>+</sup>* and *h<sup>-</sup>* strains were mated and asci were examined by staining with DAPI after 3 days at 25°C. Note that although four nuclei are present, there are no spores in the double-mutant ascus. Scale bar: 10 μm. (D) *Mug27-D dma1-D h<sup>+</sup>* and *h<sup>-</sup>* strains expressing *psy1-GFP* were mated and filmed during spore formation. The time indicated represents minutes after the first frame shown.

The finding that SPBs in the same meiotic cycle behave in a non-equivalent manner in the absence of *dma1p* strongly suggests that *dma1p* is involved in regulating behaviour of the SPB during meiosis, and raises the intriguing possibility that, as in the mitotic cycle (Grallert et al., 2004), fission yeast SPBs might have different properties according to their age. In this context, it is noteworthy that, in *Saccharomyces cerevisiae*, the SPB component *nud1p* (the orthologue of *cdc11p* in *S. pombe*) helps to distinguish the ages of the four SPBs (Gordon et al., 2006). The transient appearance of a slower migrating form of *sid2p* in during meiosis II in *dma1-D* cells suggests that *dma1p* influences the abundance of this form of *sid2p* during meiosis. *Sid2p* and *mug27p* cooperate during spore formation, so it is possible that this is an active form of *sid2p* that persists for longer than usual. However, many other models are



possible and future experiments will investigate the nature of the modification and its functional significance.

In the mitotic cycle, *dma1p* is considered to be an inhibitor of the SIN when the cell cycle is perturbed (see Introduction) but, in an unperturbed cell cycle, the main effect is an increased chromosome loss rate (Murone and Simanis, 1996). The absence of *dma1p* during meiosis results in a failure to form spores from one or more of the SPBs, implying that *dma1p* might play a more important role in meiosis. Increased expression of *mug27* slightly increases spore size but does not affect spore number (see Results). If one role for *dma1p* is to regulate *mug27p*, then, in its absence, persistent *mug27p* activity might perturb FSM development, leading to formation of bigger spores and aberrant FSM expansion events. By contrast, loss of both *dma1p* and *mug27p* strongly inhibits spore formation. Taken together with the genetic interactions of *dma1-D* with *mfr1* and the meiotic septins, our data are consistent with the view that *dma1p* plays multiple roles during meiosis in addition to regulating the meiotic SIN components such as *mug27p*.

A common feature of the meiotic localisation of *dma1p* is its association with sites of membrane remodelling, such as the point of fusion between cells and the expanding FSM. It is also noteworthy that *dma1p* is found between the two nuclei, where closure of the FSM will occur. In *Saccharomyces cerevisiae*, removal and degradation of *ssp1p* from the FSM is essential for spore formation (Maier et al., 2007). Because the RING-finger of *dma1* is required for its meiotic activity, it is tempting to speculate that *dma1p* will promote the degradation and/or inactivation of an equivalent protein. However, BLAST searches have not revealed a clear counterpart of *ssp1p* in fission yeast. The fact that *dma1p* and the SIN proteins are localised at the SPB in meiosis suggests that, as is the case in the mitotic cycle, where mitosis and cytokinesis are coordinated from the SPB, the meiotic SPB acts as a signalling centre to coordinate nuclear division and spore formation.

## Materials and Methods

### Yeast strains, media and culture conditions

Media have been described previously (Moreno et al., 1991); complete yeast extract (YE) medium or EMM2 were used as appropriate. For nitrogen starvation, cells were grown in EMM-N, which is EMM2 lacking  $\text{NH}_4\text{Cl}$ .

Fission yeast strains bearing GFP-tagged alleles of SIN genes used in this study have been described previously (Guertin et al., 2000; Krapp et al., 2001; Salimova et al., 2000; Sohrmann et al., 1998; Sparks et al., 1999). *dma1::ura4+* was constructed by (Murone and Simanis, 1996). *Dma1p* was tagged with GFP at its C-terminus according to standard techniques (Bahler et al., 1998b). FHA (R64A; H88A) and RF (C210S) mutants of *dma1* were constructed by Justine Shaw (J. Cassidy, Controlling cytokinesis in the fission yeast *Schizosaccharomyces pombe*: the role of *dma1* and *ubc8*, PhD thesis, University of Lausanne, 2005) using standard techniques and integrated into the *dma1* locus, expressed from the *dma1* promoter. A full description of the properties of these mutants is in preparation (J. Shaw, A.K. and V.S., unpublished data). GFP-tagged *spo3p*, *psy1p*, *spo15p* and *meu14p* have been described previously (Ikemoto et al., 2000; Nakamura et al., 2001; Okuzaki et al., 2003) and were obtained from the Shimoda laboratory or the Yeast National Bioresearch Project, Japan. *Plo1p*-GFP, *pcp1p*-GFP and *sad1p*-CFP have been described previously (Bahler et al., 1998a; Flory et al., 2002; Wu et al., 2003). *Mug27p*-GFP and *mug27::ura4+* were described in Perez-Hidalgo et al. (Perez-Hidalgo et al., 2008) and *mfr1-D* was described in Blanco et al. (Blanco et al., 2001); all were obtained from the Moreno laboratory (Salamanca, Spain). The *cnp1*-Cherry strain (Alvarez-Tabares et al., 2007) was obtained from the Hagan laboratory. Standard techniques (Moreno et al., 1991) were used to create the strains described in the text. For examination of GFP-*atb2p*, the *GFP-atb2* gene was expressed from its own promoter on a multicopy plasmid; the construction is not toxic to cells.

To examine the effect on spore formation and size, as well as to localise GFP-tagged proteins during meiosis, strains of opposite mating types expressing chromosomally GFP-tagged alleles were mated on EMM-N plates at 25°C for 20 hours, resuspended in EMM-N and mounted. Spore size was measured from photographs using Adobe Photoshop.

Diploid strains were obtained by mating on EMM-N plates (Blanco et al., 2001). Synchronous meiosis in *pat1-114/pat1-114* temperature-sensitive mutants was performed as follows. *h-h-pat1-114/pat1-114* diploid cells were cultured in YE medium at 25°C for 1 day and transferred to EMM plus supplements (100 µg/ml) for another day. These cells were then washed and resuspended in EMM- $\text{NH}_4\text{Cl}$  plus supplements (10 µg/ml) at a density of  $2\text{--}3 \times 10^6$  cells/ml. After 16 hours at 25°C, most cells were arrested in G1 phase and the culture was shifted to 34°C in the presence of EMM containing 0.5 g/l  $\text{NH}_4\text{Cl}$  and 10 µg/ml supplements to induce meiosis.

### RNA and protein methods

RNA was extracted using the RNeasy Kit from Qiagen. Five micrograms of total RNA were glyoxylated (Glyoxal Sample Dye, Ambion) and run on a 1.2% agarose gel. RNA was transferred onto a positively charged nylon membrane (Roche) and hybridised with a DIG-labelled probe (DIG Northern Kit, Roche) covering the *dma1* open reading frame.

Total protein extracts were made using the trichloroacetic acid (TCA) extraction protocol (Foiani et al., 1994). Protein extracts were run on SDS-PAGE gels and transferred to nitrocellulose membranes (Protran, Whatman). Primary antibodies against GFP (this laboratory), *cdc13* (Moreno laboratory, Salamanca, Spain), c-myc (Santa Cruz Biotechnology) and tubulin (TAT-1; Keith Gull, Oxford, UK) were used. Secondary antibodies conjugated to horseradish peroxidase and ECL western blotting reagents (Amersham) were used to visualise the bands.

### Microscopy

DAPI staining (1 µg/ml) was performed on cells that had been fixed with 70% ethanol as described previously (Balasubramanian et al., 1997; Moreno et al., 1991). To estimate the proportion of cells in meiosis I, meiosis II or sporulation, we determined the percentage of cells with one, two or four nuclei after DAPI (1 µg/ml) staining and the percentage of asci with mature spores with phase-contrast microscopy.

Examination of GFP-tagged proteins in living cells was performed using a Zeiss Axiovert 200 microscope equipped with a confocal scanner (CSU10; Yokogawa Electric Corporation), a coolSNAP HQ camera (Photometrics) and 63×1.4 NA plan-*apo* or 100×1.4 NA plan-*apo* objective. Images were collected using Metamorph software (Universal Imaging, version 4.5). Images were assembled in Adobe Photoshop 7 or CS and Powerpoint 2003.

We thank members of our laboratory for helpful discussions. We thank the Pollard, Moreno, Shimoda, Gould, McCollum, Hagan, Cooper, Ekwall and Davis laboratories for strains and reagents. This work was funded by the Swiss National Science Foundation and EPFL.

## References

- Alvarez-Tabares, I., Grallert, A., Ortiz, J. M. and Hagan, I. M. (2007). Schizosaccharomyces pombe protein phosphatase 1 in mitosis, endocytosis and a partnership with Wsh3/Tea4 to control polarised growth. *J. Cell Sci.* **120**, 3589-3601.
- Bahler, J., Steever, A. B., Wheatley, S., Wang, Y., Pringle, J. R., Gould, K. L. and McCollum, D. (1998a). Role of polo kinase and Mid1p in determining the site of cell division in fission yeast. *J. Cell Biol.* **143**, 1603-1616.
- 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. (1998b). Heterologous modules for efficient and versatile PCR-based gene targeting in Schizosaccharomyces pombe. *Yeast* **14**, 943-951.
- Balasubramanian, M. K., McCollum, D. and Gould, K. L. (1997). Cytokinesis in fission yeast Schizosaccharomyces pombe. *Methods Enzymol.* **283**, 494-506.
- Blanco, M. A., Pelloquin, L. and Moreno, S. (2001). Fission yeast *mfr1* activates APC and coordinates meiotic nuclear division with sporulation. *J. Cell Sci.* **114**, 2135-2143.
- Bothos, J., Summers, M. K., Venere, M., Scolnick, D. M. and Halazonetis, T. D. (2003). The Chfr mitotic checkpoint protein functions with Ubc13-Mms2 to form Lys63-linked polyubiquitin chains. *Oncogene* **22**, 7101-7107.
- Chang, L. and Gould, K. L. (2000). Sid4p is required to localize components of the septation initiation pathway to the spindle pole body in fission yeast. *Proc. Natl. Acad. Sci. USA* **97**, 5249-5254.
- 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.
- Fankhauser, C. and Simanis, V. (1993). The Schizosaccharomyces pombe *cdc14* gene is required for septum formation and can also inhibit nuclear division. *Mol. Biol. Cell* **4**, 531-539.
- Fankhauser, C. and Simanis, V. (1994). The *cdc7* protein kinase is a dosage dependent regulator of septum formation in fission yeast. *EMBO J.* **13**, 3011-3019.
- Fankhauser, C., Marks, J., Reymond, A. and Simanis, V. (1993). The S. pombe *cdc16* gene is required both for maintenance of p34cdc2 kinase activity and regulation of septum formation: a link between mitosis and cytokinesis? *EMBO J.* **12**, 2697-2704.
- Foiani, M., Marini, F., Gamba, D., Lucchini, G. and Plevani, P. (1994). The B subunit of the DNA polymerase alpha-primase complex in *Saccharomyces cerevisiae* executes an essential function at the initial stage of DNA replication. *Mol. Cell. Biol.* **14**, 923-933.

- Flory, M. R., Morphey, 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.
- Furge, K. A., Wong, K., Armstrong, J., Balasubramanian, M. and Albright, C. F. (1998). Byr4 and Cdc16 form a two-component GTPase-activating protein for the Spg1 GTPase that controls septation in fission yeast. *Curr. Biol.* **8**, 947-954.
- Furge, K. A., Cheng, Q. C., Jwa, M., Shin, S., Song, K. and Albright, C. F. (1999). Regions of Byr4, a regulator of septation in fission yeast, that bind Spg1 or Cdc16 and form a two-component GTPase-activating protein with Cdc16. *J. Biol. Chem.* **274**, 11339-11343.
- Garcia-Cortes, J. C. and McCollum, D. (2009). Proper timing of cytokinesis is regulated by *Schizosaccharomyces pombe* Etd1. *J. Cell Biol.* **186**, 739-753.
- Gordon, O., Taxis, C., Keller, P. J., Benjak, A., Stelzer, E. H., Simchen, G. and Knop, M. (2006). Nud1p, the yeast homolog of Centriolin, regulates spindle pole body inheritance in meiosis. *EMBO J.* **25**, 3856-3868.
- Grallert, A., Krapp, A., Bagley, S., Simanis, V. and Hagan, I. M. (2004). Recruitment of NIMA kinase shows that maturation of the *S. pombe* spindle-pole body occurs over consecutive cell cycles and reveals a role for NIMA in modulating SIN activity. *Genes Dev.* **18**, 1007-1021.
- Guertin, D. A. and McCollum, D. (2001). Interaction between the noncatalytic region of Sid1p kinase and Cdc14p is required for full catalytic activity and localization of Sid1p. *J. Biol. Chem.* **276**, 28185-28189.
- Guertin, D. A., Chang, L., Irshad, F., Gould, K. L. and McCollum, D. (2000). The role of the sid1p kinase and cdc14p in regulating the onset of cytokinesis in fission yeast. *EMBO J.* **19**, 1803-1815.
- Guertin, D. A., Venkatram, S., Gould, K. L. and McCollum, D. (2002). Dma1 prevents mitotic exit and cytokinesis by inhibiting the septation initiation network (SIN). *Dev. Cell* **3**, 779-790.
- Hachet, O. and Simanis, V. (2008). Mid1p/anillin and the septation initiation network orchestrate contractile ring assembly for cytokinesis. *Genes Dev.* **22**, 3205-3216.
- Hagan, I. and Yanagida, M. (1995). The product of the spindle formation gene *sad1+* associates with the fission yeast spindle pole body and is essential for viability. *J. Cell Biol.* **129**, 1033-1047.
- Hou, M. C., Salek, J. and McCollum, D. (2000). Mob1p interacts with the Sid2p kinase and is required for cytokinesis in fission yeast. *Curr. Biol.* **10**, 619-622.
- Huang, Y., Yan, H. and Balasubramanian, M. K. (2008). Assembly of normal actomyosin rings in the absence of Mid1p and cortical nodes in fission yeast. *J. Cell Biol.* **183**, 979-988.
- 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.
- Kang, D., Wong, J. and Fang, G. (2004). A Xenopus cell-free system for analysis of the Chfr ubiquitin ligase involved in control of mitotic entry. *Methods Mol. Biol.* **280**, 229-243.
- Krapp, A. and Simanis, V. (2008). An overview of the fission yeast septation initiation network (SIN). *Biochem. Soc. Trans.* **36**, 411-415.
- 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.
- Lattmann, E., Krapp, A. and Simanis, V. (2009). Cytokinesis: closure resets your SIN. *Curr. Biol.* **19**, R1040-R1042.
- Lee, B. H. and Amon, A. (2003). Role of Polo-like kinase CDC5 in programming meiosis I chromosome segregation. *Science* **300**, 482-486.
- Maier, P., Rathfelder, N., Finkbeiner, M. G., Taxis, C., Mazza, M., Le Panse, S., Haguener-Tsapir, R. and Knop, M. (2007). Cytokinesis in yeast meiosis depends on the regulated removal of Ssp1p from the prospore membrane. *EMBO J.* **26**, 1843-1852.
- Martin-Castellanos, C., Blanco, M., Rozalen, A. E., Perez-Hidalgo, L., Garcia, A. I., Conde, F., Mata, J., Ellermeier, C., Davis, L., San-Segundo, P. et al. (2005). A large-scale screen in *S. pombe* identifies seven novel genes required for critical meiotic events. *Curr. Biol.* **15**, 2056-2062.
- 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, S. and Gould, K. L. (2006). Identification of functional domains within the septation initiation network kinase, Cdc7. *J. Biol. Chem.* **281**, 9935-9941.
- Minet, M., Nurse, P., Thuriaux, P. and Mitchison, J. M. (1979). Uncontrolled septation in a cell division cycle mutant of the fission yeast *Schizosaccharomyces pombe*. *J. Bacteriol.* **137**, 440-446.
- Moreno, S., Klar, A. and Nurse, P. (1991). Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol.* **194**, 795-823.
- Morrell, J. L., Tomlin, G. C., Rajagopalan, S., Venkatram, S., Feoktistova, A. S., Tasto, J. J., Mehta, S., Jennings, J. L., Link, A., Balasubramanian, M. K. et al. (2004). Sid4p-Cdc11p assembles the septation initiation network and its regulators at the *S. pombe* SPB. *Curr. Biol.* **14**, 579-584.
- Mulvihill, D. P., Petersen, J., Ohkura, H., Glover, D. M. and Hagan, I. M. (1999). Plol1 kinase recruitment to the spindle pole body and its role in cell division in *Schizosaccharomyces pombe*. *Mol. Biol. Cell* **10**, 2771-2785.
- Murone, M. and Simanis, V. (1996). The fission yeast *dma1* gene is a component of the spindle assembly checkpoint, required to prevent septum formation and premature exit from mitosis if spindle function is compromised. *EMBO J.* **15**, 6605-6616.
- Nabeshima, K., Nakagawa, T., Straight, A. F., Murray, A., Chikashige, Y., Yamashita, Y. M., Hiraoka, Y. and Yanagida, M. (1998). Dynamics of centromeres during metaphase-anaphase transition in fission yeast: Dis1 is implicated in force balance in metaphase bipolar spindle. *Mol. Biol. Cell* **9**, 3211-3225.
- Nakamura, T., Nakamura-Kubo, M., Hirata, A. and Shimoda, C. (2001). The *Schizosaccharomyces pombe* spo3+ gene is required for assembly of the forespore membrane and genetically interacts with *psyl(+)*-encoding syntaxin-like protein. *Mol. Biol. Cell* **12**, 3955-3972.
- Nakamura, T., Asakawa, H., Nakase, Y., Kashiwazaki, J., Hiraoka, Y. and Shimoda, C. (2008). Live observation of forespore membrane formation in fission yeast. *Mol. Biol. Cell* **19**, 3544-3553.
- Nakase, Y., Nakamura-Kubo, M., Ye, Y., Hirata, A., Shimoda, C. and Nakamura, T. (2008). Meiotic spindle pole bodies acquire the ability to assemble the spore plasma membrane by sequential recruitment of sporulation-specific components in fission yeast. *Mol. Biol. Cell* **19**, 2476-2487.
- Niva, O. and Yanagida, M. (1985). Triploid meiosis and aneuploidy in *Schizosaccharomyces pombe*: an unstable aneuploid disomic for chromosome III. *Curr. Genet.* **9**, 463-470.
- Ohtaka, A., Okuzaki, D. and Nojima, H. (2008). Mug27 is a meiosis-specific protein kinase that functions in fission yeast meiosis II and sporulation. *J. Cell Sci.* **121**, 1547-1558.
- Okuzaki, D., Satake, W., Hirata, A. and Nojima, H. (2003). Fission yeast *meu14+* is required for proper nuclear division and accurate forespore membrane formation during meiosis II. *J. Cell Sci.* **116**, 2721-2735.
- Onishi, M., Koga, T., Hirata, A., Nakamura, T., Asakawa, H., Shimoda, C., Bahler, J., Wu, J. Q., Takegawa, K., Tachikawa, H. et al. (2010). Role of septins in the orientation of forespore membrane extension during sporulation in fission yeast. *Mol. Cell Biol.* **30**, 2057-2074.
- Perez-Hidalgo, L., Rozalen, A. E., Martin-Castellanos, C. and Moreno, S. (2008). Slk1 is a meiosis-specific Sid2-related kinase that coordinates meiotic nuclear division with growth of the forespore membrane. *J. Cell Sci.* **121**, 1383-1392.
- Petersen, J., Nielsen, O., Egel, R. and Hagan, I. M. (1998). F-actin distribution and function during sexual differentiation in *Schizosaccharomyces pombe*. *J. Cell Sci.* **111**, 867-876.
- Roberts-Galbraith, R. H. and Gould, K. L. (2008). Stepping into the ring: the SIN takes on contractile ring assembly. *Genes Dev.* **22**, 3082-3088.
- Rosenberg, J. A., Tomlin, G. C., McDonald, W. H., Snysman, B. E., Muller, E. G., Yates, J. R., 3rd and Gould, K. L. (2006). Ppc89 links multiple proteins, including the septation initiation network, to the core of the fission yeast spindle-pole body. *Mol. Biol. Cell* **17**, 3793-3805.
- Salimova, E., Sohrmann, M., Fournier, N. and Simanis, V. (2000). The *S. pombe* orthologue of the *S. cerevisiae* *mob1* gene is essential and functions in signalling the onset of septum formation. *J. Cell Sci.* **113**, 1695-1704.
- Schmidt, S., Sohrmann, M., Hofmann, K., Woollard, A. and Simanis, V. (1997). The Spg1 GTPase is an essential, dosage-dependent inducer of septum formation in *Schizosaccharomyces pombe*. *Genes Dev.* **11**, 1519-1534.
- Shimoda, C. (2004). Forespore membrane assembly in yeast: coordinating SPBs and membrane trafficking. *J. Cell Sci.* **117**, 389-396.
- Shimoda, C. and Nakamura, T. (2004). Control of late meiosis and ascospore formation. In *The Molecular Biology of Schizosaccharomyces pombe* (ed. R. Egel), pp. 311-327. Heidelberg: Springer-Verlag.
- Sohrmann, M., Schmidt, S., Hagan, I. and Simanis, V. (1998). Asymmetric segregation on spindle poles of the *Schizosaccharomyces pombe* septum-inducing protein kinase Cdc7p. *Genes Dev.* **12**, 84-94.
- Song, K., Mach, K. E., Chen, C. Y., Reynolds, T. and Albright, C. F. (1996). A novel suppressor of *ras1* in fission yeast, *byr4*, is a dosage-dependent inhibitor of cytokinesis. *J. Cell Biol.* **133**, 1307-1319.
- Sparks, C. A., Morphey, M. and McCollum, D. (1999). Sid2p, a spindle pole body kinase that regulates the onset of cytokinesis. *J. Cell Biol.* **146**, 777-790.
- 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., Petersen, J., MacIver, F., Mulvihill, D. P., Glover, D. M. and Hagan, I. M. (2001). The role of Plol1 kinase in mitotic commitment and septation in *Schizosaccharomyces pombe*. *EMBO J.* **20**, 1259-1270.
- Tomlin, G. C., Morrell, J. L. and Gould, K. L. (2002). The spindle pole body protein *cdc11p* links *sid4p* to the fission yeast septation initiation network. *Mol. Biol. Cell* **13**, 1203-1214.
- Wilkinson, C. R., Wallace, M., Morphey, M., Perry, P., Allshire, R., Javerzat, J. P., McIntosh, J. R. and Gordon, C. (1998). Localization of the 26S proteasome during mitosis and meiosis in fission yeast. *EMBO J.* **17**, 6465-6476.
- Wu, J. Q., Kuhn, J. R., Kovar, D. R. and Pollard, T. D. (2003). Spatial and temporal pathway for assembly and constriction of the contractile ring in fission yeast cytokinesis. *Dev. Cell* **5**, 723-734.
- Yamamoto, M. (2004). Initiation of meiosis. In *The Molecular Biology of Schizosaccharomyces pombe* (ed. R. Egel), pp. 297-309. Heidelberg: Springer-Verlag.
- Yan, H., Ge, W., Chew, T. G., Chow, J. Y., McCollum, D., Neiman, A. M. and Balasubramanian, M. K. (2008). The meiosis-specific Sid2p-related protein Slk1p regulates forespore membrane assembly in fission yeast. *Mol. Biol. Cell* **19**, 3676-3690.
- Yoo, B. Y., Calleja, G. B. and Johnson, B. F. (1973). Ultrastructural changes of the fission yeast (*Schizosaccharomyces pombe*) during ascospore formation. *Arch. Mikrobiol.* **91**, 1-10.