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COMMENTARY

Pombe's thirteen – control of fission yeast cell division by the septation initiation network

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ABSTRACT

The septation initiation network (SIN) regulates aspects of cell growth and division in *Schizosaccharomyces pombe* and is essential for cytokinesis. Insufficient signalling results in improper assembly of the contractile ring and failure of cytokinesis, generating multinucleated cells, whereas too much SIN signalling uncouples cytokinesis from the rest of the cell cycle. SIN signalling is therefore tightly controlled to coordinate cytokinesis with chromosome segregation. Signalling originates from the cytoplasmic face of the spindle pole body (SPB), and asymmetric localisation of some SIN proteins to one of the two SPBs during mitosis is important for regulation of the SIN. Recent studies have identified *in vivo* substrates of the SIN, which include components involved in mitotic control, those of the contractile ring and elements of the signalling pathway regulating polarised growth. The SIN is also required for spore formation following meiosis. This has provided insights into how the SIN performs its diverse functions in the cell cycle and shed new light on its regulation.

KEY WORDS: Cell division, Cytokinesis, Kinase, Phosphatase, Signalling, Yeast

Introduction

Schizosaccharomyces pombe cells are cylinders that grow by tip elongation. When cells enter mitosis, elongation ceases and the actin and tubulin cytoskeletons are reorganised in preparation for nuclear and cell division (Hagan and Hyams, 1988; Marks et al., 1986; McCully and Robinow, 1971; Mitchison and Nurse, 1985). A contractile actomyosin ring (CAR) is assembled at the centre of the cell during mitosis (Pollard, 2010; Pollard and Wu, 2010). At the end of anaphase, it guides synthesis of the septum, which bisects the cell (Fig. 1A) (Ishiguro, 1998; Sipiczki, 2007). The position of the division site is governed by the location of the interphase nucleus and depends on Mid1p, an anillin-related PH-domain protein, and other proteins that confer cell polarity (Oliferenko et al., 2009). Cytokinesis is regulated by the septation initiation network (SIN). If the SIN does not signal, cytokinesis fails. However, cell growth, S-phase and mitosis continue, producing elongated, multinucleated cells (Mitchison and Nurse, 1985); this is referred to hereafter as a SIN phenotype. Failure to turn SIN signalling off, or overexpression of *plo1* (Ohkura et al., 1995), *spg1* (Schmidt et al., 1997) or *cdc7* (Fankhauser and Simanis, 1994), generates multiseptated cells (Minet et al., 1979). Moreover, ectopic activation of the SIN triggers formation of a contractile ring and septum at any point in the cell cycle (Ohkura et al., 1995; Schmidt et al., 1997). These phenotypes are shown in Fig. 1B. There are 13 essential SIN components (Fig. 2A), whose roles are outlined briefly below (see also Goyal et al., 2011; Johnson et al., 2012; Simanis, 2003). The counterparts of the SIN, the mitotic exit network (MEN) in budding yeast and the mammalian HIPPO pathway, are discussed in Box 1.

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Essential components of the SIN

Scaffold proteins

Cytokinesis requires intact spindle pole bodies (SPBs) (Magidson et al., 2006). All of the core SIN proteins, except Etd1p, are located at the SPB through a tripartite scaffold. Ppc89 is essential for Sid4p localisation to the SPB, but not vice-versa (Rosenberg et al., 2006), whereas Sid4p is essential for Cdc11p association to the SPB, but not vice-versa (Krapp et al., 2001; Morrell et al., 2004), giving rise to the hierarchical order shown in Fig. 2A. Ppc89p is important for SPB function and its null allele (null alleles are denoted by the suffix *-D* in this Commentary) shows a complex, pleiotropic phenotype (Rosenberg et al., 2006) that contrasts with *cdc11-D* and *sid4-D* mutants, which do not affect mitosis (Chang and Gould, 2000; Krapp et al., 2001). Ppc89p, Sid4p (Rosenberg et al., 2006) and Sid2p (Sparks et al., 1999) localise to the cytoplasmic side of the SPB, implying that SIN signalling initiates in the cytoplasm.

The signalling GTPase Spg1p

Spg1p is an inducer of septation (Schmidt et al., 1997). Its signalling is controlled by the bipartite GTPase-activating protein (GAP) Byr4p–Cdc16p (for a review of GAP function see, for example, Cherfils and Zeghouf, 2013). Cdc16p is the catalytic subunit and resembles Rab GAPs. Cdc16p is inactive towards Spg1p in the absence of Byr4p, which acts as a scaffold to facilitate their interaction. Byr4p is unstable unless it is bound to Spg1p, which thus regulates the amount of the GAP (Krapp et al., 2008). In the absence of Cdc16p, Byr4p prevents GTP hydrolysis and release by Spg1p, but has no effect on the release of GDP (Furge et al., 1999; Furge et al., 1998). To date, no GEF for Spg1p has been identified, but it is possible that the high intrinsic GTPase activity and rate of nucleotide release (Furge et al., 1998) might suffice for nucleotide turnover in the absence of GEF activity.

Protein kinases

Plo1p is the orthologue of the conserved kinase *POLO* (Hagan, 2008; Ohkura et al., 1995). Plo1p is observed at the SPBs, the CAR and the mitotic spindle and it associates with at least three SPB proteins, Sid4p, Cut12p and Kms2p, consistent with it having multiple targets at the SPB (Grallert and Hagan, 2002; Johnson and Gould, 2011; MacIver et al., 2003; Wälde and King, 2014). Shut-down of *plo1* expression using a regulated promoter

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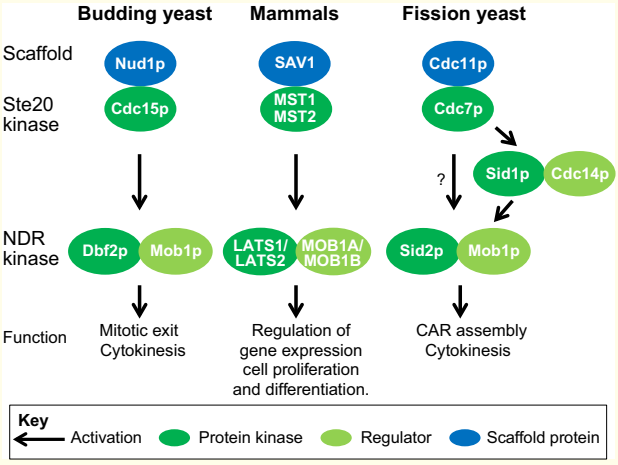
Box 1. Counterparts of the SIN in other eukaryotes

The signalling architecture of the SIN resembles the budding yeast mitotic exit network (MEN) and the mammalian HIPPO pathway. There are common core components on all the pathways, namely, a scaffold (a STE20 kinase), an NDR-family kinase and the presence of an additional module in the SIN, as shown in the figure. Kinases are denoted in green, and their regulatory subunits in light green. Scaffold proteins are shown in blue. The MEN governs both cytokinesis and mitotic exit (Hotz and Barral, 2014), and the HIPPO pathway regulates cell growth and proliferation (Gomez et al., 2014; Hergovich, 2013). Compared with these pathways, the SIN contains an additional kinase module, Sid1p–Cdc14p. The hierarchical order of SIN kinase signalling has not yet been verified biochemically, so it is unclear whether Cdc7p directly activates Sid2p, as predicted by direct comparison with the MEN and HIPPO pathways, or whether the signal passes via Sid1p–Cdc14p as is the case in the *Neurospora* SIN (Heilig et al., 2013). Analysis of the MEN has suggested that Cdc15p (the budding yeast counterpart of Cdc7p), functions as an integrator of signalling by Tem1p (Spg1p) and Cdc5p (Plo1p) (Rock and Amon, 2011). It is therefore possible that Sid1p–Cdc14p provides an input from another pathway, which is integrated with signals from Cdc7p and Spg1p to activate Sid2p–Mob1p.



produces a SIN phenotype (Ohkura et al., 1995), which is also seen in some hypomorphic *plo1* mutants (Tanaka et al., 2001). Epistasis analysis places Plo1p upstream of the SIN (Krapp et al., 2003; Tanaka et al., 2001), and increased expression of *plo1* induces septum formation (Ohkura et al., 1995), hence, Plo1p is considered an activator of the SIN.

SIN signalling downstream of Plo1p requires three protein kinases (Fig. 2A); the STE-20 family kinase Cdc7p (Fankhauser and Simanis, 1994), which interacts with Spg1p-GTP during mitosis (Mehta and Gould, 2006; Schmidt et al., 1997); the PAK-GC kinase Sid1p with its activator Cdc14p (Guertin et al., 2000; Guertin and McCollum, 2001), and the NDR-family kinase Sid2p with its activator Mob1p (Hou et al., 2004; Hou et al., 2000; Salimova et al., 2000). The kinases have been proposed to act in the order Cdc7p–(Sid1p–Cdc14p)–(Sid2p–Mob1p) (Guertin et al., 2000).

Etd1p

The sequence of Etd1p gives no clue to its biochemical function; although it has some limited similarity with *S. cerevisiae* Lte1p, which regulates mitotic exit (García-Cortés and McCollum, 2009). It is thought to act as a link between the CAR and the SIN,

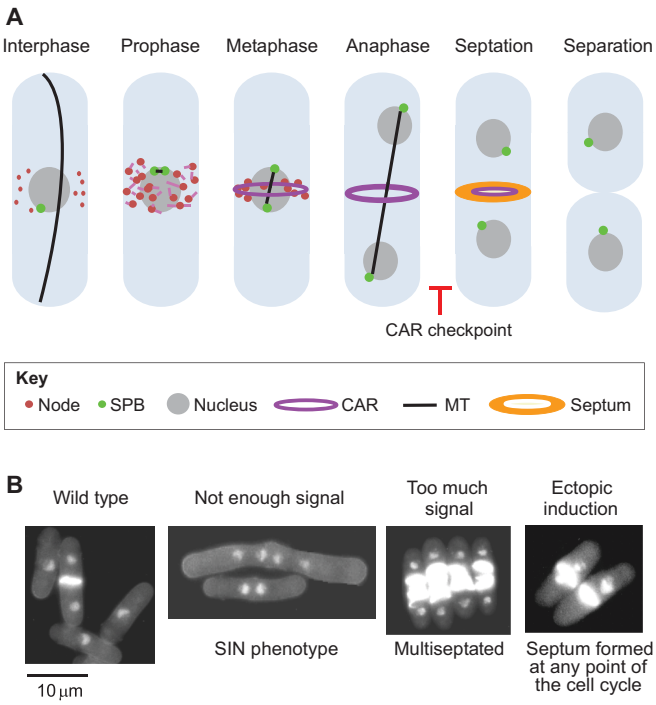


Fig. 1. *S. pombe* cytokinesis and the phenotypic effects of SIN mutants. (A) Schematic representation of cell division in *S. pombe*. In interphase, bundles of microtubules (MT) span the length of the cell and help to position the nucleus medially; for clarity a single MT bundle is shown. During interphase Mid1p is localised mainly in the nucleus, but a subset is found at the cell cortex, in structures commonly called nodes. Following entry into mitosis, a spindle is formed and chromosomes are aligned and attached to opposite SPBs. Mid1p is exported from the nucleus, and associates with the nodes, together with other CAR components. These condense together to form a CAR, which contracts at the end of mitosis, guiding septum synthesis. Reformation of interphase MTs occurs at this time (not shown). (B) The phenotypic effects of different levels of SIN signalling are shown. Cells were stained with DAPI for DNA and Calcofluor for the septum; original images are reproduced with permission from Simanis (Simanis, 2003). Note the presence of a single, medially placed septum in wild-type cells. If the SIN does not signal, cells become elongated and multinucleated, whereas the cell becomes multiseptated if SIN signalling is constitutively activated. Note that multiseptated in this context differs from the 'multiseptated' phenotype that is produced by defects in cell separation, in which each segment of the multi-compartmented cell has a nucleus (for example, see Grallert et al., 1998).

and both Spg1p and Rho1p have been suggested to be Etd1p targets. Etd1p is required for localisation of the SIN kinases to the SPB and CAR in anaphase. It has been detected at the cell tips, the medial region and at the division site during mitosis, and is degraded at the time of septation (Alcaide-Gavilán et al., 2014; Daga et al., 2005; García-Cortés and McCollum, 2009; Jimenez and Oballe, 1994; Lahoz et al., 2010).

How is the SIN regulated?

A large number of candidate regulators of SIN signalling have been identified by genetic screens and biochemical analyses of SIN proteins (Fig. 3). The localisation of SIN proteins at the SPB is also an important factor in SIN regulation. The sections below will discuss various aspects of SIN regulation.

Regulation of the signalling by SPB association of SIN proteins

During interphase, only the Spg1p-GAP complex and the scaffolding factors are seen at the SPBs whereas the kinase

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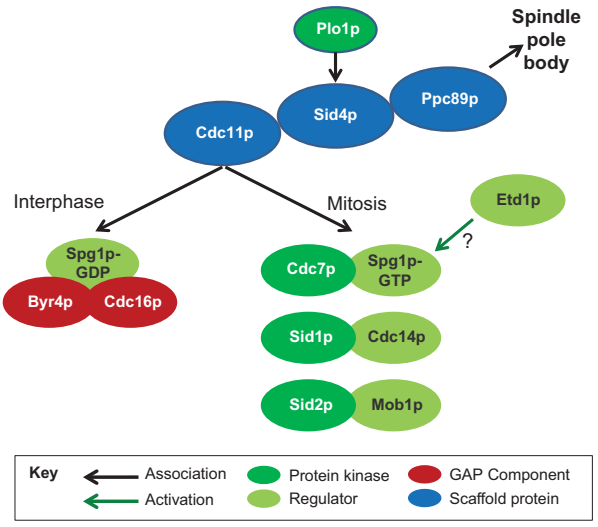

Fig. 2. The 13 essential components of the SIN. (A) Schematic representation of the key components of the SIN. Protein kinases required for signalling are shown in dark green and their regulatory subunits in light green. Scaffold proteins are shown in blue. Ppc89p links the other scaffold proteins to the SPB. All of these proteins, as well as Etd1p, are required for signal transmission and loss of function of any of them produces a SIN phenotype. Byr4p and Cdc16p are shown in red to represent their inhibitory activity towards SIN signalling.

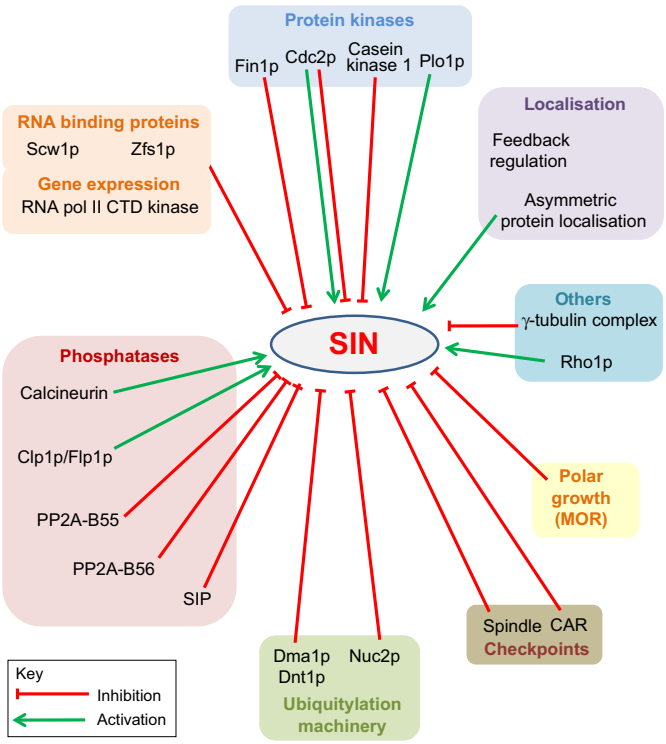

Fig. 3. Regulators of the SIN. Pathways or proteins known to impact on SIN signalling are shown here. The mechanism of regulation remains to be determined in most cases.

modules associate with the SPB only during mitosis (Fig. 2A). A notable feature of the SIN is that some of its components associate asymmetrically with the SPBs during mitosis (Fig. 4A). It is widely assumed, but not proven, that the localisation of SIN proteins, particularly Cdc7p, to the SPB in mitosis indicates that they are active.

How is the asymmetry regulated?

Both Cdc11p and Byr4p are heavily phosphorylated during mitosis (Krapp et al., 2001; Song et al., 1996), and the effects of mutating a subset of these phosphorylation sites have been investigated. Phosphorylation of Byr4p by Cdc2p and Plo1p (Rachfall et al., 2014) both contribute to the timely establishment of Cdc7p asymmetry in anaphase. Mutation of Sid2p phosphorylation sites on Cdc11p prevents Cdc7p becoming asymmetric at the SPBs during anaphase (Feoktistova et al., 2012). This has led to the proposal that a feedback loop within the SIN contributes to its spatial regulation. Consistent with this view, laser ablation of the new SPB (nSPB) in anaphase results in association of Cdc7p with the old SPB (oSPB) (Magidson et al., 2006), suggesting that the SPBs communicate during mitosis. Computational modelling (Bajpai et al., 2013) produced results consistent with the notion that asymmetric protein segregation is established by antagonistic interactions between the SIN kinases and Byr4p–Cdc16p, which act in part by regulating Cdc11p phosphorylation.

Examination of large populations of living cells points to the existence of two states of the SIN in the mitotic cycle (Fig. 4B). The transition between the states is delayed by the DYRK-family kinase Pom1p and by the spindle assembly checkpoint, and is promoted by CDK inactivation, the APC/C and the 14-3-3 protein Rad24p (Wachowicz et al., 2015). The significance of the two states is presently unclear. However, it is noteworthy that in *S. cerevisiae*, the mitotic exit network (MEN) effector Cdc14p is activated in a two-step process; it is first transiently released from the nucleolus, which becomes definitive upon MEN activation, promoting mitotic exit. If the MEN is not activated, Cdc14p release is only transient (Weiss, 2012). It is possible that the early state of the SIN reflects 'preparatory' events prior to its full activation at the end of anaphase, or it might be a manifestation of its role in CAR assembly (see below).

Is the asymmetry important?

Asymmetric localisation of SIN proteins is required for correct regulation of SIN signalling (Schmidt et al., 1997; Sohrmann et al., 1998) and also to turn it off promptly when cytokinesis has been completed (García-Cortés and McCollum, 2009). Loss of either Cdc16p or Byr4p favours the accumulation of Spg1p-GTP and association of the core SIN kinases with the SPB throughout the cell cycle, producing multiple septa. Mutants that fail to distribute SIN proteins asymmetrically in anaphase B compromise the regulation of cytokinesis (see next section; for additional discussion see Johnson et al., 2012; Simanis, 2003).

Regulators of SIN signalling

Protein phosphatases

Several phosphatases have been identified genetically as regulators of the SIN. These include the phosphatase complex PP2A (Goyal and Simanis, 2012; Jiang and Hallberg, 2001; Lahoz et al., 2010; Le Goff et al., 2001), Clp1p (Cueille et al., 2001; Trautmann et al., 2001), calcineurin (also known as PP2B) (Lu et al., 2002) and the PP2A-related SIN-inhibitory phosphatase (SIP) complex (Singh et al., 2011), which is the counterpart of the mammalian striatin interacting phosphatase and kinase (STRIPAK) complexes (Hwang and Pallas, 2014). Loss of calcineurin or Clp1p function is deleterious in SIN mutant backgrounds, suggesting that they promote SIN signalling. The opposite is true of PP2A; mutations of the regulatory subunits

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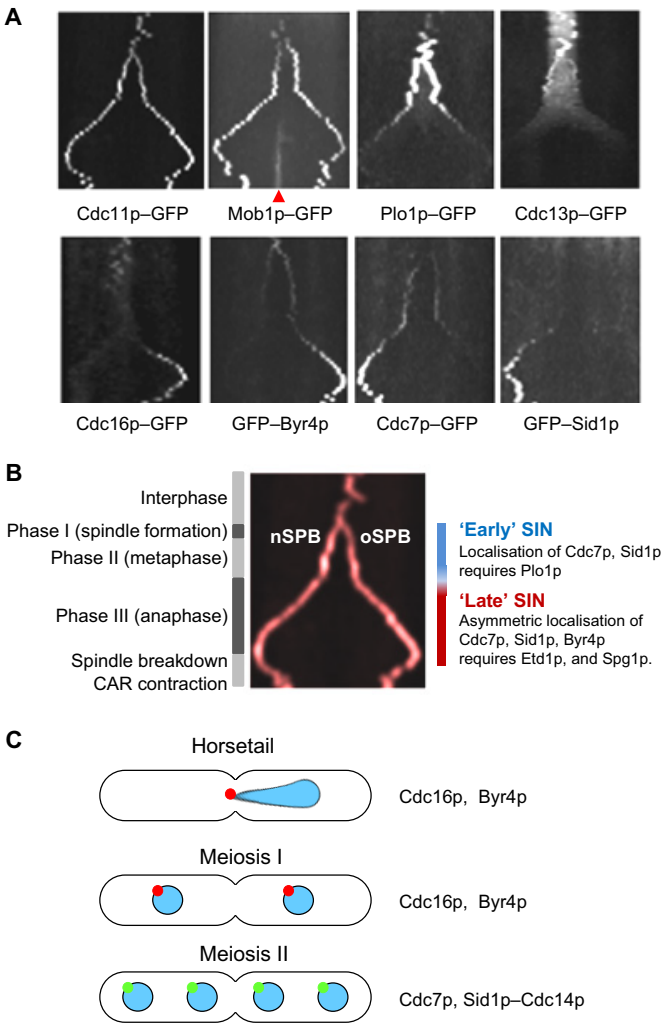

Fig. 4. SPB Localisation of SIN proteins in mitosis. (A) Localisation of representative SIN components and regulators during mitosis. Cells were recorded through mitosis and kymographs of the indicated proteins were prepared. Spg1p, Sid4p and Ppc89p localisation resembles that of Cdc11p. During interphase, only the Spg1p-GAP complex and the scaffolding proteins are seen at the SPBs. The kinase modules are only found at the SPB during mitosis. Each has its own pattern; Cdc7p is observed at both SPBs during metaphase (see B), becoming asymmetric at the new (n)SPB during anaphase B. In contrast, Sid1p associates stably with the nSPB only in anaphase B. Cdc16p leaves the SPB at the onset of mitosis, whereas Byr4p persists until metaphase. The signal of both proteins then becomes strong on the old (o)SPB during anaphase B. In contrast, Mob1p is seen at both SPBs throughout mitosis. The presence of the medial line (arrowed) in the Mob1 kymograph arises from its association with the CAR. Kymographs courtesy of Philippe Collin. (B) The coloured image of the scaffold Cdc11p–GFP illustrates progress from interphase (single SPB) to mitosis as a kymograph. The vertical axis is time, the horizontal axis represents SPB position. The inter-SPB distance decreases as the nuclei move to the middle of the daughter cells after spindle disassembly. In wild-type cells, CAR contraction begins at maximal SPB separation. The three phases of mitosis (Nabeshima et al., 1998) are shown to the right of the image. The 'early' and 'late' states of the SIN are shown to the right of the image. The 'early' state requires Plo1p activity, and is characterised by faint SIN protein signals and the unstable association of Cdc7p and Sid1p with the SPBs. The 'late' state does not require Plo1p, but depends upon Spg1p and Etd1p. It is characterised by asymmetric localisation of some SIN proteins (see A). The gradient between them indicates the fact that the precise timing of the transition varies from cell to cell. (C) Cartoon depicting the localisation of SIN proteins in meiosis. Cdc11p, Sid4p, Spg1p and Sid2p–Mob1p are associated with the SPBs throughout meiosis. Cdc16p and Byr4p are found at the SPBs during the horsetail stage and meiosis I, whereas Cdc7p and Sid1p–Cdc14p are absent from the SPBs during both these stages. During meiosis II, Cdc16p and Byr4p are replaced by Cdc7p and Sid1p–Cdc14p (Krapp et al., 2006; Krapp et al., 2010; Krapp and Simanis, 2014; Yan et al., 2008). The meiosis-specific Sid2p-related protein kinase Mug27p localises to the SPB during the meiotic nuclear divisions and associates with the forespore membrane during spore formation before being degraded at the end of meiosis II (not shown). It does not require Sid4p or Cdc11p for SPB association (Ohtaka et al., 2008). Red and green SPBs denote what is presumed to be the inactive and active SIN, respectively.

(B or B′), the major catalytic subunit Ppa2p, the activator PTPA (also known as Ypa2p) or SIP-null mutants, can rescue conditional SIN mutants (see references above), suggesting that PP2A inhibits SIN signalling. The spectrum of SIN mutant alleles that can be rescued differs according to the PP2A mutation; this might reflect the existence of multiple PP2A targets within the SIN or, alternatively, some of the rescue effects might be indirect. Mutants in PP2A and its regulators (Goyal and Simanis, 2012; Jiang and Hallberg, 2001; Lahoz et al., 2010), as well as SIP mutants (Singh et al., 2011) abrogate asymmetric localisation of SIN proteins in mitosis, and impair cytokinesis with variable phenotypic penetrance in unperturbed cells. These mutants are also very sensitive to minor perturbations of the cytokinetic machinery (Goyal and Simanis, 2012; Singh et al., 2011), suggesting that PP2A might fine-tune SIN activity to assure the fidelity of cytokinesis. Cdc11p is phosphorylated at the time of septum formation and dephosphorylated at the end of mitosis (Krapp et al., 2001), and PP2A, SIP and Clp1p all contribute to this (Chen et al., 2013; Krapp et al., 2003; Lahoz et al., 2010; Le Goff et al., 2001; Singh et al., 2011).

Protein kinases

SPB-associated mitotic protein kinases are important for controlling SIN signalling. The cyclin-dependent kinase (CDK) Cdc2p–Cdc13p (hereafter referred to as Cdc2p) influences the SIN both positively and negatively. Active Cdc2p inhibits the SIN early in mitosis and its inactivation is required for septum formation and to establish SIN protein asymmetry (Chang et al., 2001; Dischinger et al., 2008; Guertin et al., 2000; He et al., 1997; Yamano et al., 1996). Cdc2p and the Byr4p–Cdc16p GAP are thought to cooperate to prevent septation in interphase (Cerutti and Simanis, 1999). The GAP presumably prevents accumulation of Spg1p-GTP, but the relevant targets of Cdc2p are unknown. Once the cell enters mitosis, Cdc2p and Plo1p cooperate to ensure the removal of Byr4p from the SPBs and facilitate SIN signalling in anaphase (Rachfall et al., 2014). Fin1p is the sole 'never-in-mitosis' (nimA) ortholog in fission yeast (Krien et al., 1998). Fin1p inhibits SIN signalling on the oSPB and affects the asymmetric localisation of SIN proteins. Its association with the SPBs requires Byr4p and SIN activity (Grallert et al., 2004). However, the mechanism by which Fin1p regulates the SIN is still unknown.

Ubiquitylation

The ubiquitin ligase Dma1p is required to prevent septum formation in mitotically arrested cells (Murone and Simanis, 1996). Dma1p binds to and ubiquitylates Sid4p after the latter has been phosphorylated by casein kinase I (Johnson et al., 2013). Ubiquitylation of Sid4p is required for the function of a

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checkpoint that operates in parallel to the spindle assembly checkpoint, and prevents the recruitment of Plo1p to the SPB, thereby inhibiting SIN activity in mitotically arrested cells (Guertin et al., 2002; Johnson and Gould, 2011). The nucleolar protein Dnt1p (Jin et al., 2007) binds to Dma1p during mitosis and regulates its activity (Wang et al., 2012).

Gene expression

The SIN is also regulated by proteins that are associated with gene expression. For instance, mutation of the RNA polymerase II (Pol II) C-terminal domain (CTD) kinase complex Lsk1p–Lsc1p (Karagiannis and Balasubramanian, 2007; Karagiannis et al., 2005), and mutations in the CTD of Pol II itself, compromise the ability of cells to arrest division in response to perturbations of the cytokinetic machinery and also to rescue some SIN mutants. However, the relevant target genes have not yet been identified. Mutation of the transcription factor *ace2* also rescues many hypomorphic SIN alleles; in this case the important targets appears to be the cell-wall-degrading enzymes Agn1p and Eng2p and the septin ring assembly factor Mid2p (Jin et al., 2006).

Null mutants of either of the RNA-binding proteins Zfs1p and Scw1p can rescue hypomorphic SIN mutants (Beltraminelli et al., 1999; Cuthbertson et al., 2008; Jin and McCollum, 2003; Karagiannis et al., 2002), but it has not been determined which of the RNAs that are targeted by these proteins are relevant for this. Recent studies have proposed that the rescue of SIN mutants by *scw1-D* might be indirect, because cytokinesis takes longer in the *scw1-D* cells, thereby providing additional time for SIN-dependent events to be completed in hypomorphic SIN mutants (Wu et al., 2010).

Others

The importance of the SPB in SIN regulation is further highlighted by a study implicating the SPB-associated γ-tubulin complex, which acts as an inhibitor of the SIN when Cdc2p activity is high to prevent untimely septation (Vardy et al., 2002), although the underlying mechanism remains unresolved. Increased expression of *rho1* also rescues some hypomorphic SIN mutants and *etd1-D* (Alcaide-Gavilán et al., 2014; Jin et al., 2006), but the nature of the link between Rho signalling and the SIN is unresolved.

Turning the SIN off

Turning the SIN off at the end of mitosis (as assessed by the reappearance of the interphase constellation of SIN proteins at the SPB) correlates with CAR closure and SIN-dependent removal of

Table 1. Proposed functions of the SIN

Function	Target	References	Comment
Meiotic nuclear permeability	?	Arai et al., 2010	SIN functions downstream of the protein kinase Spo4p–Spo6p.
Spore formation	Secretory apparatus?	Krapp et al., 2006; Ohtaka et al., 2008; Pérez-Hidalgo et al., 2008; Yan et al., 2008	
Septum biosynthesis	?	Jin et al., 2006	
Reorganisation of endocytic machinery	?	Gachet and Hyams, 2005	
CAR checkpoint	Clp1	Chen et al., 2008	Phosphorylation is by Sid2p.
Maintenance of CAR	?	Alcaide-Gavilán et al., 2014; Mishra et al., 2004	Sid2p activity is required to maintain the CAR in checkpoint arrested cells.
Postmitotic nuclear positioning in CAR-checkpoint arrested cells	Klp2	Mana-Capelli et al., 2012	Phosphorylation is by Sid2p.
Post-anaphase microtubule organising centre formation.	?	Heitz et al., 2001	
Formation and retention of astral microtubules in anaphase	?	Krapp et al., 2001	
Equatorial retention of the CAR	?	Pardo and Nurse, 2003	
Mitotic commitment	Fin1p	Grallert et al., 2012	Sid2p phosphorylates and activates Fin1p. Does not require Cdc7p–Spg1p.
Spindle checkpoint?	Mph1	Gupta et al., 2013	Identified in screen for Sid2p substrates. Role unconfirmed.
Autoregulation	Cdc11p	Feoktistova et al., 2012	Sid2p phosphorylates Cdc11p at multiple sites. This is important for establishing Cdc7p asymmetry in anaphase. It has been proposed that this constitutes a regulatory feedback loop within the SIN.
CAR formation	Cdc12p	Bohnert et al., 2013; Hachet and Simanis, 2008; Huang et al., 2008	The formin Cdc12p is phosphorylated to regulate its F-actin bundling activity. Important for CAR assembly.
Control of polarized growth	Nak1p and Sog2p	Gupta et al., 2014; Gupta et al., 2013; Ray et al., 2010	Proposed mutual inhibition between SIN and MOR prevents conflicts between polar growth and cytokinesis.

The table lists proposed functions of the SIN. It should be noted that some of these individual 'functions' might represent different facets of others. In most cases the targets of the SIN have not been identified. Certain results are analysed further in the text. Note that Clp1p is also known as Flp1p.

Etd1p from the medial region (García-Cortés and McCollum, 2009). The mechanism has not been determined.

Increased expression of the anaphase-promoting complex (APC/C) subunit *nuc2* produces a SIN phenotype (Kumada et al., 1995) by interfering with the interaction between Spg1p and Cdc7p, perhaps by stimulating activity of Byr4p–Cdc16p (Chew and Balasubramanian, 2008). This role of Nuc2p is independent of its APC/C function and contributes to turning off the SIN at the end of mitosis.

What does the SIN do?

The SIN has been implicated in many processes (Table 1), but in most cases the SIN target remains unknown. Recent systematic searches for Sid2p substrates have exploited the fact that phosphorylation of the Sid2p consensus site, RxxS (Mah et al., 2005), creates a binding site for 14-3-3 proteins. Comparison of the proteins that associate with Rad24p when the SIN is switched on or off, has generated a list of potential Sid2p targets (Gupta et al., 2013). Some of these, such as the spindle checkpoint protein Mph1p, might point to new roles for Sid2p and/or the SIN, whose biological significance will doubtless be tested soon. Here, I shall focus on roles for the SIN in CAR assembly, the CAR checkpoint, mitotic commitment and meiosis.

Roles of the SIN at the CAR

CAR assembly

The SIN is required for condensation of the precursor nodes that form the CAR (Wu et al., 2003) to generate the compact structure seen in anaphase, and in SIN mutants the improperly assembled CAR collapses during anaphase (Hachet and Simanis, 2008; Huang et al., 2008; Mishra et al., 2004). The SIN and Mid1p cooperate in CAR formation; *mid1-D* cells assemble their CAR using an alternative mechanism that does not involve condensation of nodes. This event is dependent on the SIN because no CAR is formed if both Mid1p and SIN function are compromised (Hachet and Simanis, 2008; Huang et al., 2008). Plo1p, which regulates both Mid1p and the SIN, is thought to coordinate these two pathways (Roberts-Galbraith and Gould, 2008).

Plo1p associates transiently with the CAR in early mitosis (Bähler et al., 1998), whereas Sid2p–Mob1p associates with the CAR in mid-late anaphase (Fig. 4). As the scaffold proteins are required for CAR assembly, one or more of the SIN proteins might require activation at the SPB in early mitosis, but it is unknown whether the CAR-associated SIN proteins must pass via the SPB. CAR association of Sid2p requires every SIN protein to be functional (Daga et al., 2005; Guertin et al., 2000; Hou et al., 2000; Ohkura et al., 1995; Salimova et al., 2000; Sparks et al., 1999). It is also noteworthy that the IQGAP Rng2p, which is necessary for CAR assembly (Laporte et al., 2011; Padmanabhan et al., 2011; Takaine et al., 2014; Tebbs and Pollard, 2013), is localised at the SPBs in mitosis (Eng et al., 1998).

Sid2p substrates in the CAR

Phosphorylation of the formin Cdc12p by Sid2p during CAR formation allows it to bundle F-actin (Bohnert et al., 2013); mutation of the phosphorylation sites on Cdc12p impairs CAR formation. The PCH-domain protein Cdc15p binds to and curves membranes, and acts as a scaffold to recruit other CAR components (Roberts-Galbraith et al., 2009). Cdc15p also requires SIN function to associate with the CAR (Hachet and Simanis, 2008), but it is not known whether Cdc15p is a SIN substrate. It seems likely that there will be additional SIN targets in the CAR.

The SIN and the CAR checkpoint

The existence of a checkpoint that monitors CAR integrity was discovered through study of mutants in the β(1,3) D-glucan synthase *bgs1*. Some *bgs1* alleles complete mitosis, but cannot form a division septum and arrest with an interphase microtubule array, with nuclei positioned in the middle of what would be the daughter cells. The CAR persists at the cell cortex, Cdc7p and Sid1p are both associated with the nSPB, and resumption of tip growth is blocked (Le Goff et al., 1999; Liu et al., 2000; Liu et al., 1999). This checkpoint is also triggered by mutants in CAR components (Mishra et al., 2004). The checkpoint arrest requires SIN function (Le Goff et al., 1999; Liu et al., 2000; Liu et al., 1999), and Sid2p is required for CAR maintenance when the checkpoint is active (Alcaide-Gavilán et al., 2014) and for positioning the nuclei away from the CAR in cells arrested by the CAR checkpoint (Table 1) (Mana-Capelli et al., 2012).

Targets of Sid2p in the CAR checkpoint

The CAR checkpoint requires the function of the *CDC14*-related phosphatase Clp1p. Like its budding yeast counterpart, Clp1p is regulated in part by localisation (Cueille et al., 2001; Trautmann et al., 2001). It is sequestered to the nucleolus in interphase, before being released into the nucleus and the cytoplasm during mitosis, where it dephosphorylates numerous substrates (Chen et al., 2013). Clp1p cannot perform its checkpoint role if it is confined to the nucleus by attachment of a nuclear localisation signal (Trautmann and McCollum, 2005). Phosphorylation of Clp1p by Sid2p retains it in the cytoplasm through interaction with Rad24p (Mishra et al., 2005) and mutagenesis of Sid2p phosphorylation sites in Clp1p compromises the CAR checkpoint (Chen et al., 2008). However, a mutant Clp1p tagged with a nuclear export signal is incapable of maintaining the CAR checkpoint in a *sid2* mutant (Trautmann and McCollum, 2005). It has therefore been suggested that there is a positive feedback between the SIN and Clp1p, and that Clp1p is required to maintain SIN activity in the arrested cells (Mishra et al., 2004), although additional roles for Clp1p and Sid2p cannot be ruled out. For example, Clp1p regulates gene expression at the M/G1 transition (Papadopoulou et al., 2010), but whether this contributes to checkpoint function is unknown.

Regulation of polar growth by the SIN

The morphology orb6 network

S. pombe has a second HIPPO-related signalling pathway, called the morphology *orb6* network (MOR), which is required for polarised growth in interphase. Its main components are two protein kinase modules, the protein kinase Nak1p (Huang et al., 2003; Leonhard and Nurse, 2005) and its regulators Sog2p (also known as Lrp1p) (Gupta et al., 2013; Kume et al., 2013) and Pmo25p (Kanai et al., 2005; Kume et al., 2007; Mendoza et al., 2005), which act upstream of the kinase Orb6p (Verde et al., 1998) and its regulator Mob2p (Hou et al., 2003). MOR signalling also requires the scaffold protein Mor2p (Hirata et al., 2002). Pmo25p, Nak1p and Sog2p localise to the SPB and the division site, whereas Mob2p, Orb6p and Mor2p only localise to the cell periphery and the division site. Orb6p and Nak1p activity is high in interphase and decreases during mitosis, when tip growth ceases (Kanai et al., 2005; Mendoza et al., 2005; Ray et al., 2010).

SIN–MOR crosstalk

Activation of the SIN decreases the activity of Orb6p, and it has been proposed that the SIN inhibits the MOR during mitosis in

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order to prevent the cell from becoming 'conflicted' between CAR assembly and tip growth (Ray et al., 2010). Sid2p phosphorylates Nak1p, and mutation of the phosphorylation sites on Nak1p results in continued cell elongation during mitosis and lysis at the time of septation (Gupta et al., 2014; Gupta et al., 2013). Pmo25p localisation to the SPBs requires SIN activity (Kanai et al., 2005; Mendoza et al., 2005), and it has been suggested that SIN-dependent sequestration of Pmo25p and Nak1p at the SPB prevents MOR activation (Ray et al., 2010). It has also been proposed that the presence of Nak1p at the SPB inhibits the SIN, thereby providing crosstalk between the SIN and MOR to ensure robust switching of the actin polymerisation network and cell wall synthesis enzymes from tip growth to CAR and septum formation (Ray et al., 2010). However, earlier studies showed that Sid1p is required for maximum activation of Pmo25p-associated kinase activity (Kume et al., 2007), suggesting that additional levels of regulation remain to be discovered.

A role for Sid2p in regulating mitotic commitment

The SIN regulator Fin1p is also implicated in regulation of the G2/M transition; *fin1-D* cells are viable, but delayed in mitotic commitment. Sid2p phosphorylates and activates Fin1p at the G2/M transition (Grallert et al., 2012). This might be a SIN-independent function of Sid2p because it does not depend on Cdc7p or Spg1p. It is unknown whether activation of Fin1p by Sid2p is required for its role in regulating the SIN in anaphase. As the timing of mitotic commitment is sensitive to stress and the nutritional status of the cell (Fantes and Nurse, 1977; Fantes and Nurse, 1978; Petersen and Hagan, 2005; Petersen and Nurse, 2007), identification of the regulators of Sid2p in this context will be of interest.

The SIN in meiosis

Fission yeast meiosis and spore formation

When starved, cells of opposite mating types undergo a pheromone-dependent G1 arrest, followed by cytoplasmic and nuclear fusion and premeiotic S-phase. Homologous recombination then takes place while the nucleus migrates from end to end in the cell and adopts an elongated 'horsetail' shape (Chikashige et al., 1994; Robinow, 1977). This is followed by the two meiotic divisions. Finally, spores are formed by deposition of membranes and cell wall around the four nuclei (reviewed by Shimoda, 2004; Shimoda and Nakamura, 2004; Yamamoto, 2004).

Roles of the SIN in meiosis

The SIN has at least two roles in meiosis. First, it is thought to act downstream of the Spo4p–Spo6p protein kinase complex to effect the transient change in nuclear permeability during meiosis I (Arai et al., 2010). Second, the SIN is required for spore formation (Krapp et al., 2006; Yan et al., 2008). Leaving aside Ppc89p and Etd1p, which have not been tested, all of the mitotic SIN proteins except Sid2p are essential for spore formation. An additional Sid2p-like kinase, Mug27p (also called Ppk35p or Slk1p), is expressed specifically during meiosis. Mug27p cooperates with Sid2p; the individual mutants impair spore formation and a double mutant abolishes it (Ohtaka et al., 2008; Pérez-Hidalgo et al., 2008; Yan et al., 2008). Increased expression of the syntaxin *psy1*, a factor involved in the secretory pathway, rescues the sporulation defect of *mug27-D sid2-250* (Yan et al., 2008), suggesting that Mug27p and Sid2p cooperate to promote recruitment of components of the secretory apparatus to allow forespore membrane (FSM) expansion during spore formation.

Regulation of the SIN during meiosis

The SIN might be regulated differently during meiosis and mitosis (Krapp and Simanis, 2014). For example, generation of Spg1p-GTP is not sufficient to recruit Cdc7p to the SPB. Moreover, in addition to its GAP scaffold function, Byr4p prevents SPB association of Cdc7p before meiosis II. Proteolysis also plays an important role in SIN regulation during meiosis. Destruction of Byr4p after completion of meiosis I probably permits the association of Cdc7p with the SPBs to activate the SIN in meiosis II. Dma1p is required to form normal asci in meiosis (Krapp et al., 2010), and it mediates the degradation of several SIN proteins, including Mug27p, Cdc7p, Cdc11p and Sid4p at the end of meiosis. Finally, none of the SIN proteins localise asymmetrically to the SPBs during meiosis (Fig. 4C), consistent with the fact that all four meiosis II nuclei will be transformed into spores.

Future directions

To date, the search for SIN targets has focussed on targets of Sid2p. Several Sid2p substrates were not identified in the Rad24p affinity-trap assay, so it is likely that additional substrates remain to be identified. To understand how the SIN works, it will be necessary to identify the substrates of the SIN kinases both in meiosis and mitosis, expanding upon the studies discussed above. Once the phosphorylation sites on SIN proteins and their substrates have been determined, mutants will help us to understand their regulatory significance. Now that many of the regulatory relationships between the SIN proteins have been established, further mathematical modelling of the SIN will be useful as an aid to better describe our observations (Csikász-Nagy et al., 2007) and to make predictions that can be tested at the bench (Bajpai et al., 2013). Studies on the counterparts of the SIN in other organisms will also provide new hypotheses to test in *S. pombe*; for example, is the assembly of SIN proteins at the SPB also a phosphorylation-dependent event, as in the mitotic exit network of the budding yeast (Rock et al., 2013)? Conversely, insights into how the SIN is regulated and identification of its targets might help us to understand the MEN and HIPPO pathways.

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Competing interests

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References

Alcaide-Gavilán, M., Lahoz, A., Daga, R. R. and Jimenez, J. (2014). Feedback regulation of SIN by Etd1 and Rho1 in fission yeast. *Genetics* **196**, 455-470.

Arai, K., Sato, M., Tanaka, K. and Yamamoto, M. (2010). Nuclear compartmentalization is abolished during fission yeast meiosis. *Curr. Biol.* **20**, 1913-1918.

Bähler, J., Steever, A. B., Wheatley, S., Wang, Y., Pringle, J. R., Gould, K. L. and McCollum, D. (1998). Role of polo kinase and Mid1p in determining the site of cell division in fission yeast. *J. Cell Biol.* **143**, 1603-1616.

Bajpai, A., Feoktistova, A., Chen, J. S., McCollum, D., Sato, M., Carazo-Salas, R. E., Gould, K. L. and Csikász-Nagy, A. (2013). Dynamics of SIN asymmetry establishment. *PLOS Comput. Biol.* **9**, e1003147.

Beltraminelli, N., Murone, M. and Simanis, V. (1999). The S. pombe zfs1 gene is required to prevent septation if mitotic progression is inhibited. *J. Cell Sci.* **112**, 3103-3114.
Bohnert, K. A., Grzegorzewska, A. P., Willet, A. H., Vander Kooi, C. W., Kovar, D. R. and Gould, K. L. (2013). SIN-dependent phosphoinhibition of formin multimerization controls fission yeast cytokinesis. *Genes Dev.* **27**, 2164-2177.
Cerutti, L. and Simanis, V. (1999). Asymmetry of the spindle pole bodies and spg1p GAP segregation during mitosis in fission yeast. *J. Cell Sci.* **112**, 2313-2321.
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.
Chang, L., Morrell, J. L., Feoktistova, A. and Gould, K. L. (2001). Study of cyclin proteolysis in anaphase-promoting complex (APC) mutant cells reveals the requirement for APC function in the final steps of the fission yeast septation initiation network. *Mol. Cell. Biol.* **21**, 6681-6694.
Chen, C. T., Feoktistova, A., Chen, J. S., Shim, Y. S., Clifford, D. M., Gould, K. L. and McCollum, D. (2008). The SIN kinase Sid2 regulates cytoplasmic retention of the S. pombe Cdc14-like phosphatase Clp1. *Curr. Biol.* **18**, 1594-1599.
Chen, J. S., Broadus, M. R., McLean, J. R., Feoktistova, A., Ren, L. and Gould, K. L. (2013). Comprehensive proteomics analysis reveals new substrates and regulators of the fission yeast clp1/cdc14 phosphatase. *Mol. Cell. Proteomics* **12**, 1074-1086.
Cherfils, J. and Zeghouf, M. (2013). Regulation of small GTPases by GEFs, GAPs, and GDIs. *Physiol. Rev.* **93**, 269-309.
Chew, T. G. and Balasubramanian, M. K. (2008). Nuc2p, a subunit of the anaphase-promoting complex, inhibits septation initiation network following cytokinesis in fission yeast. *PLoS Genet.* **4**, e17.
Chikashige, Y., Ding, D. Q., Funabiki, H., Haraguchi, T., Mashiko, S., Yanagida, M. and Hiraoka, Y. (1994). Telomere-led premeiotic chromosome movement in fission yeast. *Science* **264**, 270-273.
Csikász-Nagy, A., Kapuy, O., Gyorffy, B., Tyson, J. J. and Novák, B. (2007). Modeling the septation initiation network (SIN) in fission yeast cells. *Curr. Genet.* **51**, 245-255.
Cueille, N., Salimova, E., Esteban, V., Blanco, M., Moreno, S., Bueno, A. and Simanis, V. (2001). Flp1, a fission yeast orthologue of the s. cerevisiae CDC14 gene, is not required for cyclin degradation or rum1p stabilisation at the end of mitosis. *J. Cell Sci.* **114**, 2649-2664.
Cuthbertson, B. J., Liao, Y., Birnbaumer, L. and Blackshear, P. J. (2008). Characterization of zfs1 as an mRNA binding and destabilizing protein in Schizosaccharomyces pombe. *J. Biol. Chem.* **283**, 2586-2594.
Daga, R. R., Lahoz, A., Muñoz, M. J., Moreno, S. and Jimenez, J. (2005). Etd1p is a novel protein that links the SIN cascade with cytokinesis. *EMBO J.* **24**, 2436-2446.
Dischinger, S., Krapp, A., Xie, L., Paulson, J. R. and Simanis, V. (2008). Chemical genetic analysis of the regulatory role of Cdc2p in the S. pombe septation initiation network. *J. Cell Sci.* **121**, 843-853.
Eng, K., Naqvi, N. I., Wong, K. C. and Balasubramanian, M. K. (1998). Rng2p, a protein required for cytokinesis in fission yeast, is a component of the actomyosin ring and the spindle pole body. *Curr. Biol.* **8**, 611-621.
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.
Fantes, P. and Nurse, P. (1977). Control of cell size at division in fission yeast by a growth-modulated size control over nuclear division. *Exp. Cell Res.* **107**, 377-386.
Fantes, P. A. and Nurse, P. (1978). Control of the timing of cell division in fission yeast. Cell size mutants reveal a second control pathway. *Exp. Cell Res.* **115**, 317-329.
Feoktistova, A., Morrell-Falvey, J., Chen, J. S., Singh, N. S., Balasubramanian, M. K. and Gould, K. L. (2012). The fission yeast septation initiation network (SIN) kinase, Sid2, is required for SIN asymmetry and regulates the SIN scaffold, Cdc11. *Mol. Biol. Cell* **23**, 1636-1645.
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.
Gachet, Y. and Hyams, J. S. (2005). Endocytosis in fission yeast is spatially associated with the actin cytoskeleton during polarised cell growth and cytokinesis. *J. Cell Sci.* **118**, 4231-4242.
García-Cortés, J. C. and McCollum, D. (2009). Proper timing of cytokinesis is regulated by Schizosaccharomyces pombe Etd1. *J. Cell Biol.* **186**, 739-753.
Gomez, M., Gomez, V. and Hergovich, A. (2014). The Hippo pathway in disease and therapy: cancer and beyond. *Clin Transl Med.* **3**, 22.
Goyal, A. and Simanis, V. (2012). Characterization of ypa1 and ypa2, the Schizosaccharomyces pombe orthologs of the peptidyl proyl isomerases that activate PP2A, reveals a role for Ypa2p in the regulation of cytokinesis. *Genetics* **190**, 1235-1250.
Goyal, A., Takaine, M., Simanis, V. and Nakano, K. (2011). Dividing the spoils of growth and the cell cycle: The fission yeast as a model for the study of cytokinesis. *Cytoskeleton (Hoboken)* **68**, 69-88.
Grallert, A. and Hagan, I. M. (2002). Schizosaccharomyces pombe NIMA-related kinase, Fin1, regulates spindle formation and an affinity of Polo for the SPB. *EMBO J.* **21**, 3096-3107.
Grallert, A., Grallert, B., Ribar, B. and Sipiczki, M. (1998). Coordination of initiation of nuclear division and initiation of cell division in Schizosaccharomyces pombe: genetic interactions of mutations. *J. Bacteriol.* **180**, 892-900.
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.
Grallert, A., Connolly, Y., Smith, D. L., Simanis, V. and Hagan, I. M. (2012). The S. pombe cytokinesis NDR kinase Sid2 activates Fin1 NIMA kinase to control mitotic commitment through Pom1/Wee1. *Nat. Cell Biol.* **14**, 738-745.
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.
Gupta, S., Mana-Capelli, S., McLean, J. R., Chen, C. T., Ray, S., Gould, K. L. and McCollum, D. (2013). Identification of SIN pathway targets reveals mechanisms of crosstalk between NDR kinase pathways. *Curr. Biol.* **23**, 333-338.
Gupta, S., Govindaraghavan, M. and McCollum, D. (2014). Crosstalk between NDR kinase pathways co-ordinates cytokinesis with cell separation in S. pombe. *Eukaryot. Cell.* **13**, 1104-1112.
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. M. (2008). The spindle pole body plays a key role in controlling mitotic commitment in the fission yeast Schizosaccharomyces pombe. *Biochem. Soc. Trans.* **36**, 1097-1101.
Hagan, I. M. and Hyams, J. S. (1988). The use of cell division cycle mutants to investigate the control of microtubule distribution in the fission yeast Schizosaccharomyces pombe. *J. Cell Sci.* **89**, 343-357.
He, X., Patterson, T. E. and Sazer, S. (1997). The Schizosaccharomyces pombe spindle checkpoint protein mad2p blocks anaphase and genetically interacts with the anaphase-promoting complex. *Proc. Natl. Acad. Sci. USA* **94**, 7965-7970.
Heilig, Y., Schmitt, K. and Seiler, S. (2013). Phospho-regulation of the Neurospora crassa septation initiation network. *PLoS ONE* **8**, e79464.
Heitz, M. J., Petersen, J., Valovin, S. and Hagan, I. M. (2001). MTOC formation during mitotic exit in fission yeast. *J. Cell Sci.* **114**, 4521-4532.
Hergovich, A. (2013). Regulation and functions of mammalian LATS/NDR kinases: looking beyond canonical Hippo signalling. *Cell Biosci.* **3**, 32.
Hirata, D., Kishimoto, N., Suda, M., Sogabe, Y., Nakagawa, S., Yoshida, Y., Sakai, K., Mizunuma, M., Miyakawa, T., Ishiguro, J. et al. (2002). Fission yeast Mor2/Cps12, a protein similar to Drosophila Furry, is essential for cell morphogenesis and its mutation induces Wee1-dependent G(2) delay. *EMBO J.* **21**, 4863-4874.
Hotz, M. and Barral, Y. (2014). The Mitotic Exit Network: new turns on old pathways. *Trends Cell Biol.* **24**, 145-152.
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.
Hou, M. C., Wiley, D. J., Verde, F. and McCollum, D. (2003). Mob2p interacts with the protein kinase Orb6p to promote coordination of cell polarity with cell cycle progression. *J. Cell Sci.* **116**, 125-135.
Hou, M. C., Guertin, D. A. and McCollum, D. (2004). Initiation of cytokinesis is controlled through multiple modes of regulation of the Sid2p-Mob1p kinase complex. *Mol. Cell. Biol.* **24**, 3262-3276.
Huang, T. Y., Markley, N. A. and Young, D. (2003). Nak1, an essential germinal center (GC) kinase regulates cell morphology and growth in Schizosaccharomyces pombe. *J. Biol. Chem.* **278**, 991-997.
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.
Hwang, J. and Pallas, D. C. (2014). STRIPAK complexes: structure, biological function, and involvement in human diseases. *Int. J. Biochem. Cell Biol.* **47**, 118-148.
Ishiguro, J. (1998). Genetic control of fission yeast cell wall synthesis: the genes involved in wall biogenesis and their interactions in Schizosaccharomyces pombe. *Genes Genet. Syst.* **73**, 181-191.
Jiang, W. and Hallberg, R. L. (2001). Correct regulation of the septation initiation network in Schizosaccharomyces pombe requires the activities of par1 and par2. *Genetics* **158**, 1413-1429.
Jimenez, J. and Oballe, J. (1994). Ethanol-hypersensitive and ethanol-dependent cdc- mutants in Schizosaccharomyces pombe. *Mol. Gen. Genet.* **245**, 86-95.
Jin, Q. W. and McCollum, D. (2003). Scw1p antagonizes the septation initiation network to regulate septum formation and cell separation in the fission yeast Schizosaccharomyces pombe. *Eukaryot. Cell* **2**, 510-520.

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Jin, Q. W., Zhou, M., Bimbo, A., Balasubramanian, M. K. and McCollum, D. (2006). A role for the septation initiation network in septum assembly revealed by genetic analysis of sid2-250 suppressors. *Genetics* **172**, 2101-2112.
Jin, Q. W., Ray, S., Choi, S. H. and McCollum, D. (2007). The nucleolar Net1/Cfi1-related protein Dnt1 antagonizes the septation initiation network in fission yeast. *Mol. Biol. Cell* **18**, 2924-2934.
Johnson, A. E. and Gould, K. L. (2011). Dma1 ubiquitinates the SIN scaffold, Sid4, to impede the mitotic localization of Plo1 kinase. *EMBO J.* **30**, 341-354.
Johnson, A. E., McCollum, D. and Gould, K. L. (2012). Polar opposites: fine-tuning cytokinesis through SIN asymmetry. *Cytoskeleton (Hoboken)* **69**, 686-699.
Johnson, A. E., Chen, J. S. and Gould, K. L. (2013). CK1 is required for a mitotic checkpoint that delays cytokinesis. *Curr. Biol.* **23**, 1920-1926.
Kanai, M., Kume, K., Miyahara, K., Sakai, K., Nakamura, K., Leonhard, K., Wiley, D. J., Verde, F., Toda, T. and Hirata, D. (2005). Fission yeast MO25 protein is localized at SPB and septum and is essential for cell morphogenesis. *EMBO J.* **24**, 3012-3025.
Karagiannis, J. and Balasubramanian, M. K. (2007). A cyclin-dependent kinase that promotes cytokinesis through modulating phosphorylation of the carboxy terminal domain of the RNA Pol II Rpb1p sub-unit. *PLoS ONE* **2**, e433.
Karagiannis, J., Oulton, R. and Young, P. G. (2002). The Scw1 RNA-binding domain protein regulates septation and cell-wall structure in fission yeast. *Genetics* **162**, 45-58.
Karagiannis, J., Bimbó, A., Rajagopalan, S., Liu, J. and Balasubramanian, M. K. (2005). The nuclear kinase Lsk1p positively regulates the septation initiation network and promotes the successful completion of cytokinesis in response to perturbation of the actomyosin ring in Schizosaccharomyces pombe. *Mol. Biol. Cell* **16**, 358-371.
Krapp, A. and Simanis, V. (2014). Dma1-dependent degradation of SIN proteins during meiosis in Schizosaccharomyces pombe. *J. Cell Sci.* **127**, 3149-3161.
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., Cano, E. and Simanis, V. (2003). Mitotic hyperphosphorylation of the fission yeast SIN scaffold protein cdc11p is regulated by the protein kinase cdc7p. *Curr. Biol.* **13**, 168-172.
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.
Krapp, A., Collin, P., Cano del Rosario, E. and Simanis, V. (2008). Homoeostasis between the GTPase Spg1p and its GAP in the regulation of cytokinesis in S. pombe. *J. Cell Sci.* **121**, 601-608.
Krapp, A., Del Rosario, E. C. and Simanis, V. (2010). The role of Schizosaccharomyces pombe dma1 in spore formation during meiosis. *J. Cell Sci.* **123**, 3284-3293.
Krien, M. J., Bugg, S. J., Palatsides, M., Asouline, G., Morimyo, M. and O'Connell, M. J. (1998). A NIMA homologue promotes chromatin condensation in fission yeast. *J. Cell Sci.* **111**, 967-976.
Kumada, K., Su, S., Yanagida, M. and Toda, T. (1995). Fission yeast TPR-family protein nuc2 is required for G1-arrest upon nitrogen starvation and is an inhibitor of septum formation. *J. Cell Sci.* **108**, 895-905.
Kume, K., Goshima, T., Miyahara, K., Toda, T. and Hirata, D. (2007). A method for Pmo25-associated kinase assay in fission yeast: the activity is dependent on two gC kinases Nak1 and Sid1. *Biosci. Biotechnol. Biochem.* **71**, 615-617.
Kume, K., Kubota, S., Koyano, T., Kanai, M., Mizunuma, M., Toda, T. and Hirata, D. (2013). Fission yeast leucine-rich repeat protein Lrp1 is essential for cell morphogenesis as a component of the morphogenesis Orb6 network (MOR). *Biosci. Biotechnol. Biochem.* **77**, 1086-1091.
Lahoz, A., Alcaide-Gavilán, M., Daga, R. R. and Jimenez, J. (2010). Antagonistic roles of PP2A-Pab1 and Etd1 in the control of cytokinesis in fission yeast. *Genetics* **186**, 1261-1270.
Laporte, D., Coffman, V. C., Lee, I. J. and Wu, J. Q. (2011). Assembly and architecture of precursor nodes during fission yeast cytokinesis. *J. Cell Biol.* **192**, 1005-1021.
Le Goff, X., Woollard, A. and Simanis, V. (1999). Analysis of the cps1 gene provides evidence for a septation checkpoint in Schizosaccharomyces pombe. *Mol. Gen. Genet.* **262**, 163-172.
Le Goff, X., Buvelot, S., Salimova, E., Guerry, F., Schmidt, S., Cueille, N., Cano, E. and Simanis, V. (2001). The protein phosphatase 2A B′-regulatory subunit par1p is implicated in regulation of the S. pombe septation initiation network. *FEBS Lett.* **508**, 136-142.
Leonhard, K. and Nurse, P. (2005). Ste20/GCK kinase Nak1/Orb3 polarizes the actin cytoskeleton in fission yeast during the cell cycle. *J. Cell Sci.* **118**, 1033-1044.
Liu, J., Wang, H., McCollum, D. and Balasubramanian, M. K. (1999). Drc1p/Cps1p, a 1,3-beta-glucan synthase subunit, is essential for division septum assembly in Schizosaccharomyces pombe. *Genetics* **153**, 1193-1203.
Liu, J., Wang, H. and Balasubramanian, M. K. (2000). A checkpoint that monitors cytokinesis in Schizosaccharomyces pombe. *J. Cell Sci.* **113**, 1223-1230.
Lu, Y., Sugiura, R., Yada, T., Cheng, H., Sio, S. O., Shuntoh, H. and Kuno, T. (2002). Calcineurin is implicated in the regulation of the septation initiation network in fission yeast. *Genes Cells* **7**, 1009-1019.
MacIver, F. H., Tanaka, K., Robertson, A. M. and Hagan, I. M. (2003). Physical and functional interactions between polo kinase and the spindle pole component Cut12 regulate mitotic commitment in S. pombe. *Genes Dev.* **17**, 1507-1523.
Magidson, V., Chang, F. and Khodjakov, A. (2006). Regulation of cytokinesis by spindle-pole bodies. *Nat. Cell Biol.* **8**, 891-893.
Mah, A. S., Elia, A. E., Devgan, G., Ptacek, J., Schutkowski, M., Snyder, M., Yaffe, M. B. and Deshaies, R. J. (2005). Substrate specificity analysis of protein kinase complex Dbf2-Mob1 by peptide library and proteome array screening. *BMC Biochem.* **6**, 22.
Mana-Capelli, S., McLean, J. R., Chen, C. T., Gould, K. L. and McCollum, D. (2012). The kinesin-14 Klp2 is negatively regulated by the SIN for proper spindle elongation and telophase nuclear positioning. *Mol. Biol. Cell* **23**, 4592-4600.
Marks, J., Hagan, I. M. and Hyams, J. S. (1986). Growth polarity and cytokinesis in fission yeast: the role of the cytoskeleton. *J. Cell Sci. Suppl.* **5**, 229-241.
McCully, E. K. and Robinow, C. F. (1971). Mitosis in the fission yeast Schizosaccharomyces pombe: a comparative study with light and electron microscopy. *J. Cell Sci.* **9**, 475-507.
Mehta, S. and Gould, K. L. (2006). Identification of functional domains within the septation initiation network kinase, Cdc7. *J. Biol. Chem.* **281**, 9935-9941.
Mendoza, M., Redemann, S. and Brunner, D. (2005). The fission yeast MO25 protein functions in polar growth and cell separation. *Eur. J. Cell Biol.* **84**, 915-926.
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.
Mishra, M., Karagiannis, J., Trautmann, S., Wang, H., McCollum, D. and Balasubramanian, M. K. (2004). The Clp1p/Flp1p phosphatase ensures completion of cytokinesis in response to minor perturbation of the cell division machinery in Schizosaccharomyces pombe. *J. Cell Sci.* **117**, 3897-3910.
Mishra, M., Karagiannis, J., Sevugan, M., Singh, P. and Balasubramanian, M. K. (2005). The 14-3-3 protein rad24p modulates function of the cdc14p family phosphatase clp1p/flp1p in fission yeast. *Curr. Biol.* **15**, 1376-1383.
Mitchison, J. M. and Nurse, P. (1985). Growth in cell length in the fission yeast Schizosaccharomyces pombe. *J. Cell Sci.* **75**, 357-376.
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.
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.
Ohkura, H., Hagan, I. M. and Glover, D. M. (1995). The conserved Schizosaccharomyces pombe kinase plo1, required to form a bipolar spindle, the actin ring, and septum, can drive septum formation in G1 and G2 cells. *Genes Dev.* **9**, 1059-1073.
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.
Oliferenko, S., Chew, T. G. and Balasubramanian, M. K. (2009). Positioning cytokinesis. *Genes Dev.* **23**, 660-674.
Padmanabhan, A., Bakka, K., Sevugan, M., Naqvi, N. I., D'souza, V., Tang, X., Mishra, M. and Balasubramanian, M. K. (2011). IQGAP-related Rng2p organizes cortical nodes and ensures position of cell division in fission yeast. *Curr. Biol.* **21**, 467-472.
Papadopoulou, K., Chen, J. S., Mead, E., Feoktistova, A., Petit, C., Agarwal, M., Jamal, M., Malik, A., Spanos, A., Sedgwick, S. G. et al. (2010). Regulation of cell cycle-specific gene expression in fission yeast by the Cdc14p-like phosphatase Clp1p. *J. Cell Sci.* **123**, 4374-4381.
Pardo, M. and Nurse, P. (2003). Equatorial retention of the contractile actin ring by microtubules during cytokinesis. *Science* **300**, 1569-1574.
Pérez-Hidalgo, L., Rozalén, A. E., Martín-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. and Hagan, I. M. (2005). Polo kinase links the stress pathway to cell cycle control and tip growth in fission yeast. *Nature* **435**, 507-512.
Petersen, J. and Nurse, P. (2007). TOR signalling regulates mitotic commitment through the stress MAP kinase pathway and the Polo and Cdc2 kinases. *Nat. Cell Biol.* **9**, 1263-1272.
Pollard, T. D. (2010). Mechanics of cytokinesis in eukaryotes. *Curr. Opin. Cell Biol.* **22**, 50-56.
Pollard, T. D. and Wu, J. Q. (2010). Understanding cytokinesis: lessons from fission yeast. *Nat. Rev. Mol. Cell Biol.* **11**, 149-155.
Rachfall, N., Johnson, A. E., Mehta, S., Chen, J. S. and Gould, K. L. (2014). Cdk1 promotes cytokinesis in fission yeast through activation of the septation initiation network. *Mol. Biol. Cell* **25**, 2250-2259.
Ray, S., Kume, K., Gupta, S., Ge, W., Balasubramanian, M., Hirata, D. and McCollum, D. (2010). The mitosis-to-interphase transition is coordinated by

cross talk between the SIN and MOR pathways in Schizosaccharomyces pombe. *J. Cell Biol.* **190**, 793-805.
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.
Roberts-Galbraith, R. H., Chen, J. S., Wang, J. and Gould, K. L. (2009). The SH3 domains of two PCH family members cooperate in assembly of the Schizosaccharomyces pombe contractile ring. *J. Cell Biol.* **184**, 113-127.
Robinow, C. F. (1977). The number of chromosomes in Schizosaccharomyces pombe: light microscopy of stained preparations. *Genetics* **87**, 491-497.
Rock, J. M. and Amon, A. (2011). Cdc15 integrates Tem1 GTPase-mediated spatial signals with Polo kinase-mediated temporal cues to activate mitotic exit. *Genes Dev.* **25**, 1943-1954.
Rock, J. M., Lim, D., Stach, L., Ogrodowicz, R. W., Keck, J. M., Jones, M. H., Wong, C. C., Yates, J. R., III, Winey, M., Smerdon, S. J. et al. (2013). Activation of the yeast Hippo pathway by phosphorylation-dependent assembly of signaling complexes. *Science* **340**, 871-875.
Rosenberg, J. A., Tomlin, G. C., McDonald, W. H., Snydsman, B. E., Muller, E. G., Yates, J. R., III 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 Spg1p 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.
Simanis, V. (2003). Events at the end of mitosis in the budding and fission yeasts. *J. Cell Sci.* **116**, 4263-4275.
Singh, N. S., Shao, N., McLean, J. R., Sevugan, M., Ren, L., Chew, T. G., Bimbo, A., Sharma, R., Tang, X., Gould, K. L. et al. (2011). SIN-inhibitory phosphatase complex promotes Cdc11p dephosphorylation and propagates SIN asymmetry in fission yeast. *Curr. Biol.* **21**, 1968-1978.
Sipiczki, M. (2007). Splitting of the fission yeast septum. *FEMS Yeast Res.* **7**, 761-770.
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., Morphew, M. and McCollum, D. (1999). Sid2p, a spindle pole body kinase that regulates the onset of cytokinesis. *J. Cell Biol.* **146**, 777-790.
Takaine, M., Numata, O. and Nakano, K. (2014). Fission yeast IQGAP maintains F-actin-independent localization of myosin-II in the contractile ring. *Genes Cells* **19**, 161-176.
Tanaka, K., Petersen, J., MacIver, F., Mulvihill, D. P., Glover, D. M. and Hagan, I. M. (2001). The role of Plo1 kinase in mitotic commitment and septation in Schizosaccharomyces pombe. *EMBO J.* **20**, 1259-1270.
Tebbs, I. R. and Pollard, T. D. (2013). Separate roles of IQGAP Rng2p in forming and constricting the Schizosaccharomyces pombe cytokinetic contractile ring. *Mol. Biol. Cell* **24**, 1904-1917.
Trautmann, S. and McCollum, D. (2005). Distinct nuclear and cytoplasmic functions of the S. pombe Cdc14-like phosphatase Clp1p/Flp1p and a role for nuclear shuttling in its regulation. *Curr. Biol.* **15**, 1384-1389.
Trautmann, S., Wolfe, B. A., Jorgensen, P., Tyers, M., Gould, K. L. and McCollum, D. (2001). Fission yeast Clp1p phosphatase regulates G2/M transition and coordination of cytokinesis with cell cycle progression. *Curr. Biol.* **11**, 931-940.
Vardy, L., Fujita, A. and Toda, T. (2002). The gamma-tubulin complex protein Alp4 provides a link between the metaphase checkpoint and cytokinesis in fission yeast. *Genes Cells* **7**, 365-373.
Verde, F., Wiley, D. J. and Nurse, P. (1998). Fission yeast orb6, a ser/thr protein kinase related to mammalian rho kinase and myotonic dystrophy kinase, is required for maintenance of cell polarity and coordinates cell morphogenesis with the cell cycle. *Proc. Natl. Acad. Sci. USA* **95**, 7526-7531.
Wachowicz, P., Chasapi, A., Krapp, A., Cano Del Rosario, E., Schmitter, D., Sage, D., Unser, M., Xenarios, I., Rougemont, J. and Simanis, V. (2015). Analysis of *S. pombe* SIN protein SPB-association reveals two genetically separable states of the SIN. *J. Cell Sci.* **128**, 741-754.
Wälde, S. and King, M. C. (2014). The KASH protein Kms2 coordinates mitotic remodeling of the spindle pole body. *J. Cell Sci.* **127**, 3625-3640.
Wang, Y., Li, W. Z., Johnson, A. E., Luo, Z. Q., Sun, X. L., Feoktistova, A., McDonald, W. H., McLeod, I., Yates, J. R., III, Gould, K. L. et al. (2012). Dnt1 acts as a mitotic inhibitor of the spindle checkpoint protein dma1 in fission yeast. *Mol. Biol. Cell* **23**, 3348-3356.
Weiss, E. L. (2012). Mitotic exit and separation of mother and daughter cells. *Genetics* **192**, 1165-1202.
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.
Wu, J. Q., Ye, Y., Wang, N., Pollard, T. D. and Pringle, J. R. (2010). Cooperation between the septins and the actomyosin ring and role of a cell-integrity pathway during cell division in fission yeast. *Genetics* **186**, 897-915.
Yamamoto, M. (2004). Initiation of meiosis. In *The Molecular Biology of Schizosaccharomyces Pombe* (ed. R. Egel), pp. 297-309. Heidelberg: Springer-Verlag.
Yamano, H., Gannon, J. and Hunt, T. (1996). The role of proteolysis in cell cycle progression in Schizosaccharomyces pombe. *EMBO J.* **15**, 5268-5279.
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.

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