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Regulation of the Cdc14-like Phosphatase CLP1 in *Schizosaccharomyces pombe* and Identification of SID2 Kinase Substrates: A Dissertation

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REGULATION OF THE CDC14-LIKE PHOSPHATASE CLP1 IN SCHIZOSACCHAROMYCES POMBE, AND IDENTIFICATION OF SID2 KINASE SUBSTRATES

A Dissertation Presented

By

CHUN-TI CHEN

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To whom I love most

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Abstract

Coordination of mitosis and cytokinesis is crucial to generate healthy daughter cells with equal amounts of genetic and cytoplasmic materials. In the fission yeast *Schizosaccharomyces pombe*, an evolutionarily conserved Cdc14-like phosphatase (Clp1) functions to couple mitosis and cytokinesis by antagonizing CDK activity. The activity of Clp1 is thought to be regulated in part by its subcellular localization. It is sequestered in the nucleolus and the spindle pole body (SPB) during interphase. Upon mitotic entry, it is released into the cytoplasm and localized to the kinetochores, the actomyosin ring, and the mitotic spindle to carry out distinct functions. It is not clear how Clp1 is released from the nucleolus, however, once released, a conserved signaling pathway termed Septation Initiation Network (SIN) functions to retain Clp1 in the cytoplasm until completion of cytokinesis. The SIN and Clp1 function together in a positive feedback loop to promote each other's activity. That is, the SIN promotes cytoplasmic retention of Clp1, and cytoplasmic Clp1 antagonizes CDK activity and reverses CDK inhibition on the SIN pathway to promote its function and activity. However, at the start of this thesis, the mechanism by which the SIN regulated Clp1 was unknown. The SIN pathway is also required to promote constriction of the actomyosin ring, and the septum formation. However, its downstream targets were still uncharacterized. In two separate studies, we studied how Clp1 is released from the nucleolus at mitotic entry (Chapter II) and how the SIN kinase Sid2 acts to retain Clp1 in the cytoplasm (Chapter III). In Chapter IV, we

identified several Sid2 candidate substrates, and revealed other functions of the SIN pathway in coordinating mitotic events.

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CHAPTER I

General Introduction

Cell cycle progression is tightly regulated and monitored to ensure faithful reproduction of daughter cells. Cells have developed different checkpoints in response to defects that occur during the cell cycle, and function to delay cell cycle progression until the defect is repaired. Numerous studies have been done to elucidate the crosstalk between these regulatory machineries and cellular events. In this thesis, I'm interested in understanding how the fission yeast *Schizosaccharomyces pombe* coordinates mitosis and cytokinesis at the end of the cell cycle. *S. pombe* is a rod-shaped organism that grows by tip elongation and divides by medial fission. Several factors make this unicellular eukaryote an ideal model system, including the evolutionary conservation of regulatory machinery, a fully sequenced genome, availability of a complete gene deletion set, and availability of many conditionally lethal mutations. Also, multiple techniques can be easily applied to this organism, such as biochemistry, genetics, and live-cell imaging.

Oscillation of the cyclin-dependent kinases (CDKs) governs the cell cycle transitions in eukaryotic cells. Each transition is regulated by a specific set of CDKs and its activating cyclin subunits. For example, entry of mitosis requires activation of mitotic Cdk1, which is regulated by accumulation of B-type cyclin, and activating dephosphorylation and phosphorylation of Cdk1. On the other hand, exit from mitosis

requires inactivation of Cdk1, which is regulated by ubiquitylation and degradation of B-type cyclin, and reversal of Cdk1 specific phosphorylation events (Figure1-1, red line). The evolutionarily conserved phosphatase Cdc14 has been described as such a Cdk1 site-specific phosphatase. The Cdc14-family of phosphatases plays a conserved role to dephosphorylate Cdk1 substrates and accelerate Cdk1 inactivation to promote mitotic exit and cytokinesis. The onset of cytokinesis must correlate with mitotic exit, Cdk1 inactivation, and reversal of Cdk1 phosphorylation sites (Figure 1-1, green line). This regulatory mechanism ensures that cytokinesis does not occur prior to chromosome segregation and prevents genetic instability. Therefore, studying the regulation of the Cdc14 phosphatase becomes an important task to gain better understanding of timely regulation of Cdk1 activity.

Cytokinesis is the last stage in the cell cycle, when physical barriers are built to separate two daughter cells. Cytokinesis must be temporally and spatially regulated to ensure its fidelity. In the fission yeast *S. pombe*, cytokinesis is executed by the constriction of an actomyosin-based ring (equivalent to the mammalian ingression furrow), followed by septum formation. The spatial cue for cytokinesis is provided by an anillin-related protein Mid1 (Chang et al., 1996, Sohrmann et al., 1996). Mid1 accumulates in the nucleus during interphase, and shuttles between the nucleus and adjacent cortex. Its localization determines the future division site together with regulation of a DYRK family kinase Pom1 (Bahler and Pringle, 1998; Celton-Morizur et

Figure 1-1

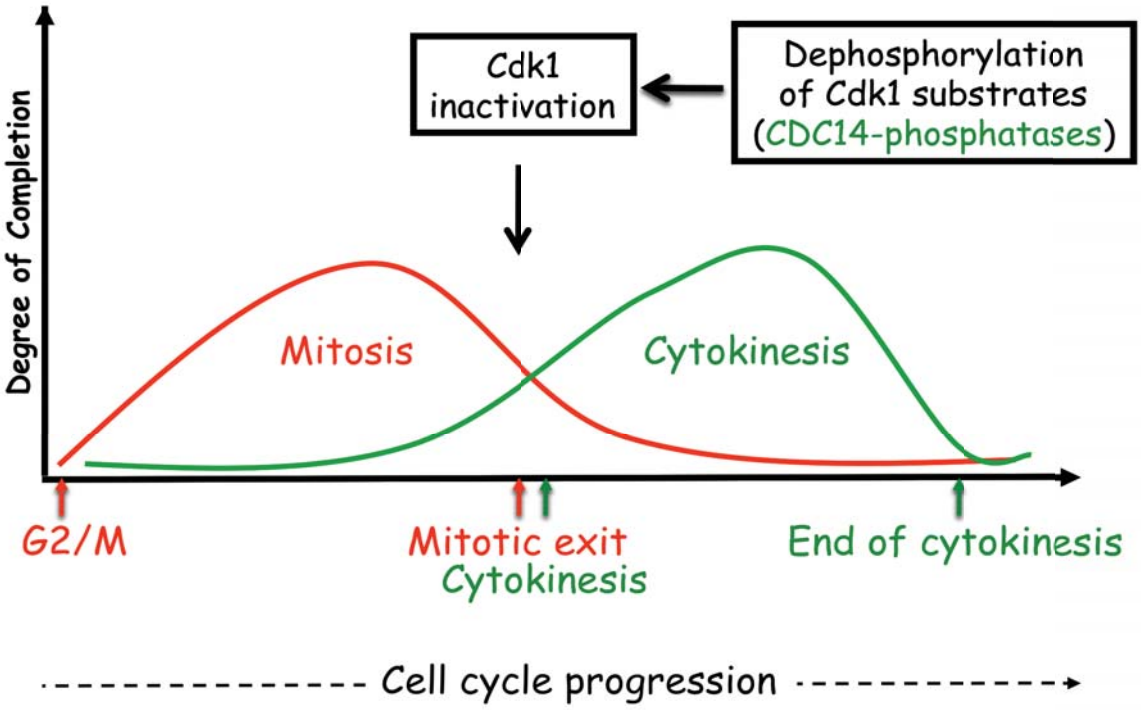


Figure 1-1 Coordination of mitosis and cytokinesis is regulated by oscillation of the cyclin-dependent kinase (CDK).
The X-axis represents the timeline of cell cycle progression. The Y-axis represents the degree of completion of cell cycle events, such as mitosis and cytokinesis. The red line indicates the timely activation of Cdk1, and the green line indicates the timely activation of cytokinesis.

al, 2006; Padte et al., 2006), the Polo-like kinase Plk1 (Paoletti and Chang, 2000; Bähler et al., 1998) and Cdr2 (Almonacid et al., 2009). At the onset of mitosis, Mid1 is released from the nucleus and forms a node-like broad band surrounding the medial cortex for recruiting other ring components. Assembly of the ring proteins occurs in a timely ordered manner (Wu et al., 2003). Myo2 (type II myosin heavy chain), Cdc4 and Rlc1 (regulatory light chain of type II myosin) were first recruited to the broad band (McCollum et al., 1995 Kitayama et al., 1997; Le Goff et al., 2000), followed by three actin regulators: the IQGAP protein Rng2 (Eng et al., 1998), the formin Cdc12 (Chang et al., 1997), and finally the PCH family protein Cdc15 (Fankhauser et al., 1995). Upon actin polymerization, the tropomyosin Cdc8 is recruited (Balasubramanian et al., 1992), followed by α-actinin Ain1 (Wu et al., 2001), and Myp2 (Bezanilla et al., 1997). The proteins were firstly recruited as a broad band, and condensed into a homogenously compacted ring prior to constriction. Upon contraction of the actomyosin ring, synthesis of the division septum occurs at the same time. After the cell wall is built to partition the mother cell, the primary septum and adjacent cell wall is degraded by the action of α- and ß-glucanases (Martin-Cuadrado et al., 2003; Dekker et al., 2004).

The temporal cue provided to initiate cytokinesis in the fission yeast is triggered by activation of the Septation Initiation Network (SIN) that coordinates other mitotic events with cytokinesis (McCollum and Gould, 2001). The SIN is a GTPase regulated kinase cascade that is assembled at the spindle pole body (equivalent to the mammalian centrosome). The SIN is composed of one GTPase (Spg1), four kinases (Plo1, Cdc7,

Sid1 and Sid2), two binding partners of Sid1 and Sid2 (Cdc14 and Mob1, respectively), two scaffold proteins (Sid4 and Cdc11), a binary GTPase-activating protein (Cdc16/Byr4), and a putative GTPase-exchange factor (Etd1) (Krapp et al., 2004; García-Cortes, and McCollum, 2009). The SIN is activated when the upstream SPB-bound Spg1 is brought into proximity with its activator Etd1 at the cell tip during anaphase B, ensuring that cytokinesis is initiated when chromosomes are fully segregated. Once activated, GTP-bound Spg1 recruits the Cdc7 kinase to the newly formed SPB (Shormann et al., 1998). Like Cdc7, Sid1-Cdc14 kinase complex is also recruited to the new SPB in response to Spg1 activation (Guertin et al., 2000). Finally, the activation of the upstream kinases activates the Sid2-Mob1 kinase complex and activated Sid2-Mob1 translocates from the SPB to the cell division site (Sparks et al., 1999). The targets of Sid2-Mob1 kinase complex at the cell division site are still not clear. Other then promoting cytokinesis, the SIN pathway is also important to coordinate the nuclear division cycle and the cell division cycle. When the division apparatus is perturbed, the SIN functions together with a CDK antagonist protein Clp1 (see below) to prevent further rounds of the nuclear division cycle until the cytokinesis is completed (Cueille, et al., 2001; Trautmann et al., 2001; Mishra et al., 2004; Mishra et al., 2006).

Timely activation and inactivation of the mitotic cyclin-dependent kinase (Cdk1) is crucial for cells to maintain the integrity of genomic materials in the daughter cells. Cdc14 family phosphatases play a conserved role to antagonize CDK activity. Cdc14 was first identified in the budding yeast *Saccharomyces cerevisiae* and had multiple

functions that correlate with its localization. It is sequestered in the nucleolus during interphase as part of the RENT complex by binding to its inhibitor Net1/Cfi1 (Visintin et al., 1999; Shou et al., 1999; Traverso et al., 2001). Upon entry into anaphase, Cdc14 is released from the nucleolus by two signaling pathway termed Cdc fourteen Early Anaphase Release (FEAR) network and the Mitotic Exit Network (MEN). FEAR-dependent release of Cdc14 is transient, and important for Cdc14 functions in activation of the MEN, segregation of the rDNA, translocation of chromosomal passenger proteins to the mitotic spindle, and the regulation of anaphase spindle dynamics and the positioning of the nucleus (Stegmeiier and Amon, 2004). Sustained release of Cdc14 from the nucleolus requires MEN activity, and the MEN-dependent release of Cdc14 plays an essential role in promoting cytokinesis and mitotic exit by reversing CDK phosphorylation, promoting mitotic cyclin degradation, and accumulation of the CDK inhibitor Sic1. (Morgan, 1999; Ohi and Gould, 1999; Simanis 2003).

Unlike Cdc14, its homologous protein in fission yeast, Clp1, is released from the nucleolus upon entry into mitosis through an unknown mechanism (Chapter II). As cells progress through mitosis, Clp1 localizes to the kinetochores, contractile actomyosin ring, mitotic spindle and cytoplasm, where it carries out distinct functions (Figure 1-2A) (Cueille et al., 2001; Trautmann et al., 2001; Krapp et al., 2004). It has been reported that the phosphatase activity of Clp1 is inhibited by CDK phosphorylation, and it promotes its own activity by autodephosphorylation (Wolfe et al., 2006). Clp1 has been shown to reverse several CDK phosphorylation events, such as Cdk1 phosphorylation of the

monopolin subunit Mde4, and the kinesin-6 protein Klp9, which are important to regulate chromosome biorientation and promote proper spindle elongation during metaphase-anaphase transition (Choi et al., 2009; Fu et al., 2009). Clp1 also dephosphorylates a PHC family protein Cdc15, and stabilizes the actomyosin ring after being recruited to the actomyosin ring by the scaffold protein, Mid1 (Clifford et al., 2008). In addition, Clp1 antagonizes Cdk1 activity by promoting degradation of the Cdk1 activator Cdc25 phosphatase (Esteban et al., 2004; Wolfe and Gould, 2004). Clp1 facilitates activation of the Septation Initiation Network (SIN) by antagonizing Cdk1 activity, and both function together to promote cytokinesis (Trautmann et al, 2001).

It has been proposed that Clp1 is retained in the cytoplasm through a Sid2-dependent interaction with a 14-3-3 protein Rad24, which becomes especially important during a cytokinesis delay caused by perturbation of the actomyosin ring (Cueille et al, 2001; Trautmann et al., 2001, Mishra et al. 2004). Sid2 kinase is the most downstream component of the SIN pathway (Krapp et al., 2004). In Chapter III, we will describe the molecular mechanism of how Sid2 phosphorylation on Clp1 promotes its cytoplasmic retention through binding to Rad24. Like Clp1, it has been reported that cytoplasmic localization of Cdc14 is regulated by Dbf2 kinase which is the Sid2 homolog in budding yeast. However, the Dbf2 phosphorylation on Cdc14 is not thought to promote 14-3-3 protein binding but inhibits the function of a nuclear localization signal (NLS) at the C-terminus of Cdc14 (Mohl et al., 2009).

The full activation of SIN pathway is triggered only after proper chromosome segregation. This regulatory mechanism prevents the non-segregated chromosomes from being damaged by the constriction of actomyosin ring and the formation of the division septum. During normal cytokinesis, the nuclei stay away from the division apparatus after nuclear division; presumably some other mechanisms function to prevent the segregated nuclei from being damaged by the ingressing septum. Interestingly, if cytokinesis is delayed, the cells maintain active SIN and the nuclei remain apart until cytokinesis is completed. However, if the SIN is inactive, the nuclei are not kept apart, and gradually become clustered in the middle of the cell. This observation suggests that the SIN pathway may function not only to promote continuous repair of the defective ring when cytokinesis is delayed, but also to regulate nuclear positioning to prevent the nuclei being damaged by the septum. How the SIN regulates nuclear positioning during late mitosis is not clear. In other words, the downstream targets of the SIN pathway are not yet identified.

According to the result found in Chapter III, we showed that Sid2 phosphorylation on one of its substrates Clp1 creates binding sites for the 14-3-3 protein Rad24. Furthermore, Clp1, Rad24 and Sid2 kinase colocalize to the spindle pole body and the actomyosin ring in late anaphase (Figure 1-2) (Trautmann et al., 2001; Cueille, et al., 2001; Sparks et al., 1999; Mishra et al., 2005). These observations suggest that Rad24 may bind to more uncharacterized Sid2 substrates at these subcellular localizations, and by purifying Rad24 binding complexes we may identify

uncharacterized Sid2 substrates. In Chapter IV, we used Rad24 as a trap of Sid2 kinase substrates and have identified several potential Sid2 substrates. We were interested in studying two of the Sid2 candidate substrates, Ase1 and Klp2, that could be involved in regulation of nuclear positioning when cytokinesis is delayed. Ase1 is the yeast homolog of PRC1 that functions to bundle anti-parallel microtubules in interphase and mitosis (Loiodice et al., 2005; Yamashita et al., 2005); Klp2 is a minus-end directed kinesin (kinesin-14) that can induce formation of parallel microtubule bundles (Sharp et al., 1997; Braun et al., 2009), and is essential for karyogamy (Troxell et al., 2001; Okazaki and Niwa, 2008). Ase1 and Klp2 are shown to be important for microtubule organization: Ase1 contributes to formation of bipolar microtubule bundles, and Klp2 functions to slide newly formed microtubules that are nucleated from the γ-tubulin complexes along the sides of preexisting microtubules (Janson et al., 2007). Deletion of *ase1* inhibits formation of antiparallel microtubule bundles (Loiodice et al., 2005; Yamashita et al., 2005), whereas deletion of *klp2* inhibits microtubule sliding (Carazo-Salas and Nurse, 2005). Interestingly, loss of *ase1* and *klp2* suppressed the nuclear clustering phenotype in SIN mutants, suggesting that the SIN may negatively regulate these two proteins to prevent nuclear clustering during cytokinesis.

Figure 1-2

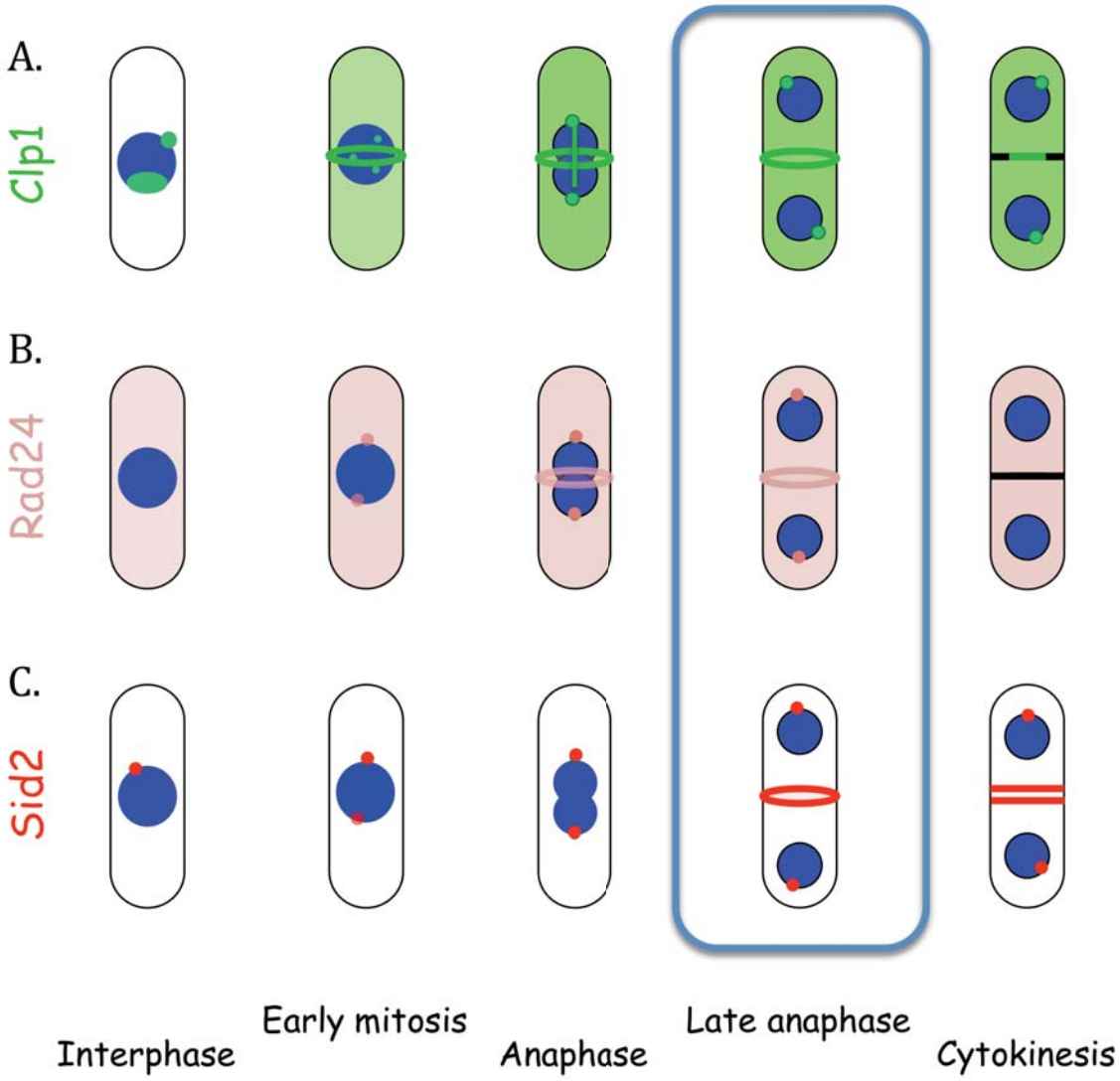


Figure 1-2 Localization of Clp1, Rad24, and Sid2 during mitosis.
This cartoon summarizes the localization of Clp1 (in green), Rad24 (in pink), and Sid2 kinase (in red) during mitosis. The colocalization of these three proteins in late anaphase is circled by a blue square.

CHAPTER II

S. POMBE ORTHOLOGUES OF THE FEAR PROTEINS ARE NOT REQUIRED FOR RELEASE OF CDC14-FAMILY PHOSPHATASE CLP1 FROM THE NUCLEOLUS DURING MITOSIS

Figure 2-3, figure 2-4, table 2-1, and table 2-2 were contributed by Dr. Marie-Pierre Peli-Gulli and Dr. Viesturs Simanis.

Summary

Cdc14-family phosphatases are highly conserved regulators of cell cycle progression. Two of the best studied members of this family are budding yeast Cdc14 and its fission yeast homolog Clp1. The function of both *Saccharomyces cerevisiae* Cdc14 and *Schizosaccharomyces pombe* Clp1 are controlled in part by their regulated sequestration and release from the nucleolus. In the budding yeast *S. cerevisiae* a set of proteins collectively termed the FEAR network promote nucleolar and telomeric DNA segregation by triggering the release of the conserved Cdc14 phosphatase from the nucleolus. Here we show that FEAR homologs in *S. pombe* do not promote release of the Cdc14 homolog Clp1 from the nucleolus, and Clp1 is not required for nucleolar and telomeric DNA segregation suggesting that this aspect of Cdc14 regulation and function may not be universally conserved.

Introduction

Timely activation and inactivation of Cyclin dependent kinases (CDKs) regulate most cell cycle transitions. For example, entry into mitosis requires CDK activation and exit from mitosis and cytokinesis requires loss of CDK activity and dephosphorylation of CDK substrates. In the budding yeast *S. cerevisiae*, the phosphatase Cdc14 seems to be the key phosphatase required to dephosphorylate CDK substrates and promote exit from mitosis and cytokinesis (Jaspersen et al., 1998; Visintin et al., 1998). Cdc14-family phosphatases are conserved in all eukaryotes examined, but have been best studied in yeast (for review see D'Amours and Amon, 2004; Krapp et al., 2004). Budding yeast Cdc14 and its fission yeast homolog Clp1 are regulated in part by their localization, with both proteins thought to be sequestered and inactive in the nucleolus in interphase. They are released from the nucleolus in mitosis, and in late mitosis a conserved signaling pathway *m*itotic *e*xit *n*etwork (MEN) and *s*eptation *i*nitiation *n*etwork (SIN) act to keep Cdc14 and Clp1, respectively, out of the nucleolus (Cueille et al., 2001; Shou et al., 1999; Trautmann et al., 2001; Visintin et al., 1999). In budding yeast, Cdc14 is released from the nucleolus in early anaphase by a separate pathway known as the FEAR network (Cdc-*f*ourteen *e*arly *a*naphase *r*elease), consisting of polo kinase (Cdc5), separase (Esp1), the Esp1-associated protein (Slk19) and Spo12 (Pereira et al., 2002; Stegmeier et al., 2002; Sullivan and Uhlmann, 2003; Yoshida and Toh-e, 2002). FEAR-dependent release of Cdc14 is essential for several mitotic events including proper segregation of rDNA and telomeres (D'Amours et al., 2004; Sullivan et al., 2004; Torres-Rosell et al., 2004). In

contrast to *S. cerevisiae* Cdc14, both *S. pombe* Clp1 (Cueille et al., 2001) (Trautmann et al., 2001) and mammalian Cdc14B (Cho et al., 2005; Mailand et al., 2002; Nalepa and Harper, 2004) are released from the nucleolus upon entry into mitosis, though it is not known how this is regulated. FEAR pathway components (separase/Esp1, polo kinase/Cdc5, Slk19, and Spo12) are conserved in *S. pombe* and other species. Here, we examine whether FEAR pathway components function to promote Clp1 release in *S. pombe*, and whether Clp1 is required for segregation of the nucleolus and telomeric DNA.

Results

To examine the role of FEAR homologs in the early release of Clp1 from the nucleolus in *S. pombe*, we tested whether Clp1p could be released from the nucleolus in mutants defective for homologs of FEAR components. In each case, the *sin* mutant *sid2-250* was also present in each strain, to rule out any influence of the SIN in promoting release of Clp1 from the nucleolus. Cells were synchronized by elutriation, then shifted to 36°C to inactivate *sid2-250* as well as ts alleles of FEAR mutants where used. Release of Clp1 from the nucleolus was monitored over time. We first examined whether the *S. pombe* Polo kinase Plo1 is required for release of Clp1 from the nucleolus. Using the *plo1-25* allele (Figure 2-1C), we found that Clp1p was released normally in cells going through mitosis similar to *sid2-250* control cells (Figure 2-1A). We also tested the *plo1-24C* allele and found similar results (data not shown). We performed similar experiments using cells deleted for *spo12* (Samuel et al., 2000) (Figure 2-1B), *alp7/mia1* (a putative *SLK19* homolog(Oliferenko and Balasubramanian, 2002; Sato et al., 2004)) (Figure 2-1D), and a ts allele of separase (*cut1-205*)(Figure 2-1E). Although there are some differences in the time it takes each strain to enter mitosis because of variation intrinsic to the elutriation synchronization procedure, Clp1 was released normally in each of these mutant backgrounds as the cells entered mitosis (Figure 2-1B-D). As another way to test for a role for separase in Clp1 release from the nucleolus, we induced expression of non-degradable securin Cut2, which inhibits separase, and then scored for Clp1 nucleolar

Figure 2-1

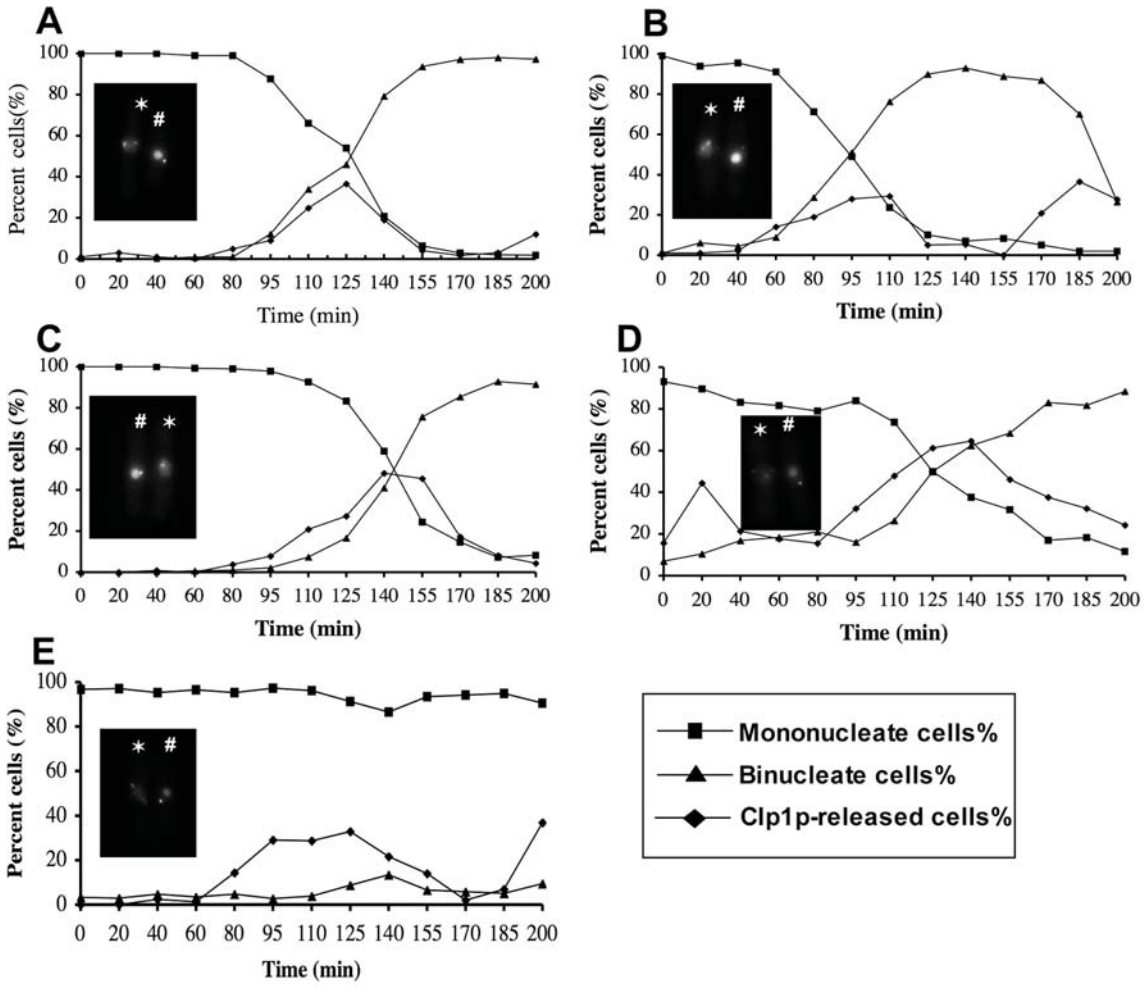


Figure 2-1. Clp1 nucleolar release in cells carrying mutions in FEAR pathway homologs.
sid2-250 clp1-GFP cells (A) or *sid2-250 clp1 -GFP* cells carrying the *spo12*Δ (B), *plo1-25* (C), *alp7*Δ (D), or *cut1-205* (E) mutations were grown at 25°C, then synchronized by centrifugal elutriation. Cells were then shifted to 36°C. Samples were fixed every 20min in methanol, stained with DAPI, and scored for number of nuclei, and Clp1-GFP localization. At least 100 cells were scored for each time point. Inset images show Clp1-GFP signal in cells of each strain with Clp1-GFP released (*) or not released (#).

release in cells with separated SPBs. Expression of non-degradable Cut2 in *sid2-250* cells at the restrictive temperature did not significantly interfere with Clp1 release (74% released) when compared with cells with control plasmid (70% released), further demonstrating that separase is not important for release of Clp1 from the nucleolus.

In budding yeast, overexpression of polo kinase and Spo12 promotes release of Cdc14 from the nucleolus (Shou et al., 2002; Sullivan and Uhlmann, 2003; Visintin et al., 2003; Yoshida and Toh-e, 2002). In *S. pombe*, Clp1 is released from the nucleolus coincident with mitotic entry (Cueille et al., 2001; Trautmann et al., 2001). To examine the effects of overexpression of $plo1^{+}$ and $spo12^{+}$ in *S. pombe*, we arrested cells immediately before mitotic entry using the *cdc25-22* mutation and tested whether overexpression of Plo1 or Spo12 can promote release of Clp1 (Figure 2-2). Cells overexpressing Plo1 (92% nucleolar) and Spo12 (96% nucleolar) did not display increased release of Clp1 from the nucleolus compared with control cells (92% nucleolar). Thus overexpression of Plo1 and Spo12 does not promote nucleolar release of Clp1.

In budding yeast, the FEAR functions to release Cdc14 in early anaphase and this release is required for a number of functions including: M1 exit in meiosis (Buonomo et al., 2003; Marston et al., 2003), nuclear positioning (Ross and Cohen-Fix, 2004), rDNA segregation (D'Amours et al., 2004; Sullivan et al., 2004; Torres-Rosell et al., 2004), MEN activation (Pereira et al., 2002; Stegmeier et al., 2002; Tinker-Kulberg and Morgan,

Figure 2-2

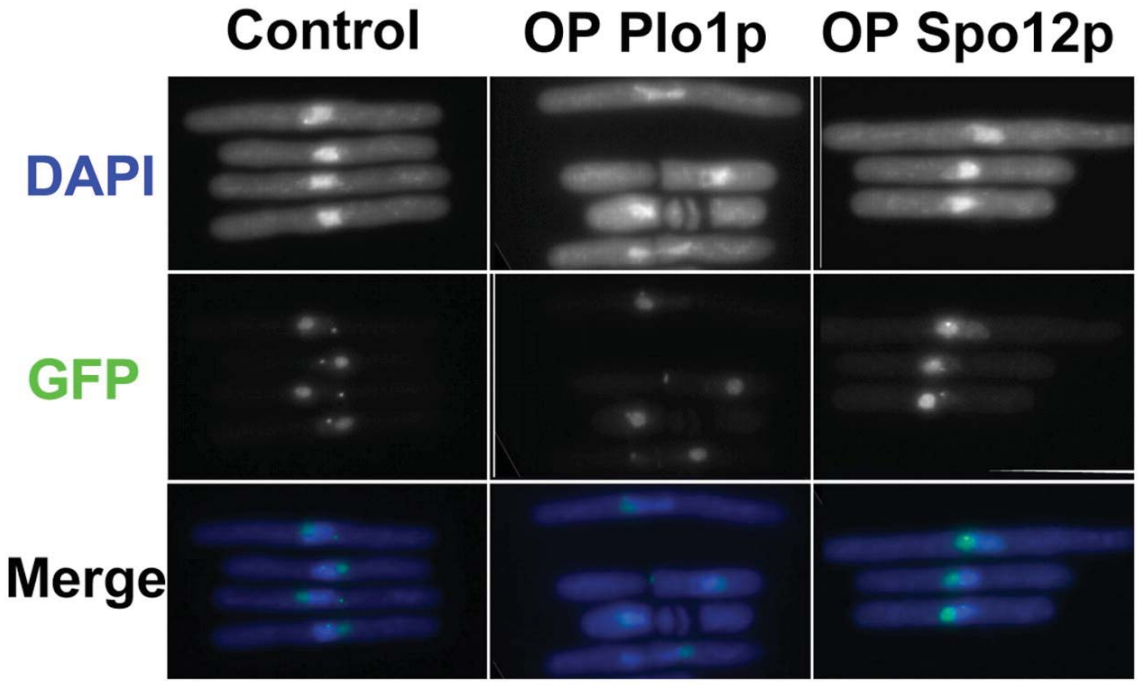


Figure 2-2. Overproduction of Plo1 and Spo12 do not cause release of Clp1.
cdc25-22 clp1-GFP cells carrying either a plasmid expressing *plo1*$^{+}$ or *spo12*$^{+}$ from the thiamine-repressible *nmt1* promoter were grown at 25°C in the absence of thiamine for 12 hours. The cells were then shifted to 36°C for an additional 4 hours in the absence of thiamine to inactivate the Cdc25-22 mutant protein and arrest cells in G2 phase. The cells were then fixed, stained with DAPI, and representative DAPI, GFP, and merged images are shown. Note that the *nmt1* promoter does not become active until 12 hours after removal of thiamine.

1999; Visintin et al., 2003), and passenger protein localization to the spindle (Pereira and Schiebel, 2003). We have examined whether any of these functions may be conserved in *S. pombe*. We find that nuclear positioning, passenger protein localization to the spindle all seem normal in the *clp1* deleted cells (Trautmann et al., 2001; Cueille et al., 2001; Trautmann et al., 2004) (data not shown). As in budding yeast, Clp1 helps activate the SIN, and *sin clp1*Δ double mutants display negative interactions (Trautmann et al., 2001; Cueille et al., 2001). However, we did not observe any synthetic interactions between FEAR components and the SIN (data not shown). Therefore these proteins may not contribute to the ability of Clp1 to activate the SIN as is observed in *S. cerevisiae*.

Because FEAR-dependent release of Cdc14 is essential for progression from meiosis I to meiosis II, we examined whether Clp1 was similarly important for meiotic progression in *S. pombe*. Meiotic progression in wild-type and *clp1*Δ cells was initiated in diploid cells using the *pat1-114* mutation. This experiment showed that homozygous *clp1*Δ cells progressed through meiosis I and II with almost identical kinetics to that of wild-type cells (Figure 2-3A, B). In addition, self-matings between h^+ and h^- wild-type or h^+ and h^- *clp1*Δ cells showed similar numbers of four-spored asci (Figure 2-3C, D). The slight decrease in complete asci in *clp1*Δ cells may reflect weakened SIN signaling in the *clp1*Δ cells, since SIN signaling is important for spore formation (Krapp et al., 2006). In both experiments, *clp1*Δ cells showed a slight but reproducible increase in the number of asci with two or three nuclei (Figure 2-3A, B, D), suggesting that similar to mitosis, Clp1p has a role in the fidelity of the process. In addition, examination of Clp1p

Figure 2-3

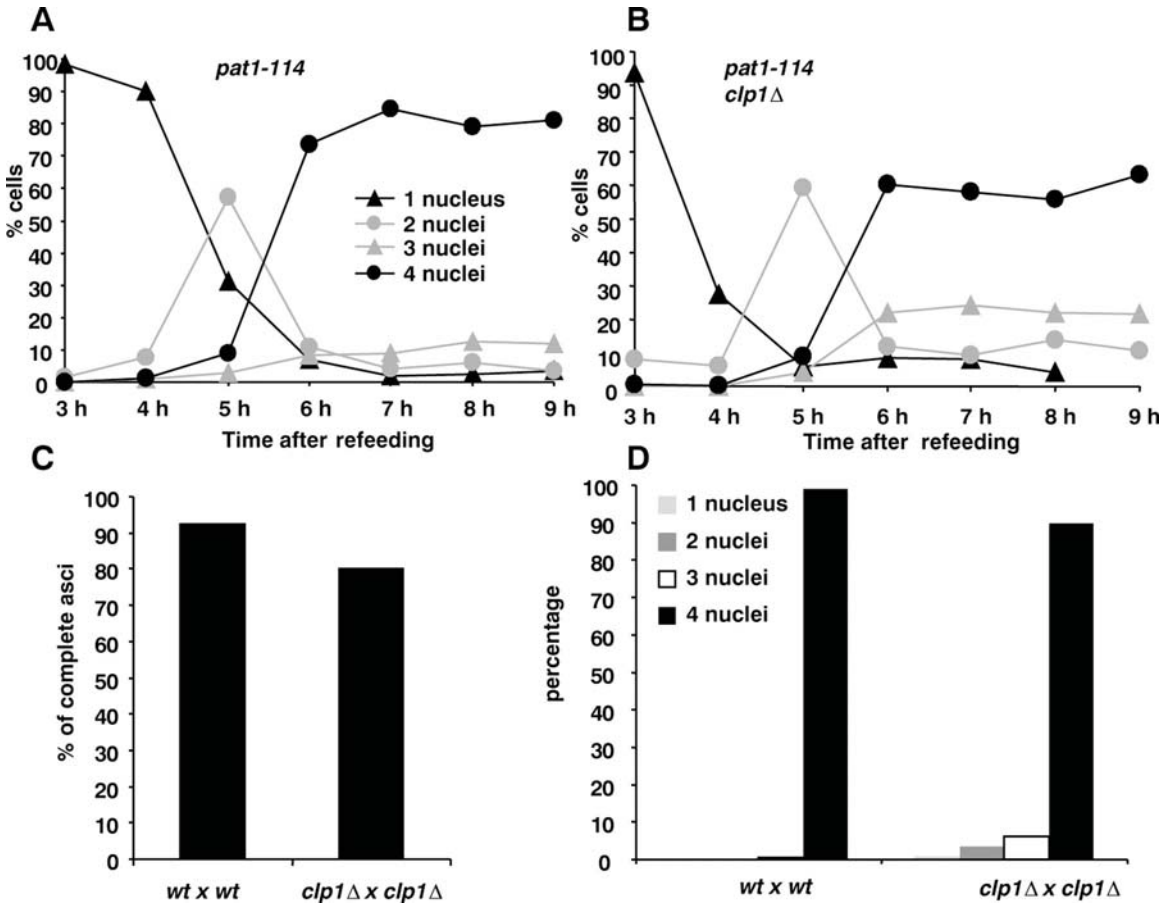


Figure 2-3. Meiosis and spore formation in *clp1Δ* cells.
(A, B) Diploid cells of the genotype h^+/h^+ *ade6-M210/ade6-M216 pat1-114/pat1-114* and h^+/h^+ *ade6-M210/ade6-M216 pat1-114/pat1-114 clp1::kanMX6/clp1::kanMX6* were grown to mid-exponential phase and then transferred to minimal medium without ammonium chloride to starve cells in G1. Cells were inoculated into complete medium at 33°C to induce meiosis. Samples were fixed at intervals and the number of nuclei per cell was determined. The key shown in A also applies to B. (C, D) Wild-type h^+ and h^- cells were mated on minimal medium lacking ammonium chloride. Cells were taken from the mating mixture and the percentage of complete asci (C) and number of nuclei per meiotic cell was determined (D).

localization in meiosis showed no difference between wild-type and *spo12*Δ cells (data not shown). Together, these and previous results (Samuel et al., 2000) show that Clp1 and Spo12 do not play an essential role in meiotic progression in fission yeast as observed in budding yeast.

We next examined whether Clp1 functioned in rDNA and telomere segregation. Nucleolar and telomere segregation was monitored using Nuc1-GFP to label the nucleolus, and a LacO array integrated at the *sod2* locus near the telomere in cells expressing LacI-GFP (Ding et al., 2004). The separation of each signal was analyzed by comparing the amount of time after SPB separation (mitotic entry) before separation of the GFP signals, as well as the distance between SPBs when the nucleolar or telomere GFP signals separate. Interestingly, Nuc1-GFP signals separated at almost the same time post SPB separation. In addition, when the SPBs were separated, the same overall distance was measured in wild-type and *clp1Δ* cells (Figure 2-4, Table 2-1). Similarly, telomere separation was not delayed relative to wild-type cells in *clp1Δ* cells (Table 2-2). These results show that Clp1 does not play a significant role in segregation of the nucleolus and telomeres.

Table 2-1

	Time of Nuc1-GFP Separation (min.)	SPB separation at Nuc1-GFP separation (μm)
wt	13.97 +/- 0.36 (18)	4.49 +/- 0.05
*clp1*Δ	13.76 +/- 0.76 (28)	4.45 +/- 0.06

Table 2-1. Segregation of nucleolar markers in *clp1*Δ and wild-type cells.
Cells expressing Cdc11-GFP to label spindle pole bodies, and Nuc1-GFP to label the nucleolus, were grown at 24°, synchronized by centrifugal elutriation and examined using time-lapse microscopy. The time of Nuc1-GFP separation was measured from the time of SPB (Cdc11-GFP) separation. The distance between SPBs, in micrometers, at the time of nucleolar separation is also measured. The number of cells analyzed is shown in parenthesis. The standard error of the mean is shown for each measurement.

Table 2-2

	Time of telomere Separation (min.)	SPB separation at telomere separation (μm)
wt	31.3 +/- 0.85 (20)	4.5 +/- 0.1
*clp1*Δ	26.2 +/- 1.3 (11)	4.4 +/- 0.59

Table 2-2. Segregation of telomeres in *clp1*Δ and wild-type cells.
Cells expressing Cdc11-GFP to label spindle pole bodies, and LacI-GFP in cells containing a LacO array integrated at the *sod2* locus near the telomere were grown at 21.5°, synchronized by centrifugal elutriation and examined using time-lapse microscopy. The time of telomere (LacI-GFP) separation was measured from the time of SPB (Cdc11-GFP) separation. The distance between SPBs, in micrometers, at the time of telomere separation is also measured. The number of cells analyzed is shown in parenthesis. The standard error of the mean is shown for each measurement.

Figure 2-4

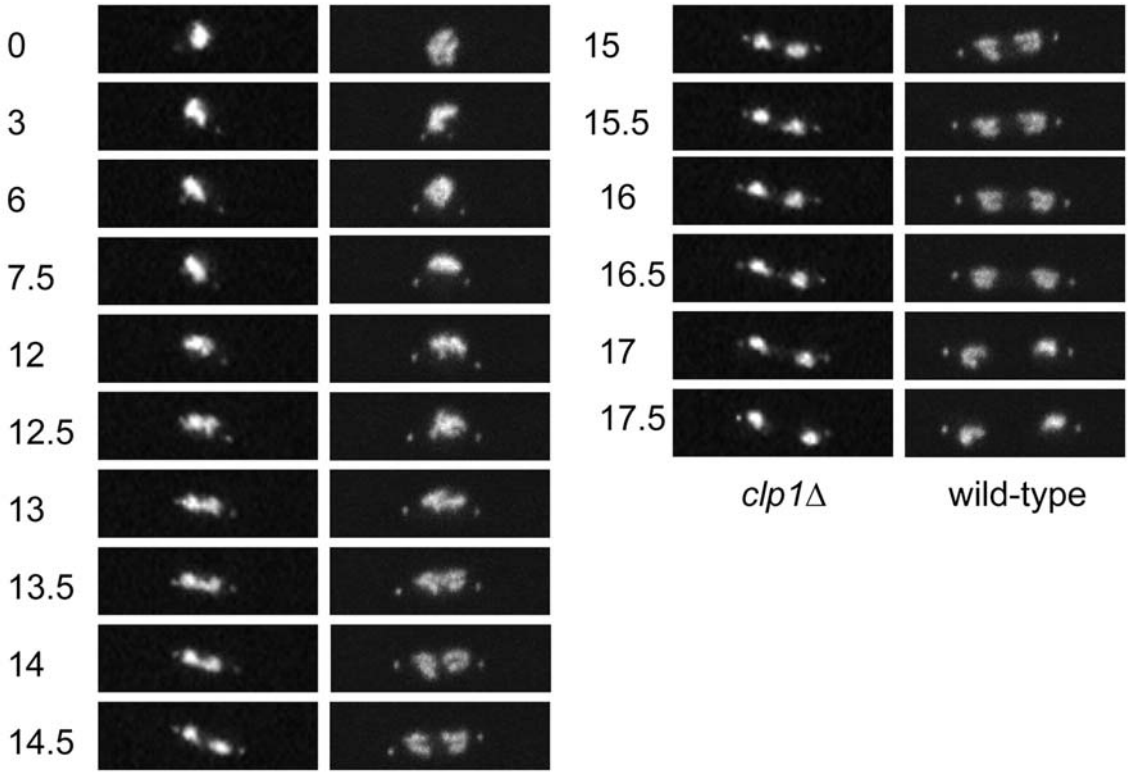


Figure 2-4. Segregation of a nucleolar marker (Nuc1-GFP) in *clp1Δ* cells.
Time-lapse series of wt and *clp1Δ* cells expressing the nucleolar marker Nucl-GFP and the spindle pole body marker Cdc11-GFP. Stacks of 11 *z*-sections of 0.5 μm were taken at 30-second intervals and projected as 2D images. Cells are shown at the indicated times. The first time SPBs labeled with Cdc11-GFP appeared as separate dots was defined as time zero.

Discussion

Studies in yeast suggest that a key mechanism for Cdc14 phosphatase regulation is through regulated nucleolar sequestration (for review see D'Amours and Amon, 2004; Krapp et al., 2004). Thus it is important to understand how their nucleolar localization is regulated. Two conserved signaling networks in budding and fission yeast, the MEN and SIN respectively seem to play a conserved role in maintaining the phosphatase outside the nucleolus in late mitosis. By contrast, initial release of the phosphatase from the nucleolus seems to be governed differently. In budding yeast the FEAR pathway and Cdk1 promotes release of Cdc14 from the nucleolus in early anaphase (Azzam et al., 2004; Pereira et al., 2002; Stegmeier et al., 2002; Sullivan and Uhlmann, 2003; Yoshida and Toh-e, 2002). However the *S. pombe* Cdc14 homolog Clp1 is released in early mitosis, and as we show here, this release does not depend on homologs of the FEAR network. In budding yeast, FEAR-dependent release of Cdc14 is important to allow Cdc14 to function in segregation of the rDNA, nucleolus and telomeres (D'Amours et al., 2004; Sullivan et al., 2004; Torres-Rosell et al., 2004). However, we found that Clp1 is not essential for these functions in *S. pombe*. One reason for the additional functions of Cdc14 in anaphase might be that budding yeast maintains high Cdk activity through anaphase unlike most other eukaryotes, which lose Cdk activity upon anaphase onset. Therefore dephosphorylation of mitotic CDK substrates by Cdc14 may be especially important for anaphase events in budding yeast. Although the FEAR pathway does not play a conserved role in regulating the Cdc14 homolog in *S. pombe*, it remains a possibility that Cdk activity might play a conserved role in promoting release of the

phosphatase from the nucleolus in both organisms. Given the similar timing of release from the nucleolus of *S. pombe* Clp1 (Cueille et al., 2001; Trautmann et al., 2001) and human Cdc14B (Cho et al., 2005; Mailand et al., 2002; Nalepa and Harper, 2004) it seems likely that they may be regulated through a conserved, FEAR-independent pathway.

CHAPTER III

THE SIN KINASE SID2 REGULATES CYTOPLASMIC RETENTION OF THE CDC14-LIKE PHOSPHATASE CLP1 IN *S. POMBE*

Figure 3-1A-B were contributed by Dr. Young-Sam Shim.
Figure 3-1C-G were contributed by Dr. Anna Feoktistova, Jun-Song Chen, Dawn M. Clifford, and Dr. Kathleen L. Gould

Summary

Cdc14-family phosphatases play a conserved role in promoting mitotic exit and cytokinesis by dephosphorylating substrates of cyclin dependent kinase (Cdk). Cdc14-family phosphatases have been best studied in yeast (for review see D. D'Amours and A. Amon, 2004; Krapp et al., 2004), where budding yeast Cdc14 and its fission yeast homolog Clp1 are regulated in part by their localization, with both proteins thought to be sequestered in the nucleolus in interphase. Cdc14/Clp1 are released from the nucleolus in mitosis, and in late mitosis a conserved signaling pathway termed the MEN/SIN acts through an unknown mechanism to keep Cdc14 and Clp1 respectively out of the nucleolus (Shou et al., 1999; Visintin et al., 1999; Cueille et al., 2001; Trautmann et al., 2001). Here we show that the most downstream SIN component, the Ndr-family kinase Sid2, acts to maintain Clp1 in the cytoplasm in late mitosis by phosphorylating Clp1 directly and thereby creating binding sites for the 14-3-3 protein Rad24. Mutation of the Sid2 phosphorylation sites on Clp1 disrupts the interaction between Clp1 and Rad24, and causes premature return of Clp1 to the nucleolus during cytokinesis. Loss of Clp1 from the cytoplasm in telophase renders cells sensitive to perturbation of the actomyosin ring, but does not affect other functions of Clp1. Because all components of this pathway are conserved, this might be a broadly conserved mechanism for regulation of Cdc14-family phosphatases.

Introduction

Coordination of mitosis and cytokinesis is important to maintain genomic stability in every cell cycle. To ensure production of daughter cells with correct ploidy, cytokinesis must occur after proper chromosome segregation, and occur only once every cell cycle. Therefore, the timely activation and inactivation of CDKs (cycling-dependent kinases) becomes crucial to coordinate several cellular events (Bloom and Cross, 2007). Cdc14-family phosphatases are evolutionarily conserved among eukaryotes and function to antagonize CDK activity by reversing CDK phosphorylation. In budding yeast, Cdc14 is essential to promote mitotic exit and its homolog in fission yeast, Clp1, plays an important role in promoting cytokinesis (D. D'Amours and A. Amon, 2004; Krapp et al., 2004). Both Cdc14 and Clp1 phosphatase activity are regulated in part by subcellular localization, which is thought to involve sequestration in the nucleolus during interphase, and released into the nucleus and cytoplasm during mitosis. A conserved signaling pathway termed mitotic exit network (MEN), and septation initiation network (SIN) function to keep Cdc14 and Clp1, respectively, out of the nucleolus in late mitosis. However, the regulatory mechanism is unclear at the molecular level.

It has been shown that Clp1 is kept in the cytoplasm by the nuclear-cytoplasmic transport protein Rad24 (Mishra et al., 2005; Trautmann and McCollum, 2005). Rad24 is a 14-3-3 protein, which is known to bind phosphopeptides, particularly the RXXpS motif (Yaffe et al., 1997). Interestingly, Clp1 and Rad24 showed direct interaction, and this

interaction depends on the most downstream SIN kinase Sid2 (Mishra et al, 2005), whose its consensus phosphorylation site has been identified as RXXS (Mah et al., 2005). In this study, we describe a molecular mechanism by which the SIN promotes retention of Clp1 in the cytoplasm through modification of these RXXS sites.

Results

Rad24 Binding to Clp1 Depends on Sid2 Phosphorylation of Clp1

Despite considerable work on the SIN/MEN pathways in fission and budding yeast the key question of how each pathway acts to keep its respective Cdc14-family phosphatase out of the nucleolus has remained unknown. Previous studies showed that in late mitosis the SIN maintains Clp1 in the cytoplasm until cytokinesis is completed by regulating the nuclear shuttling of Clp1, perhaps through the action of the 14-3-3 protein Rad24 (Mishra et al., 2005; Trautmann and McCollum, 2005). Binding of Rad24 to Clp1 depends on the most downstream SIN pathway kinase Sid2 (Mishra et al., 2005). 14-3-3 proteins are known to bind phosphopeptides, particularly the RXXpS motif (Yaffe et al., 1997), and RXXpS matches the predicted consensus phosphorylation site for Sid2 family kinases (Mah et al., 2005). Because Rad24 is restricted to the cytoplasm, we hypothesized that Sid2 phosphorylation of Clp1 might allow Rad24 to bind to and retain Clp1 in the cytoplasm. Therefore, we tested whether Sid2 could phosphorylate Clp1 directly, and whether Sid2 phosphorylation of Clp1 created binding sites for the 14-3-3 protein Rad24. We found that Sid2 kinase purified by tandem affinity purification (TAP) from yeast cells was capable of directly phosphorylating bacterially produced Clp1 (Figure 3-1A). Furthermore, Clp1 only bound Rad24 when it had been pre-phosphorylated by Sid2 kinase (Figure 3-1B).

Figure3-1

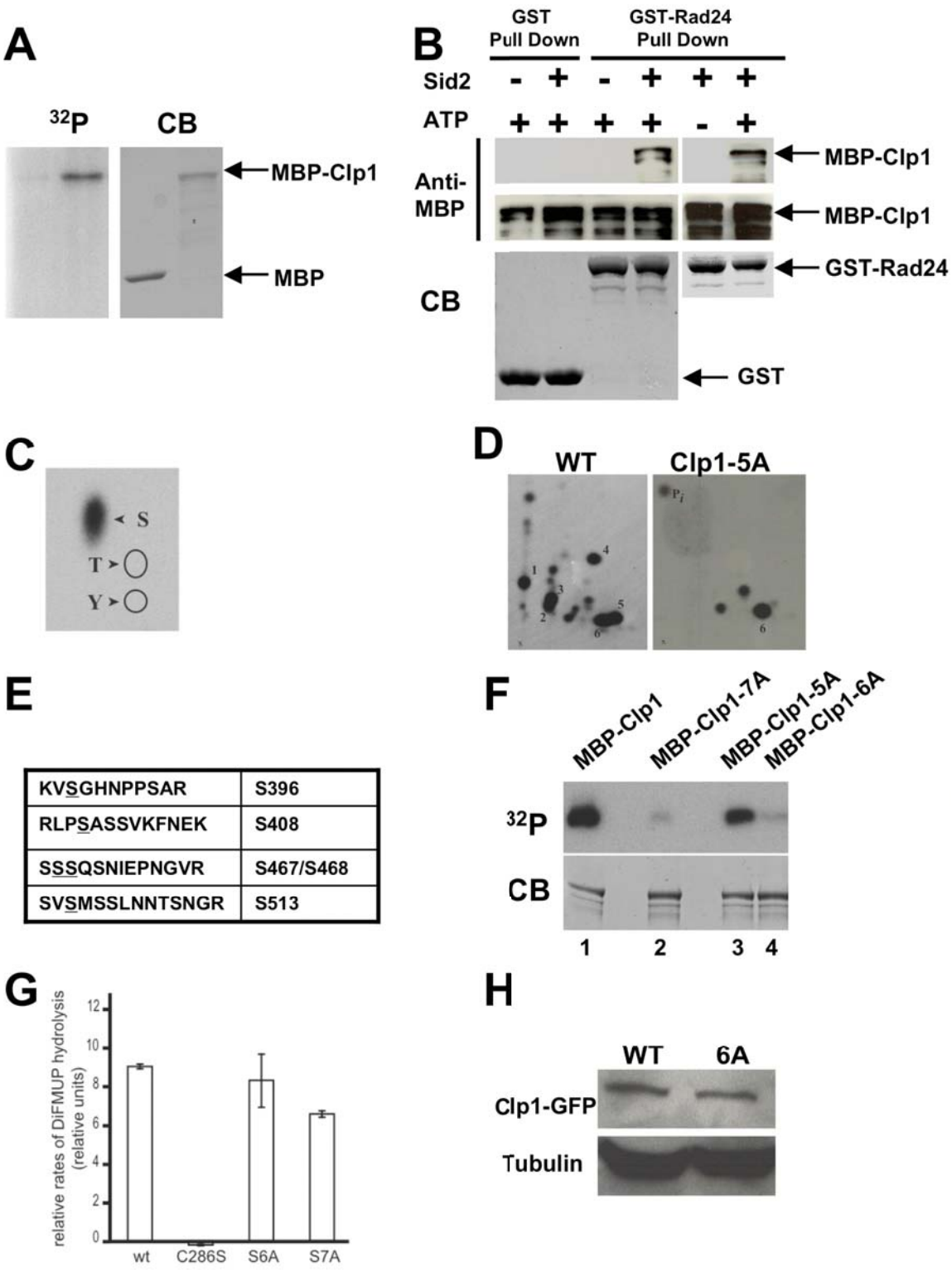

A
32P
CB
MBP-Clp1
MBP
B
GST Pull Down
GST-Rad24 Pull Down
Sid2
ATP
Anti-MBP
MBP-Clp1
MBP-Clp1
GST-Rad24
CB
GST
C
S
T
Y
D
WT
Clp1-5A
E
KVSGHNPPSAR S396
RLPSASSVKFNEK S408
SSSQSNIEPNGVR S467/S468
SVSMSSLNNTSNGR S513
F
MBP-Clp1
MBP-Clp1-7A
MBP-Clp1-5A
MBP-Clp1-6A
32P
CB
1 2 3 4
G
relative rates of DiFMUP hydrolysis (relative units)
wt
C286S
S6A
S7A
H
WT
6A
Clp1-GFP
Tubulin

Figure 3-1. Sid2 phosphorylation of Clp1 promotes binding of Rad24 (14-3-3) to Clp1 in vitro.

(A) In vitro kinase assays (Sparks et al., 1999) were performed by using Sid2 kinase complexes from TAP (tandem-affinity purification) eluates from *S. pombe* cells, and bacterially expressed MBP-Clp1. Protein labeled by γ-^{32}P was detected using a Phospho Imager (Molecular Dynamics), and the gel was stained with Coomassie Blue (CB) as loading control. (B) MBP-Clp1 was pre-incubated with Sid2 kinase in the presence or absence of unlabeled ATP, and then incubated with bacterial lysates expressing GST or GST-Rad24. Glutathione sepharose resin was added, and the precipitates were detected by Western blot using anti-MBP antiserum (New England BioLabs). (C) Phosphoamino acid analysis of MBP-Clp1 phosphorylated by Sid2 kinase. The positions of the phospho-threonine and phospho-tyrosine standards are indicated by circles. (D) Phospho-tryptic peptide analysis of MBP-Clp1 and MBP-Clp1-5A phosphorylated by Sid2 kinase. The positions of six major phosphopeptides are numbered. The position of the origin was indicated with an "x". The anode is on the left. (E) In vitro phosphorylation sites of Clp1 by Sid2 kinase identified by mass spectrometry are listed. (F) MBP-Clp1, MBP-Clp1-5A, MBP-Clp1-6A, and MBP-Clp1-7A were purified from bacterial lysates, and phosphorylated with Sid2 kinase purified using anti-Myc antibody from *cdc16-116 sid2-13Myc* cells. (G) Phosphatase activity of MBP-Clp1, MBP-Clp1-C286S (phosphatase inactive allele), MBP-Clp1-6A, and MBP-Clp1-7A were determined by their ability to hydrolyze DiFMUP (6,8-difluoro-4-methylumbelliferyl phosphate) (Wolfe et al., 2006). Reactions were performed in triplicate for standard error analysis. Data are representative of two independent experiments. (H) Cell lysates of *clp1-GFP* and *clp1-6A-GFP* were prepared in NP-40 buffer (supplemental methods). The Clp1-GFP and tubulin protein levels were determined by Western blot using anti-GFP (Santa Cruz Biotechnology), and anti-TAT1 antibodies.

To ascertain the significance of Clp1 phosphorylation by Sid2 in vivo, we sought to identify and mutate sites on Clp1 phosphorylated by Sid2. Phosphoamino acid analysis of in vitro phosphorylated Clp1 showed that it was phosphorylated exclusively on serine residues (Figure 3-1C). In vitro phosphorylated Clp1 was analyzed by two-dimensional phosphopeptide mapping, which identified 6 major tryptic peptides and a number of less abundant spots (Figure 3-1D). Analysis of in vitro phosphorylated Clp1 using mass spectrometry identified 5 sites of phosphorylation in Clp1 that were all within the C-terminal half (Figure 3-1E). Analysis of Clp1 purified from yeast cells using mass spectrometry identified the same 5 sites (Figure 3-S1). Mutation of the 5 sites to alanine (Clp1-5A) significantly reduced the overall levels of Clp1 phosphorylation in vitro (Figure 3-1F, lane 3) and eliminated 5 of the 6 major tryptic phosphopeptides (Figure 3-1D). Through a combination of mutagenesis of additional sites followed by in vitro phosphorylation and 2 dimensional phosphopeptide analyses, we identified serine 493 as the last remaining site of significant phosphorylation. Mutation of S493 in addition to the previously identified 5 sites (Clp1-6A) eliminated the last major phosphopeptide, and caused almost complete elimination of phosphorylation of Clp1 by Sid2 in vitro (Figure 3-1F, lane 4, and data not shown). Mutation of any site singly, including S493, did not cause a major reduction in Clp1 phosphorylation in vitro, or binding to Rad24 in vitro (data not shown), suggesting that no single site is crucial. Bacterially expressed Clp1-6A retained wild-type in vitro phosphatase activity suggesting that the mutations did not grossly affect the structure of the protein (Figure 3-1G). All 6 sites of phosphorylation fit the consensus RXXS motif predicted for Sid2 family kinases (Mah et al., 2005).

Mutation of an additional single RXXS motif at amino acid 499 (Clp1-7A) did not cause further reduction of overall level of phosphorylation (Figure 3-1F, lane 2), and resulted in reduced in vitro phosphatase activity of recombinant Clp1 and therefore was not pursued further (Figure 3-1G).

Loss of Sid2 Phosphorylation Sites in Clp1 Causes Premature Return of Clp1 to the Nucleolus in Late Mitosis and Failure of Rad24 Binding

To determine the role of Clp1 phosphorylation by Sid2, Clp1-6A-GFP was integrated into the *clp1*$^{+}$ locus such that it was expressed from the endogenous promoter, and was the only expressed copy of *clp1* in the cell. The level of Clp1-6A protein was similar to wild-type Clp1 (Figure 3-1H and data not shown). Like wild-type Clp1-GFP, Clp1-6A-GFP localized in interphase to the SPB and nucleolus, was released from the nucleolus as cells enter mitosis, and localized to the kinetochores and actomyosin ring in early mitosis (Figure 3-S2A). In anaphase cells Clp1-6A-GFP localized to the spindle, often appearing somewhat brighter than wild-type Clp-GFP (Figure 3-2A and Figure 3-S2A). In telophase cells where the spindle has broken down but cells have not completed cytokinesis, wild-type Clp1 remained out of the nucleolus in the cytoplasm and faintly at the contractile ring until cytokinesis was completed. In contrast, Clp1-6A appeared to return to the nucleolus prematurely and was observed only faintly if at all in the contractile ring (Figure 3-S2A-B). To examine the timing of Clp1-6A release more carefully, we performed time-lapse analysis of Clp1-GFP and Clp1-6A-GFP cells

Figure 3-2

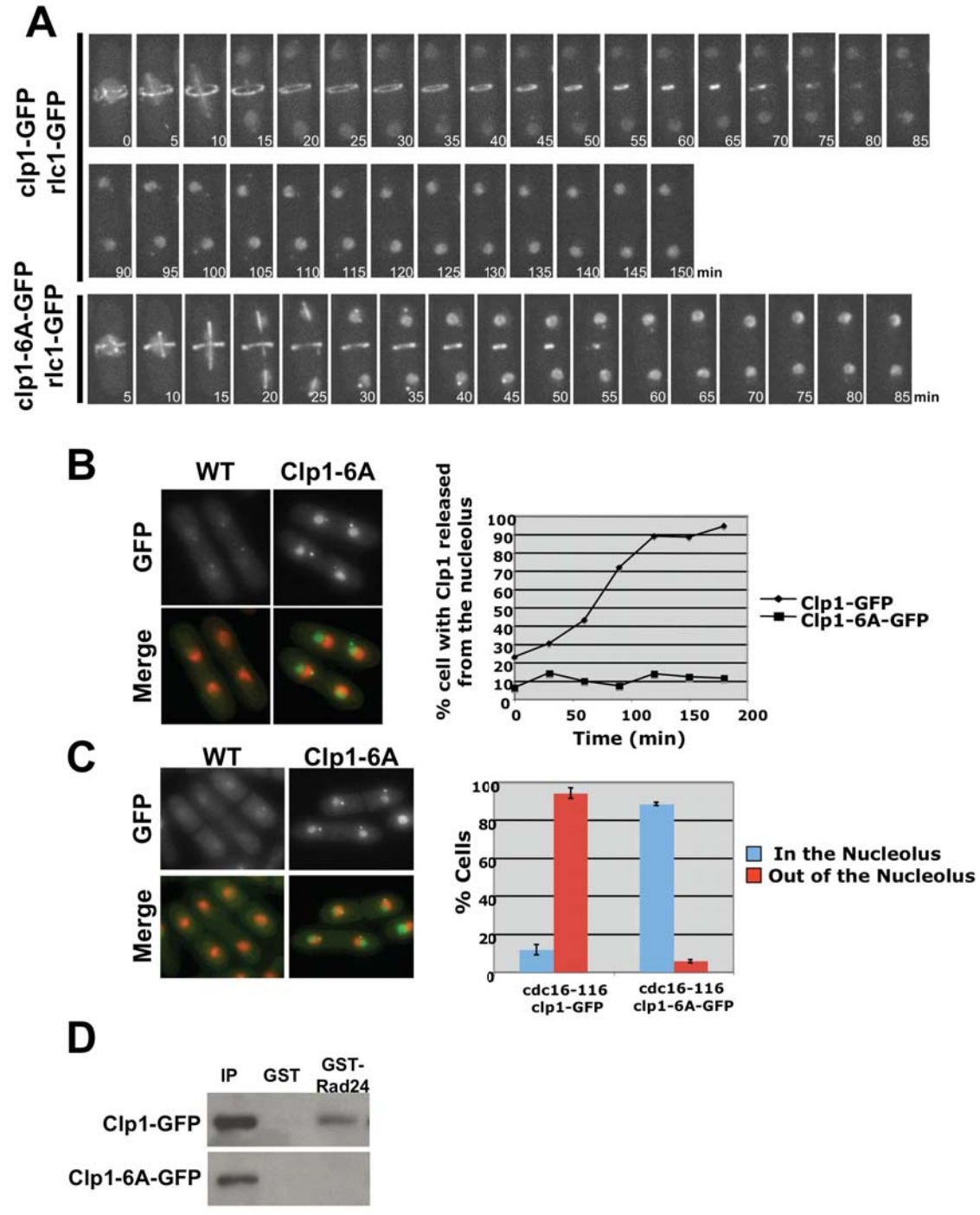

A
clp1-GFP rlc1-GFP
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85
90 95 100 105 110 115 120 125 130 135 140 145 150 min
clp1-6A-GFP rlc1-GFP
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 min
B
WT
Clp1-6A
GFP
Merge
% cell with Clp1 released from the nucleolus
Time (min)
Clp1-GFP
Clp1-6A-GFP
C
WT
Clp1-6A
GFP
Merge
% Cells
In the Nucleolus
Out of the Nucleolus
cdc16-116 clp1-GFP
cdc16-116 clp1-6A-GFP
D
IP
GST
GST-Rad24
Clp1-GFP
Clp1-6A-GFP

Figure 3-2. The *clp1-6A* mutation disrupts SIN regulation of Clp1 nucleolar localization.
(A) Time-lapse images of *clp1-GFP* and *clp1-6A-GFP* cells both expressing Rlc1-GFP as an actomyosin ring marker were collected every 5 minutes, using a spinning disc confocal microscope. Ten stacks of images were captured for each time point, with a step size of 0.55 μm between focal planes. Nucleolar to cytoplasmic ratios were calculated and shown in Figure 3-S2C. (B) *clp1-GFP* and *clp1-6A-GFP* cells were grown to mid-log phase and treated with 4μM Latrunculin B (Sigma). Cells were collected every 30 minutes, and subjected to methanol fixation and DAPI staining (shown in red). Localization of Clp1-GFP and Clp1-6A-GFP are shown after 180 min (left panel). Cells with nucleolar or dispersed GFP localization were quantified over time (right panel). (C) *clp1-GFP* and *clp1-6A-GFP* in a *cdc16-116* temperature sensitive background were cultured to mid-log phase at 25°C, then shifted to 36°C for 2 hr. The cells were subjected to methanol fixation and DAPI staining (shown in red). Quantification of nucleolar or dispersed localization of Clp1-GFP and Clp1-6A-GFP was scored in binucleate septated *cdc16-116* cells. (D) Protein lysates prepared from *clp1-GFP* and *clp1-6A-GFP* cells grown at 30°C were split 3 ways. Clp1 was immunoprecipitated from one sample (IP) using a mouse monoclonal anti-GFP antibody (Molecular Probes), and the other 2 samples were mixed with bacterially produced GST (GST), or GST-Rad24 (GST-Rad24) (supplemental methods). The complexes were precipitated with glutathione sepharose resin and probed, along with the immunoprecipitated sample, by Western blot using anti-GFP antibodies.

expressing a marker for the actomyosin ring (Rlc1-GFP) (Figure 3-2A) and quantified the nucleolar/cytoplasmic ratios of the GFP signal (Figure 3-S2C). This analysis showed that Clp1-6A re-accumulated in the nucleolus as soon as the spindle broke down prior to actomyosin ring constriction (Figure 3-2A (30 min.), and Figure 3-S2C). In contrast wild-type Clp1 did not re-accumulate in the nucleolus until 75 minutes later after the actomyosin ring had finished constriction and disappeared (Figure 3-2A (95 min.), and Figure 3-S2C).

When cytokinesis is perturbed by low doses of the actin depolymerizing drug Latrunculin B, Clp1 remains cytoplasmic during the resulting cytokinesis delay (Figure 3-2B). In contrast, Clp1-6A returns to the nucleolus (Figure 3-2B, Figure 3-S3A). This relocalization is similar to the behavior of wild-type Clp1 in SIN mutants (Trautmann et al., 2001). Interestingly, unlike *clp1*Δ cells, and like wild-type cells, *clp1-6A* cells halt further rounds of nuclear division when cytokinesis is delayed by Latrunculin B treatment and remain in a binucleate state with interphase microtubules (Figure 3-S3B-C). Similarly, when the cytokinesis checkpoint is activated using the *cps1-191* mutant defective in septum assembly and actomyosin ring constriction (Liu et al., 2000), *clp1-6A cps1-191* mutant cells arrest like *cps1-191* single mutant cells at restrictive temperature as binucleates with active SIN, interphase microtubules, and actomyosin rings consistent with the cytokinesis checkpoint being intact in *clp1-6A* cells (Figure 3-S4).

We previously showed that when the SIN is constitutively activated in telophase by inactivating a component of its GTPase activating protein, Cdc16, cells undergo repeated rounds of cytokinesis and Clp1 persists in the cytoplasm once it is released from the nucleolus in the first mitosis (Trautmann et al., 2001). However, constitutive activation of the SIN in telophase is unable to keep Clp1-6A in the cytoplasm, and the mutant protein returns to the nucleolus (Figure 3-2C). Interestingly Clp1-6A-GFP appears to localize more strongly to the SPB than the wild-type protein in both *cdc16* cells and cells arrested by the cytokinesis checkpoint (Figure 2B-C). Since the SIN is active in both situations, it suggests that the SIN may antagonize both nucleolar and SPB localization of Clp1.

We also expected that loss of Sid2 phosphorylation sites on Clp1 would disrupt binding of Rad24 to Clp1. To test this hypothesis, we examined whether bacterially produced GST-Rad24 would bind to Clp1-6A from yeast lysate. Unlike wild-type Clp1, Clp1-6A failed to bind to Rad24 (Figure 3-2D, Figure 3-S5) suggesting that the cause of premature return of Clp1-6A to the nucleolus might be loss of Rad24 binding. To try to make a phosphomimetic version of Clp1 that bound Rad24 independently of Sid2, we mutated the six Sid2 phosphorylation sites on Clp1 to aspartate residues, generating *clp1-6D*. However Clp1-6D did not bind Rad24 (data not shown) suggesting that aspartic acid residues cannot substitute in Clp1 for phosphorylated serines for 14-3-3 binding. The *clp1-6D* cells also displayed a general loss of function phenotype (data not shown)

indicating that the asparate mutations caused defects in the structure of the protein and therefore this mutant was not analyzed further (data not shown).

Absence of Clp1 from the Cytoplasm Causes Defects in Cytokinesis

The *clp1-6A* mutant allowed us to test the function of SIN mediated retention of Clp1 in the cytoplasm during telophase. We assayed whether the *clp1-6A* strain displayed any of the defects found in *clp1*Δ cells. Clp1 has roles in chromosome segregation, cytokinesis, the cytokinesis checkpoint, and regulation of cell size (Cueille et al., 2001; Trautmann et al., 2001; Mishra et al., 2004; Trautmann et al., 2004). Unlike *clp1*Δ, the *clp1-6A* mutation does not have negative interactions with mutations in genes involved in chromosome segregation such as *dis1* (Figure 3-S6A, and data not shown). Clp1 negatively regulates Cdc25 explaining both why *clp1*Δ cells have a reduced cell size and why overexpression of Clp1 causes a block in mitotic entry and cell elongation (Cueille et al., 2001; Trautmann et al., 2001; Wolfe and Gould, 2004; Esteban et al., 2004). Clp1-6A presumably is able to regulate Cdc25 normally since *clp1-6A* cells have a wild-type cell size and overexpression of Clp1-6A blocks mitotic entry like wild-type Clp1 (Figure 3-S6B-C). As shown earlier, *clp1-6A* is also wild type for the cytokinesis checkpoint. It has been previously shown that the main function of Clp1 in the cytokinesis checkpoint is to promote SIN activity (Mishra et al., 2004). Consistent with this, *clp1-6A*, unlike *clp1*Δ, did not show any negative interactions with the SIN mutants *sid1-239, sid4-A1, cdc11-136, sid2-250, cdc14-118, spg1-B8,* or *mob1-R4* (data not shown). However, we did find that *clp1-6A* is sensitive to perturbations of the actomyosin ring, showing

Figure3-3

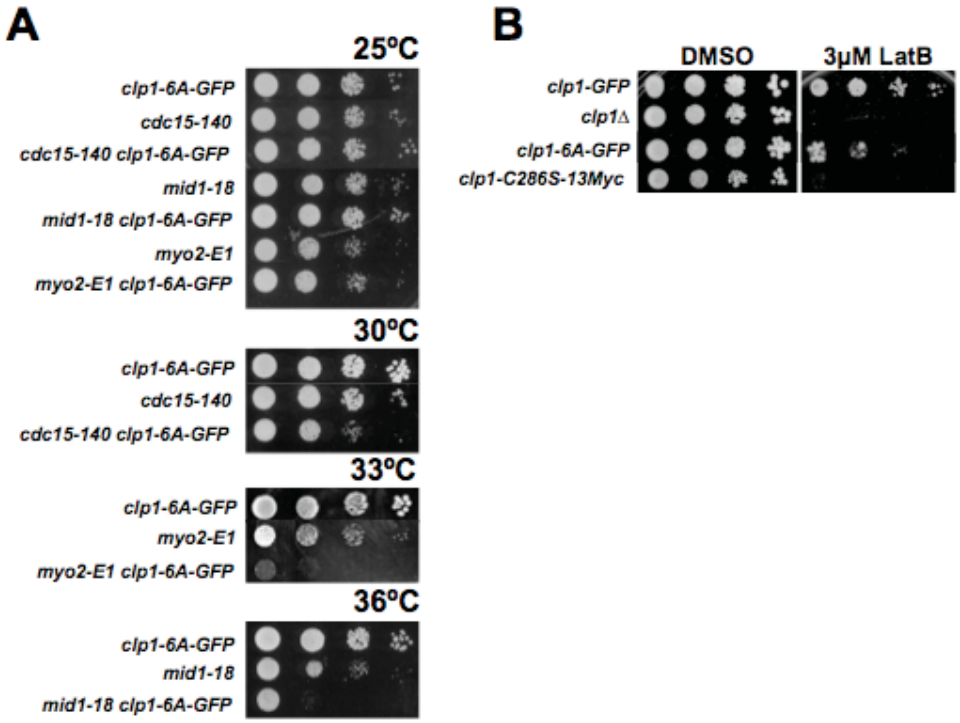


Figure 3-3. Functional analysis of Clp1-6A in cytokinesis.
(A) *clp1-6A-GFP* in different actomyosin ring mutant backgrounds (*cdc15-140*, *myo2-E1*, and *mid1-18*) were grown to mid-log phase, spotted on YE plates in 10-fold serial dilutions, and incubated at 25°C, 30°C, 33°C, and 36°C as indicated. (B) *clp1-GFP, clp1Δ*, *clp1-6A-GFP*, and *clp1-C286S-13Myc* were grown to mid-log phase, and spotted in 10-fold serial dilutions on YE plates containing 3μM LatB or DMSO (solvent control). The plates were incubated at 30°C for 3 days.

sensitivity to low doses of the actin inhibitor Latrunculin B, and negative genetic interactions with several mutations affecting actomyosin ring assembly and cytokinesis (Figure 3-3). In particular, *clp1-6A* cells had negative interactions with the actomyosin ring assembly mutants *cdc15-140*, *mid1-18*, and *myo2-E1*, with the double mutants showing synthetic growth defects at semi-permissive temperatures (Figure 3-3A). Examination of double mutant cells in liquid culture at semi-permissive temperatures showed enhanced cytokinetic defects (Figure 3-S7). For example, after 8 hours at 30°C both *myo2-E1* and *clp1-6A myo2-E1* cells showed single nuclei separated by relatively complete but misformed septa. In contrast, *clp1*Δ *myo2-E1* cells, which lack the cytokinesis checkpoint, have only occasional partial septa and are highly multinucleate (Figure 3-S8). However, the *myo2-E1* single mutant, unlike *clp1-6A myo2-E1*, was able to complete cytokinesis since there was a significant number of mononucleate cells and fewer tetranucleate cells (Figure 3-S7). Overall, these results suggest that maintenance of Clp1 in the cytoplasm is important for completion of cytokinesis when the cell division apparatus is perturbed.

Discussion

Previous studies suggested that SIN-dependent cytoplasmic retention of Clp1 was an essential part of a cytokinesis checkpoint that, in response to perturbation of the cell division apparatus, halts further cell cycle progression until cytokinesis can be completed (Trautmann et al., 2001; Mishra et al., 2004). However we found that cytoplasmic retention of Clp1 is not required to halt cell cycle progression when the actomyosin ring is damaged (Figure 3-S3, and 3-S4), but it is required to complete cytokinesis (Figure 3-3), presumably by maintaining the cell division apparatus. This is consistent with recent results showing that inability to target Clp1 to the actomyosin ring causes similar cytokinetic defects when the ring is perturbed but not cytokinesis checkpoint defects (Clifford et al., 2008).

Although many studies have shown that the SIN and MEN pathways regulate the conserved phosphatases Clp1 and Cdc14 respectively to keep them out of the nucleolus during late mitosis, the mechanism has been unclear. Here we show that the most downstream kinase in the SIN pathway, Sid2, phosphorylates Clp1 to promote binding of the 14-3-3 protein Rad24 (Figure 3-4). Binding to Rad24 results in cytoplasmic retention of Clp1. A recent study showed that the Cds1 kinase phosphorylates Clp1 on similar residues to promote cytoplasmic retention of Clp1 in response to blocks in DNA replication (Diaz-Cuervo and Bueno, 2008), suggesting that the same mechanism could be used by multiple inputs to regulate Clp1. In addition, the Sid2 homolog in animal

cells, the Lats1/2 tumor suppressor, might regulate targets using a similar strategy. Lats1/2 phosphorylates the oncogene YAP1 causing it to bind a 14-3-3 protein and be retained in the cytoplasm (Hao et al, 2007; Lei et al., 2008; Zhao et al., 2007; Oh and Irvine, 2008; Dong et al., 2007). Given that mammalian cells have at least two Cdc14 homologs, it is tempting to speculate that they too may be regulated through Lats1/2 phosphorylation and 14-3-3 binding as we observe in yeast.

Figure 3-4

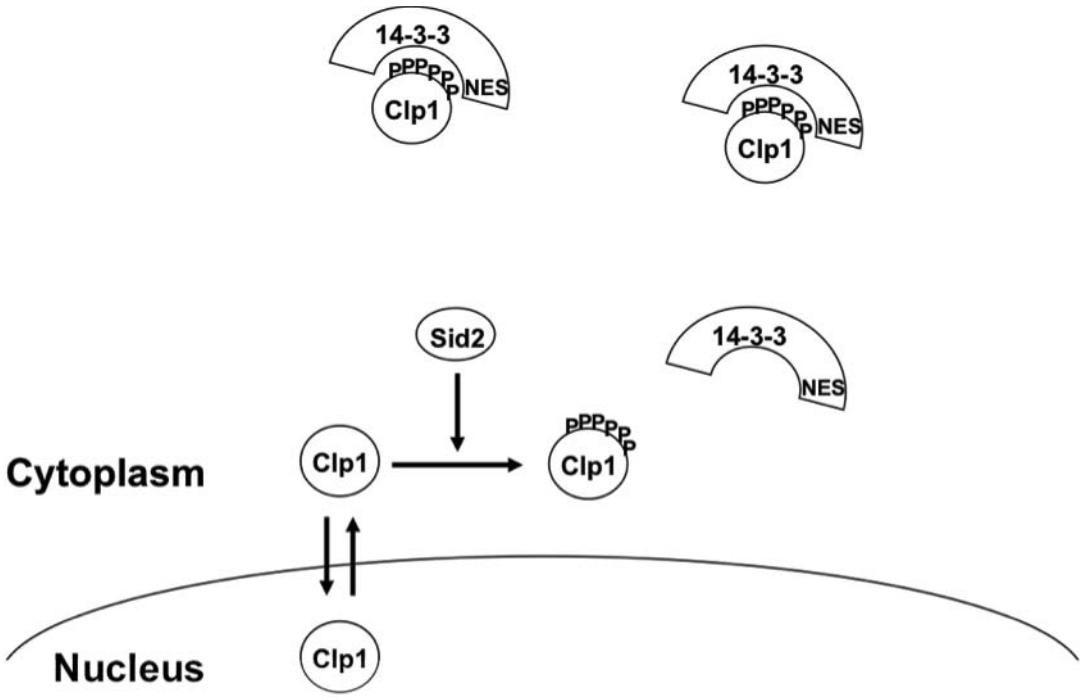


Figure 3-4. A model of Clp1 regulation by Sid2 kinase and Rad24.

CHAPTER IV

IDENTIFICATION OF SID2 KINASE SUBSTRATES: ASE1 AND KLP2 ARE INHIBITED TO KEEP NUCLEI AWAY FROM THE INGRESSING CLEAVAGE FURROW

Summary

The septation initiation network (SIN) functions to coordinate multiple mitotic events in *Schizosaccharomyces pombe*, including constriction of the actomyosin ring, formation of the division septum, inhibition of interphase polarity growth, and prevention of nuclear damage by the forming septum. However, the downstream targets of the SIN pathway involved in these regulations remain elusive. Here, we report a strategy to identify substrates of the SIN pathway, using a tandem affinity purification (TAP) approach. As shown in Chapter III, Sid2 phosphorylation on one of its substrates, Clp1, creates potential Rad24 binding sites. Therefore, we hypothesized that we could identify uncharacterized Sid2 substrates by purifying Rad24-interacting complexes, and analysing the purified complexes by mass spectrometry. Several proteins were identified to be possible Sid2 substrates. Further genetic and biochemical studies showed that a microtubule-associating protein Ase1 and a kinesin-14 motor protein Klp2 were negatively regulated by the SIN pathway to prevent nuclear clustering during cytokinesis. These results suggest that this method successfully identified SIN substrates, and has high potential to identify substrates of other kinases. More importantly, these results implied that the SIN functions not only to promote cytokinesis but also to regulate several cellular events during late mitosis, including microtubule organization, and inhibition of polarity growth before completion of cytokinesis.

Introduction

Cytokinesis is the terminal stage of cell cycle, which is responsible to separate cytoplasmic material into two genetically identical daughter cells. In the fission yeast *Schizosaccharomyces pombe*, a conserved signaling pathway termed the Septation Initiation Network (SIN) functions to promote constriction of a contractile actomyosin ring followed by the septum formation to complete cytokinesis. Most, if not all, of the SIN components are localized at the spindle pole body (SPB), which is equivalent to the mammalian centrosome. The most downstream SIN kinase Sid2 was the only component in the pathway that translocates from the SPB to the division site, and was thought to be responsible for carrying the upstream signal to the division site (Sparks et al., 1999). However, little is known about its downstream target(s) at the actomyosin ring. The conserved phosphorylation sites for Sid2 family of kinases, and 14-3-3 binding motifs share the common sequence motif, RXXpS (Yaffe et al., 1997; Mah et al., 2005). In Chapter III, it has been described that the 14-3-3 protein Rad24 binds to one of the Sid2 kinase substrates, Clp1, to regulate its subcellular localization during late mitosis (Chen, et al., 2008). Furthermore, Sid2 kinase and Rad24 co-localize to the division site and the SPB, suggesting that Sid2 kinase might create Rad24 binding sites on its substrates where they co-localized. Therefore, we suspected that purifying Rad24 interacting proteins might identify more Sid2 kinase substrates. To ensure the purified proteins are specific substrates of the SIN pathway, we repeated the purification in different SIN activity

backgrounds, and the proteins whose amount bound to Rad24 oscillated with the SIN activity were determined to be candidate substrates.

During normal cytokinesis, the two separated nuclei stay away from the division apparatus to prevent damage caused by the ingressing furrow. When cytokinesis is delayed, cells maintain active SIN and the two nuclei remain apart. However, if the SIN is inactive, the nuclei are not kept apart during cytokinesis and cluster in the middle of the cell, suggesting a role for the SIN in keeping nuclei apart during prolonged cytokinesis (Figure 4-1, 4-2A). In the presence of the anti-microtubule drug thiabendazole (TBZ), this nuclear clustering phenotype is suppressed (Hagan and Yanagida, 1997), suggesting that SIN activity may function to regulate nuclear positioning, probably through regulating microtubule organization. It has been reported that the SIN activity is required for equatorial microtubule organizing center (eMTOC) formation in *S. pombe*, which is a structure that nucleates microtubules from the medial cell division site during late mitosis, and one of its functions may be to maintain the position of the actomyosin ring (Pardo and Nurse, 2003). However, it is not clear if this is a direct effect, since the eMTOC assembly requires the actomyosin ring, which disassembles in SIN mutants. Thus, how SIN signaling affects the microtubule cytoskeleton during late mitosis is not clear. In this study, we showed that the SIN signaling may prevent nuclear clustering by negatively regulating Ase1 and a minus-end directed kinesin, Klp2.

Result

Candidate SIN substrate proteins

To identify candidate substrates of Sid2 kinase, Rad24-3HA-TAP was integrated into the *rad24*$^{+}$ locus such that it was regulated by the endogenous promoter, and was the only copy of *rad24*$^{+}$ expressed in the cell. The strain expressing Rad24-3HA-TAP was then crossed to different SIN mutants, including *cdc16-116*, *sid1-239*, and *cdc11-123*. The cells were grown at 25ºC to OD_{595} 0.4 and shifted to 36ºC for four hours. Six liters of yeast (OD_{595} 0.6-0.8) were used in total. The TAP-purification was performed as described in Gould et al., 2004. The final eluates were TCA precipitated and analysed using mass spectrometry, by our collaborators in the laboratory of Dr. Kathy Gould at Vanderbilt University. Rad24-binding proteins were considered candidate substrates if they bound Rad24 in a SIN dependent manner. For example, Clp1 serves as the best positive control since it has been shown that Sid2 phosphorylation promotes its binding to Rad24 (Chapter III). Results from mass spectrometry analysis showed that Rad24 pulled down more Clp1 in ectopic activating SIN background (using a *ts* mutant allele *cdc16-116*), and less in a SIN inactive background (using a *ts* mutant allele *sid1-239* and *cdc11-123*) compared to the result from asynchronous culture. We failed to perform Rad24-3HA-TAP purification in *sid2-250 ts*-mutant background since the Rad24-3HA-TAP and sid2-250 mutation were synthetic lethal when combined. This suggests that the Rad24-3HA-TAP is not fully functional, even though the strain has normal morphology, unlike the *rad24*Δ strain, which is round in shape. The observed genetic interaction is

consistent with a previous report that *rad24*$^{+}$ was involved in maintaining SIN activity during late mitosis (Mishra et al., 2005).

35 proteins fell into this category by comparing the change in amount of peptide count in three different SIN activity conditions (Appendix B), such as Clp1, Ase1, Nak1, Sog2, Cdc11, Scw1, and four uncharacterized proteins SPAC3G9.05, SPBC3B8.10c, SPAC16E8.08 and SPAC23C11.05. However, some of them were found with very low coverage rate and/or peptide count. More experiments need to be done to confirm whether these proteins are real Sid2 kinase substrates.

Nevertheless, several potential SIN substrates help us to explore and predict more possible SIN functions during late mitosis. The finding of Nak1 and Sog2 being Sid2 candidate substrates lead us to speculate a crosstalk between the SIN and the morphology pathway, for which the molecular mechanism is still unclear. Nak1 is an essential GC family kinase and has been shown to regulate cell morphology and polarity, probably through regulating the actin cytoskeleton (Huang et al., 2003; Huang et al 2005; Leonhard and Nurse, 2005). Sog2 was less described, but was thought to be one of the members in the morphology pathway. Studies from our lab and others have suggested that the SIN may inhibit the morphology pathway during cytokinesis to prevent the titration of actin from the division site to the cell tips (Ray and McCollum, unpublished data). These results imply that the SIN inhibits the morphology pathway through direct phosphorylation on the components of morphology pathway.

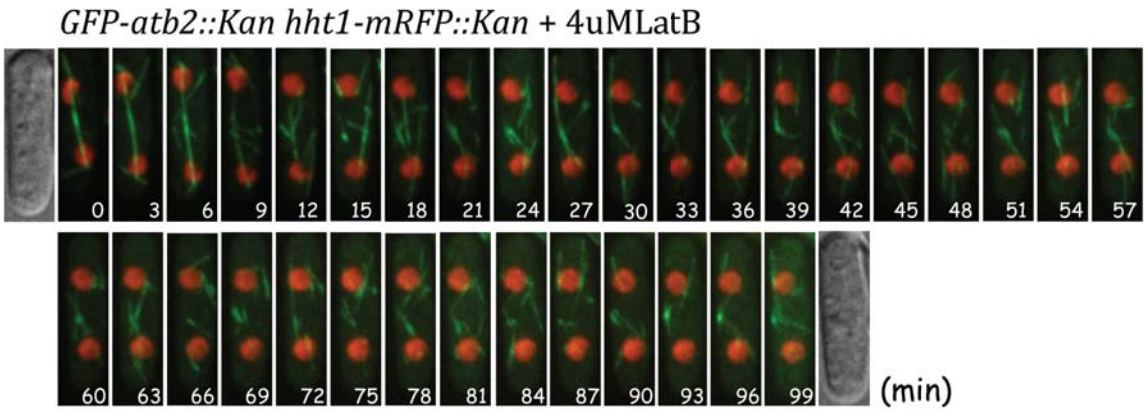
Cdc11 and Scw1 being Sid2 kinase substrates suggest that SIN may promote cytokinesis in a different manner. Cdc11 has been shown to function as a scaffold protein together with Sid4 to recruit SIN components to the SPB (Morrell et al., 2004). It is tempting to speculate that Sid2 phosphorylation on the scaffold protein followed by Rad24 binding may stabilize the protein complex and maintain the SIN activity. It has been shown that the RNA-binding protein Scw1 is a negative regulator of cell-wall formation and antagonizes SIN activity (Karagiannis et al., 2002; Jin and McCollum, 2003). We suspected that SIN might promote septum formation by removing this negative regulator through Sid2 phosphorylation. More experiments need to be done to test these hypotheses.

SIN regulates nuclear positioning through Ase1 and Klp2 when cytokinesis is delayed.

When wild-type cells are treated with a low dose of Latrunculin B to slow down cytokinesis, the SIN stays active and maintains the actomyosin ring until the completion of cytokinesis. On the other hand, SIN compromised cells (*sid2-250* mutant strain) failed to maintain the actomyosin ring and end up with cytokinesis failure. One interesting phenomenon was observed (Figure 4-1) that in SIN mutants the two separated nuclei clustered together, suggesting that active SIN not only promotes ring stability but also prevents nuclear congression. This result suggested that the SIN may play a role other than promoting actomyosin ring assembly and constriction. We speculate that the SIN keeps the two separated nuclei apart to prevent them from been damaged by the

Figure 4-1

A.



B.

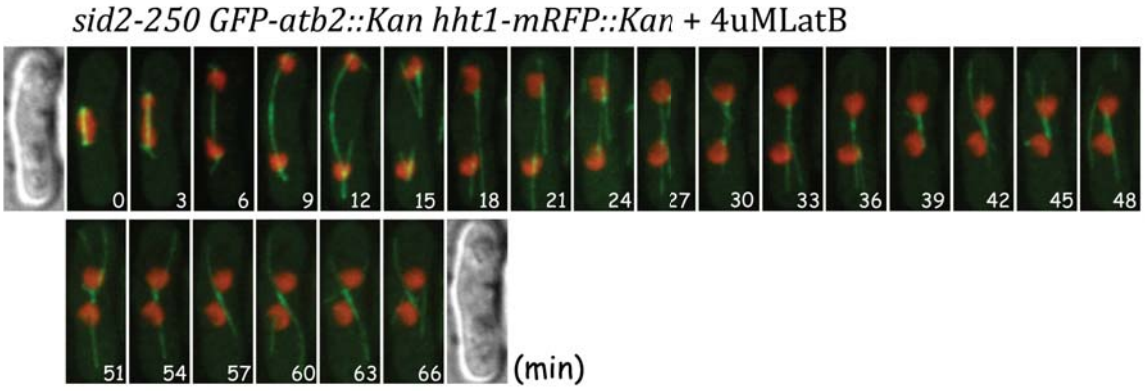


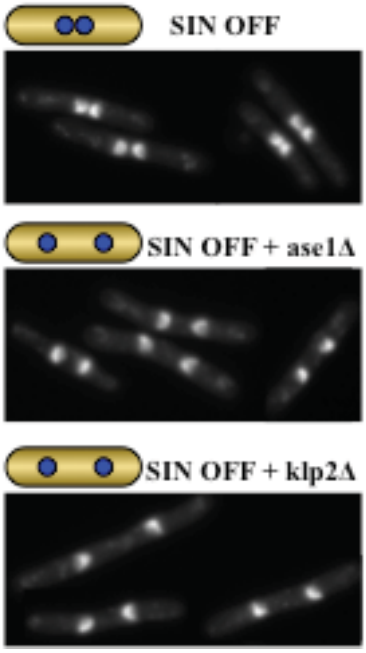
Figure 4-1. Nuclear congression in SIN compromised cells.
Time-lapse images of *wild-type* (A) and *sid2-250* (B) cells both expressing GFP-atb2 and hht1-mRFP as an microtubule and nuclear marker, respectively. Cells were grown to mid-log phase, and treated with 4 μM LatB to slightly perturb the cytokinesis. *Wild-type* cells eventually overcomes the perturbation and finishes cytokinesis. However, the *sid2-250* cells shows SIN defective phenotype under permissive temperature (25ºC). The images were collected every 3 minutes, using a spinning disc confocal microscope. Ten images were captured for each time point, with a step size of 0.55 μm between focal planes.

ingressing septum. However the regulatory mechanism by which the SIN promotes nuclear separation is not understood.

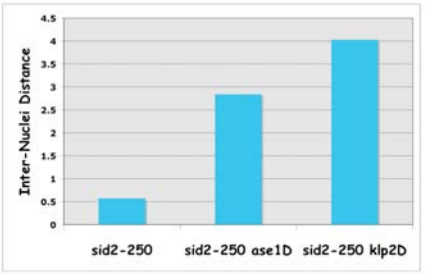
Work from our lab and others (Trautmann and McCollum unpublished observation; Okazaki and Niwa, 2008) showed that the deletion of a minus-end directed kinesin Klp2 suppressed the nuclear congression phenotype in SIN mutants (Figure 4-2). Because Ase1 was identified in our screen for Sid2 substrates, we wondered if Ase1 could also be involved in nuclear positioning during cytokinesis. Consistent with this notion, we found that deletion of Ase1 could also rescue the nuclear positioning defect in SIN mutants (Figure.4-2). Together, these results suggest that the SIN prevents nuclear congression during cytokinesis by regulating Ase1 and Klp2. To test if Ase1 and Klp2 are Sid2 kinase substrates, an in vitro kinase assay was performed using immunoprecipitated Sid2 kinase and recombinant Ase1 (Fu et al., 2009) and Klp2 (Bruan et al., 2009). This experiment showed that His-tagged Ase1 was a good substrate of Sid2 kinase, consistent with the result from the Rad24-TAP purification. Surprisingly, His-tagged Klp2 can also be phosphorylated by Sid2 kinase, especially the tail domain of this protein (Figure 4-3). Klp2 was not found in the Rad24-TAP purification. One reason could be that Sid2 phosphorylation of Klp2 does not promote Rad24 binding; in other words, there may be Sid2 substrates that do not bind to Rad24. Thus, the Rad24-TAP purification method of finding Sid2 kinase substrates may identify some but not all Sid2 kinase substrates. Alternatively, Klp2 may not be directly regulated by Sid2.

Figure 4-2

A.



B.



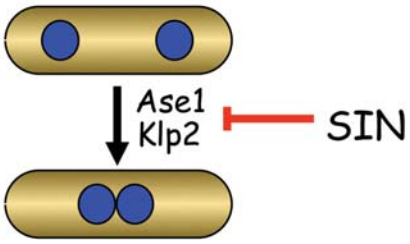


Figure 4-2 *ase1*Δ and *klp2*Δ rescue the nuclear clustering phenotype in *sid2-250*.
(A) *ase1*Δ *sid2-250*, *klp2*Δ *sid2-250* and *sid2-250* cells were grown to mid-log phase and shifter to 36°C for 2 hr. The cells were fixed with methanol and DAPI stained. (B) The distance between two nuclei was quantified. The cartoon shows a model of SIN regulation on Ase1 and Klp2.

Figure 4-3

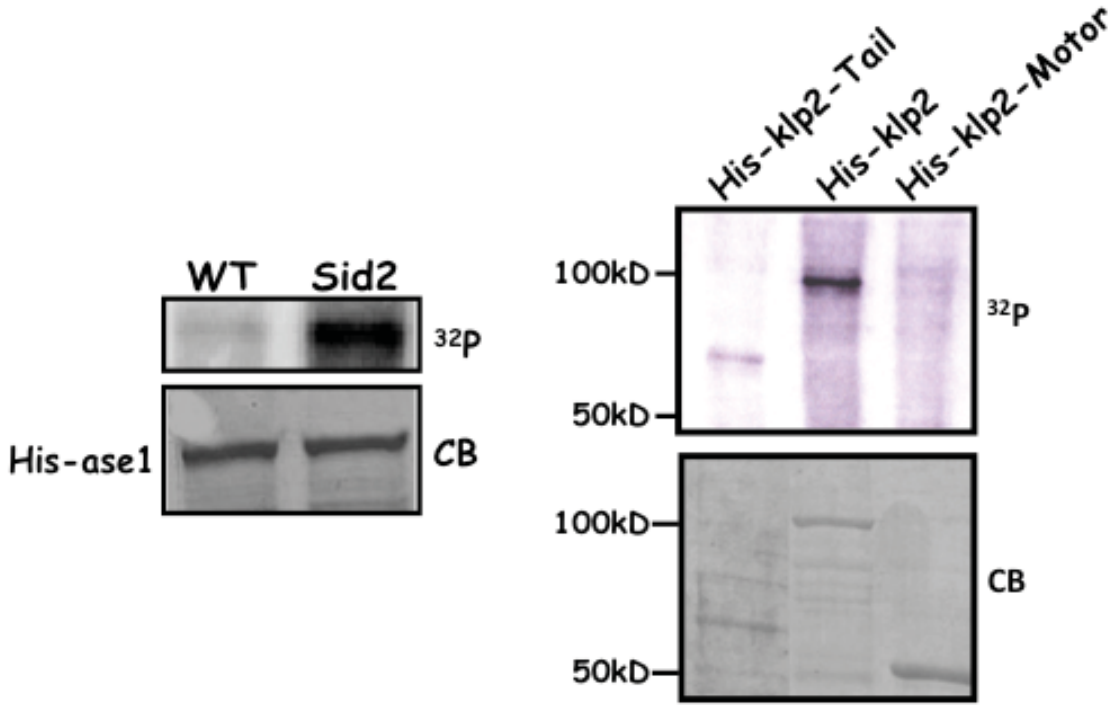


Figure 4-3 His-ase1 and His-Klp2 can be phosphorylated by Sid2 kinase in vitro.
In vitro kinase assays were performed by using immunoprecipitated Sid2 kinase and bacterially expressed His-ase1 and His-Klp2. The Sid2 kinase was immunoprecipitated using anti-Myc antibody from *cdc16-116 sid2-13Myc* (Sid2) and *wild-type* cells (WT) with untagged Sid2 as negative control. Protein labeled by γ-^{32}P was detected using a Phospho Imager (Molecular Dynamics), and the gel was stained with Coomassie Blue (CB) as loading control.

Homologues of Ase1 and Klp2 serve as microtubule organizers in many cell types. However, in the fission yeast, their function and localization have only been intensively studied in interphase cells. From the genetic analysis observed in Figure 4-2, and biochemical analysis in Figure 4-3, we hypothesized that the SIN activity negatively regulates Ase1 and Klp2, probably through Sid2 phosphorylation. To test this hypothesis we examined the localization of Ase1-GFP and Klp2-GFP in wild type and *sid2-250 ts*-mutant background. A nuclear envelope protein Uch2-GFP and an alpha tubulin protein Atb2-mCherry were also incorporated into the examined strain as a marker of nuclear position and microtubule dynamics. The cells were treated with a low dose of actin depolymerizing drug Latrunculin B for 2 hours to slow down cytokinesis. As shown in Figure 4-4, Ase1-GFP localization was not obviously different between wild type, and cells with compromised SIN (*sid2-250*), where in both cases, Ase1-GFP still localized to the overlapping microtubule bundles around the nucleus, and co-localized with microtubule (in red). Ase1-GFP was still able to localize to the microtubule when we examined its localization in a constitutively activated SIN background using *cdc16-116 ts*-mutant (data not shown), which is consistent with our observation. This result showed that the SIN activity does not affect Ase1-GFP localization, and suggested that Sid2 phosphorylation may regulate Ase1 through some other mechanism.

SIN activity inhibits Klp2 localization to the microtubule

Klp2 is a minus-end directed kinesin that has been shown to be required for karyogamy and dikaryon formation (Troxell et al., 2001; Okazaki and Niwa, 2008) and

Figure 4-4

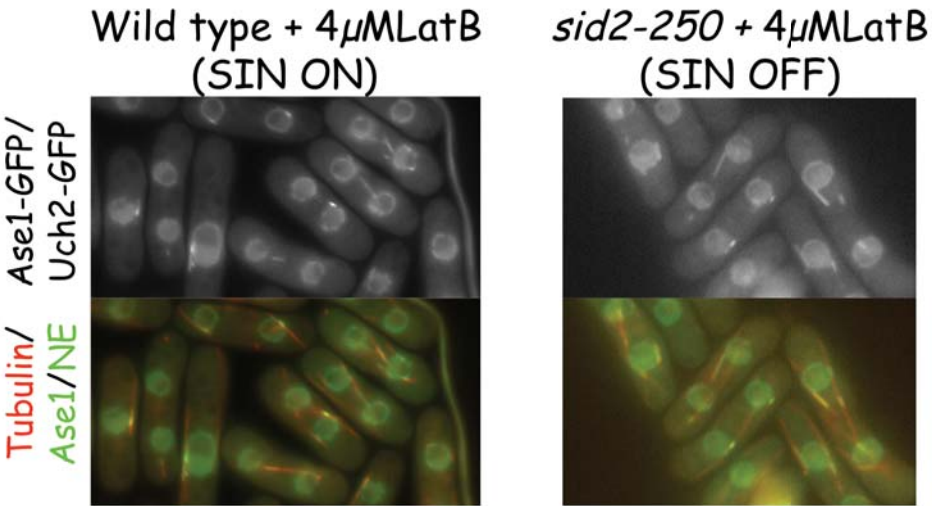


Figure 4-4 SIN activity does not affect Ase1-GFP localization. *Wild type* and *sid2-250* cells both expressing Ase1-GFP, Atb2-mCherry (tubulin, in red), and Uch2-GFP (nuclear envelope, in green) were grown to mid-log phase and treated with 4μM Latrunculin B (Sigma) for 2 hours. Live cell images were taken on a fluorescence microscope.

functions to organize interphase microtubules in fission yeast (Carazo-Salas and Nurse, 2006; Janson et al., 2007; Bruan et al., 2009). This kinesin-14 motor protein possesses an ability to slide anti-parallel microtubules. Therefore, we suspected that Klp2 provides the physical force to bring the two separated nuclei to the cell center in the absence of SIN activity.

To determine how the SIN signaling negatively regulates Klp2, Klp2-GFP localization was examined in the presence Latrunculin B, so that cytokinesis is delayed in both wild type and *sid2-250* cells, where the wild type cells arrested as binucleate cells with active SIN, and *sid2-250* showed compromised SIN activity. The results showed that in all mononucleate (interphase) wild-type and *sid2-250* cells, which have inactive SIN, Klp2-GFP localized as several linear arrays of small spots, presumably localized on the microtubule (Figure 4-5). In the binucleate mitotic wild-type cells, however, Klp2-GFP was only seen on mitotic spindle during early mitosis (data not shown) but not on anaphase or post-anaphase cells, which have active SIN (SIN ON). Interestingly, in the SIN compromised *sid2-250* cells (SIN OFF), Klp2-GFP localized on the microtubule in late mitosis like interphase cells. These results showed that SIN signaling prevents Klp2-GFP from localizing to the microtubule. To confirm this observation, Klp2-GFP localization was examined in cells where the SIN had been ectopically activated using *cdc16-116 ts* mutant. The *cdc16-116* mutant undergoes multiple rounds of septation at restrictive temperature without entering mitosis. Therefore, it causes septum formation in interphase cells, which creates cells with one compartment with a nucleus and SPB, and

Figure 4-5

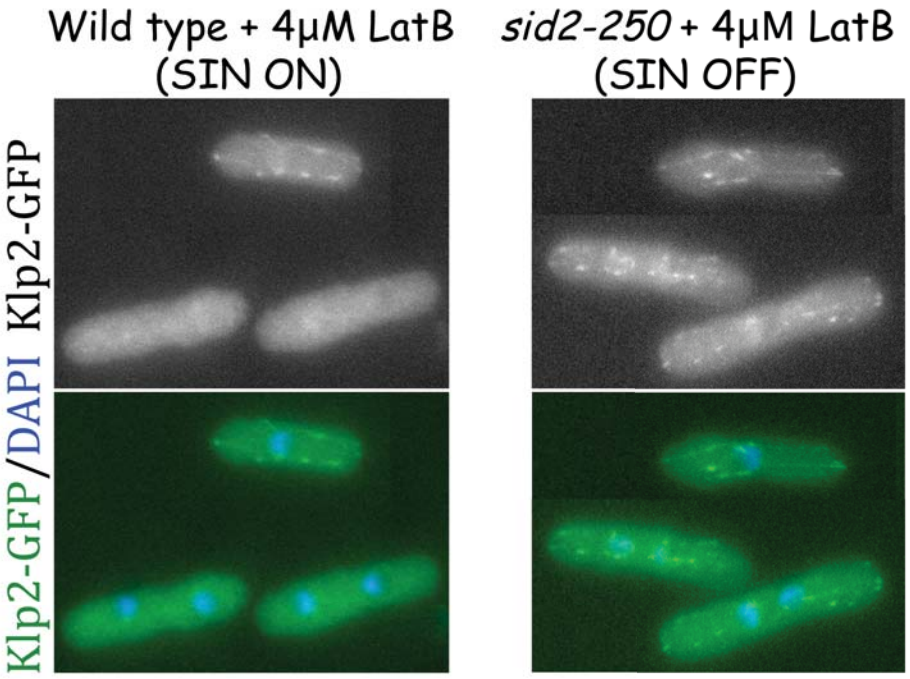


Figure 4-5 Active SIN prevents Klp2-GFP from localizing to the microtubule. *Klp2-GFP* and *Klp2-GFP sid2-250* cells were grown to mid-log phase and treated with 4 µM LatB for 2 hr. The cells were fixed with methanol and DAPI stained.

one lacking both nucleus and SPB. Because the SIN localizes to the SPB, the compartment without a nucleus and SPB lacks SIN signaling, whereas SIN signaling continues in the other compartment (García-Cortes, and McCollum, 2009). As shown in Figure 4-6, in all the mononucleate cells, Klp2-GFP was only been seen as cytoplasmic dots in the compartments created by *cdc16-116* at restrictive temperature lacking an SPB and active SIN. Therefore, this result suggested that active SIN keeps Klp2 from localizing to the microtubule. To examine if the level of Klp2-GFP protein is affected by different SIN activity, a Western Blot was performed to detect immunoprecipitated Klp2-GFP protein level in wild type and *cdc16-116 cdc3-124* background. The *cdc3-124* mutation was used to prevent the septum formation due to ectopically activated SIN, so that the regulated Klp2-GFP by the SIN pathway would be universal, and not limited in a small compartment of the septated cell. The result showed that the Klp2-GFP level did not change when the SIN was activated (Figure 4-7C), and the reason we didn't observe Klp2-GFP forming dot-like arrays on the microtubules was not due to the difference of protein level. The Klp2-GFP localization was also examined in the wild-type and *cdc16-116 cdc3-124* mutant cells. The SIN kinase Cdc7-mCherry was used as a marker of active SIN; when the SIN is activated Cdc7-mCherry is recruited to the SPB by the GTP-bound Spg1. Consistent with previous results, no Klp2-GFP localization on the microtubule was observed both in interphase and mitotic cells when the SIN had been ectopically activated, whereas the Klp2-GFP localization was intact in interphase but not in mitotic cells in wild-type cells (Figure 4-7A). The amount of cells with Klp2-GFP localization on the microtubules (klp2+) and Cdc7-mCherry at the SPB (cdc7+) was

Figure 4-6

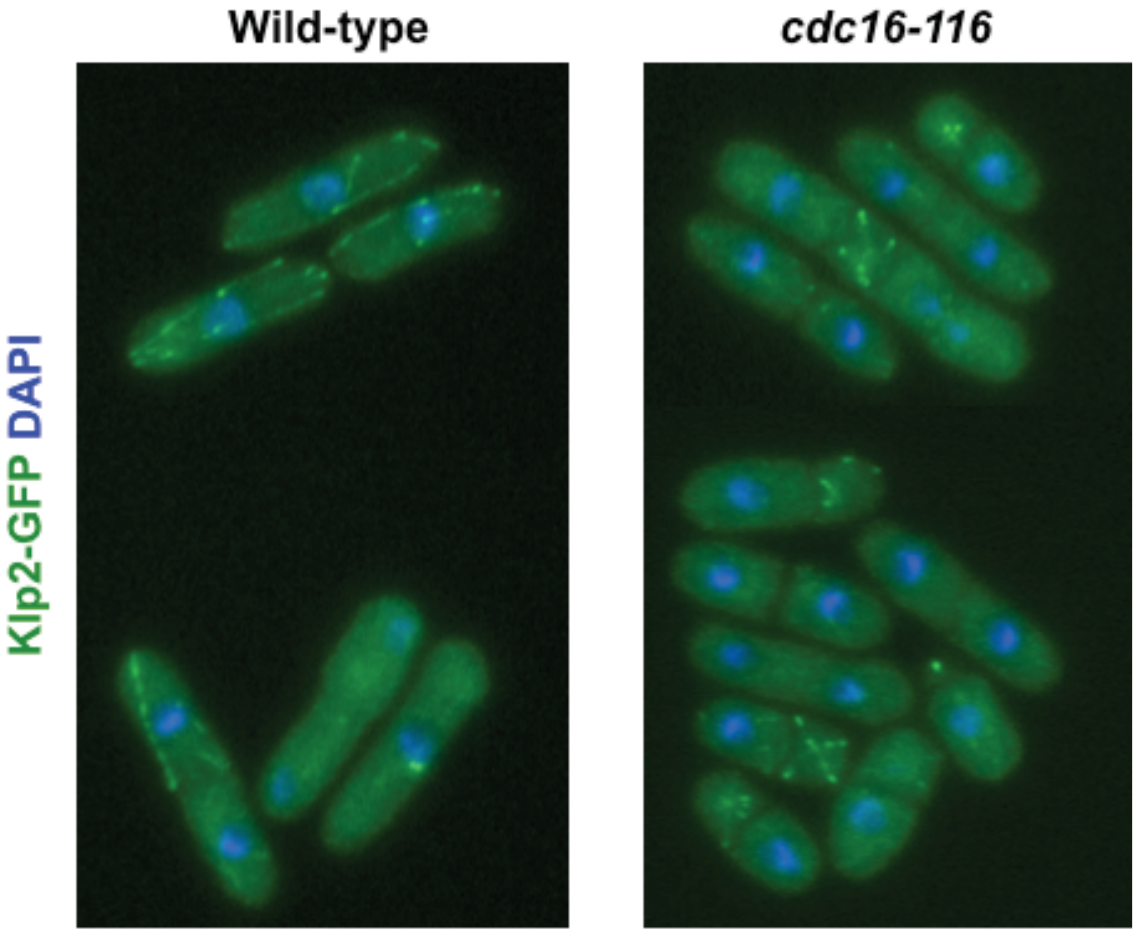


Figure 4-6 Ectopic activation of the SIN pathway prevents Klp2-GFP from localizing to the microtubule. *Klp2-GFP* and *Klp2-GFP cdc16-116* cells were grown to mid-log phase and shifted to 36°C for 2 hours. The cells were fixed with methanol and DAPI stained.

Figure 4-7

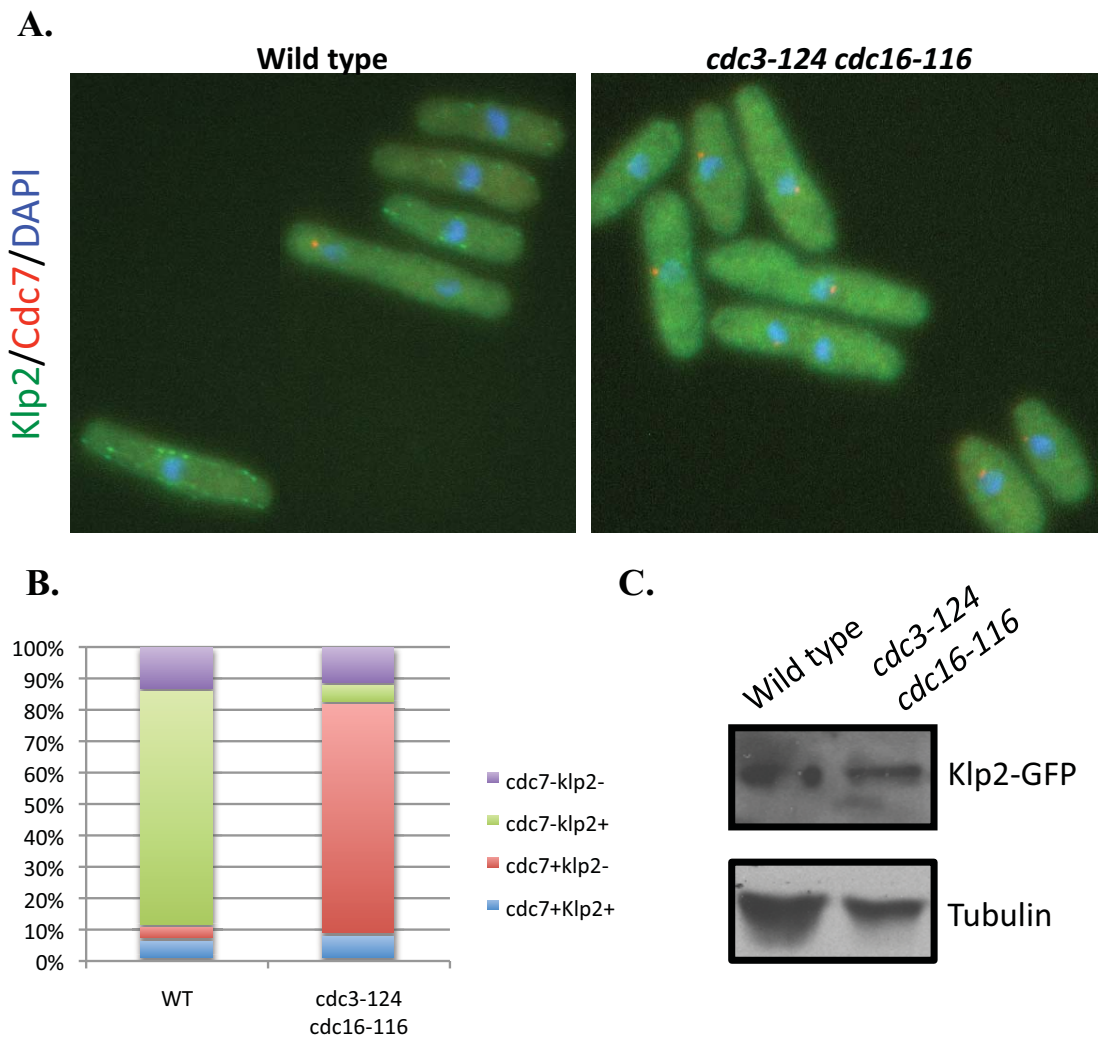


Figure 4-7 Examination of Klp2-GFP localization and protein level in the *cdc3-124 cdc16-116* mutant cells. (A) *Klp2-GFP Cdc7-mCherry* and *Klp2-GFP Cdc7-mCherry cdc3-124 cdc16-116* cells were grown to mid-log phase and shifted to 36°C for 2 hours. The cells were fixed with methanol and DAPI stained. (B) The localization of Klp2-GFP and Cdc7-mCherry to the microtubules (klp2+) and the SPB (cdc7+), respectively, in the *wild-type* and the *cdc3-124 cdc16-116* mutant cells were quantified. (C) Klp2-GFP was immunoprecipitated using a mouse monoclonal anti-GFP antibody (Molecular Probes) from protein lysates prepared from *wild-type* and *cdc3-124 cdc16-116* cells grown at 36°C for 2 hours. Klp2-GFP and tubulin were detected by a western blot using anti-GFP antibodies (Santa Cruz) and anti-tubulin TAT1 antibodies.

quantified in Figure 4-7B. In conclusion, these results showed that when the SIN is activated, it keeps Klp2-GFP from localizing to the microtubules, suggesting that the SIN might prevent nuclear clustering by inhibiting the localization of the motor protein.

Discussion

In this chapter, we proposed a strategy to identify downstream SIN targets. Our results suggested that a conserved microtubule bundling protein Ase1 could be one of the Sid2 kinase substrates, and the SIN pathway may inhibit nuclear congression during cytokinesis by negatively regulating Ase1 and a kinesin-14 protein, Klp2. Klp2 was not identified in our Rad24-TAP purification, but can be phosphorylated by Sid2 kinase in vitro. Our result showed that the SIN keeps Klp2-GFP from localizing to microtubules, suggesting that this could be how cells prevent the separated nuclei from moving inwards and becoming damaged by the ingressing septum. The significance of Sid2 phosphorylation on Ase1 in vivo is not clear. Using an in vitro binding assay, we showed that Klp2-GFP from yeast cells interacted with bacterial expressed 6His-ase1 (Figure 4-8). We hypothesized that maybe Ase1 recruits Klp2 to microtubules and that Sid2 phosphorylation of Ase1 affects the ability of Ase1 to recruit Klp2 to microtubules. However, in *ase1*Δ cells, Klp2-GFP was still able to localize to microtubules, suggesting that Ase1 is not required for Klp2 loading to microtubules (Figure 4-9). We don't know if these two proteins do interact in vivo, and if so, whether this interaction is affected by SIN signaling. A co-immunoprecipitation between Ase1 and Klp2, or an Ase1-TAP

Figure 4-8

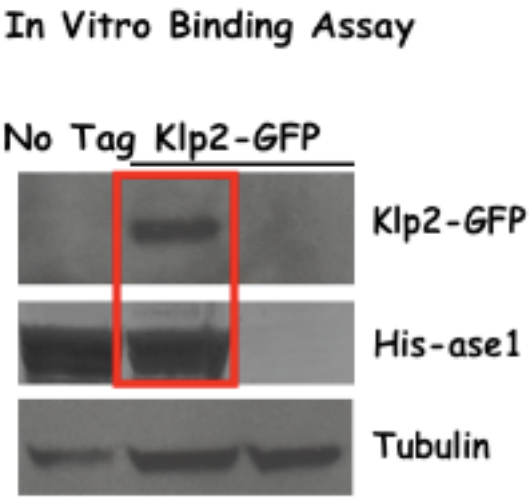


Figure 4-8 Klp2-GFP interacts with His-ase1. Yeast lysate of Klp2-GFP was prepared and incubated with crude lysate of bacterial expressed His-ase1 at 4ºC for 2 hours. 50 µl of Nickel beads were added and incubated for another 1 hour. The precipitated protein complexes were washed three times with NP-40 buffer and subjected to western blot.

purification may answer this question, and reveal more interacting proteins that could be involved in the function or regulation of these two proteins. Like Clp1, Ase1 has a cluster of RXXS sites within a small area at the C-terminal domain. It would be interesting to map all the Sid2 phosphorylation sites on Ase1 and mutagenize them to alanines or acidic residues to see if the mutated Ase1 protein causes any functional defect in microtubule organization, or could bypass the requirement for the SIN to inhibit nuclear congression during cytokinesis.

Klp2 was not identified from the Rad24-TAP purification, but can be phosphorylated by Sid2 kinase in vitro, suggesting that maybe not all the Sid2 kinase substrates interact with Rad24. However, we cannot exclude the possibility of Klp2 being a substrate of another SIN kinase, such as Sid1, or Cdc7. Klp2 also contains a cluster of RXXS motif in the tail domain (Figure 4-3). If Sid2 kinase regulates Klp2-GFP localization by phosphorylating these RXXS sites, changing these serines to alanines might re-locate mutated Klp2 protein to the microtubule, and bypass the SIN inhibition of nuclear clustering. Recent results from Sebastían Mana-Capelli in our lab showed that an end-binding 1 (EB1) protein Mal3 is required to load Klp2-GFP on to microtubules, since Klp2-GFP no longer localizes to microtubules in *mal3*Δ cells. Interestingly, *mal3*Δ also rescues the nuclear clustering phenotype in *sid2-250* cells. A Klp2-TAP purification was also proposed by Mana-Capelli, and hopefully the result can identify more Klp2 interacting proteins and reveal the molecular mechanism of SIN regulation on microtubule dynamics.

Figure 4-9

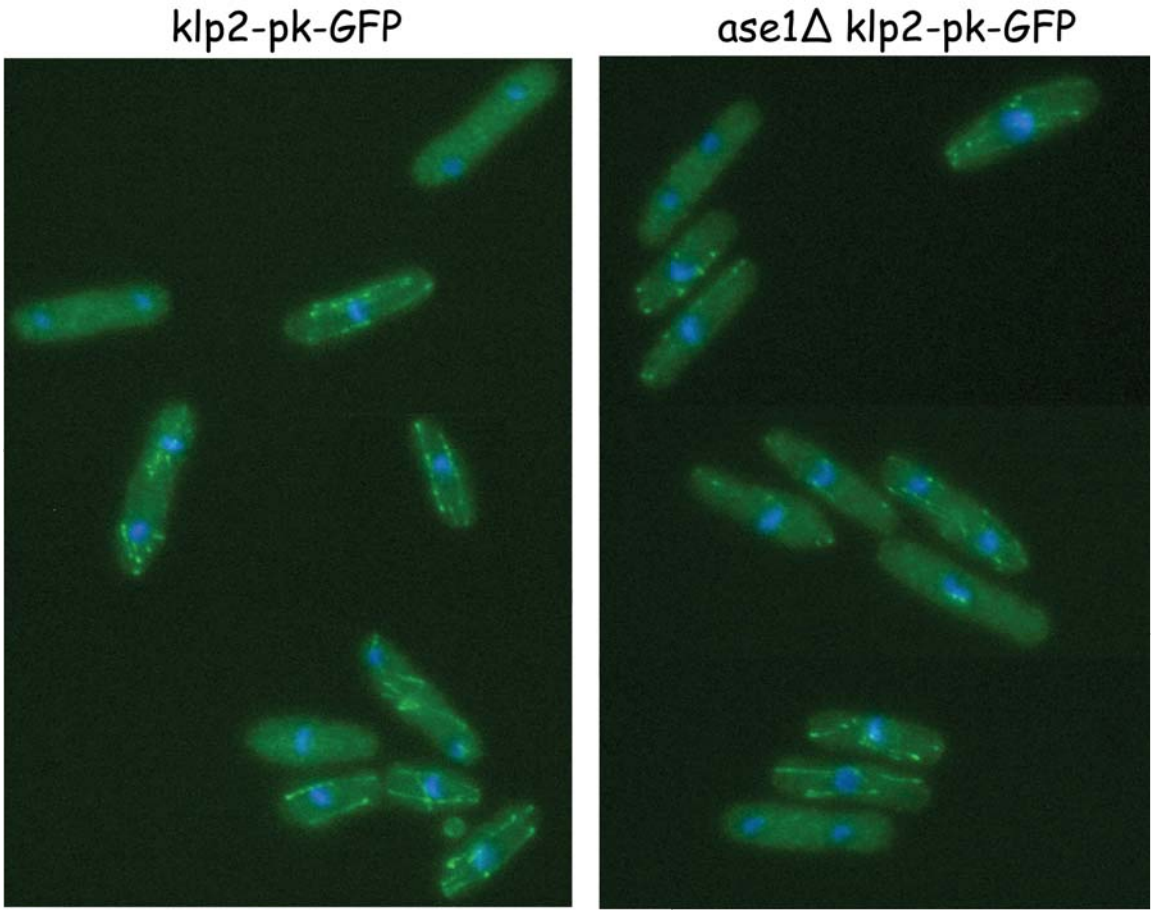


Figure 4-9 Localization of Klp2-GFP to the microtubules does not require Ase1.
klp2-GFP::ura^{+} and *ase1Δ klp2-GFP::ura*$^{+}$ cells were grown to mid-log phase at 25°C. The cells were fixed with methanol and DAPI stained.

CHAPTER V

General Discussion

Coordination of the nuclear and cytoplasmic division cycle is crucial to produce progeny with intact genetic material. Failure of proper chromosome separation prior to cytokinesis causes uneven distribution of chromosomes. Failure of cytokinesis prior to the next nuclear division cycle causes production of multinucleate cells. Either situation results in genetic instability, which is often associated with development of cancer. In the fission yeast *Schizosaccharomyces pombe*, a conserved signaling pathway termed the Septation Initiation Network (SIN) functions together with a Cdc14-like phosphatase Clp1 to couple mitosis and cytokinesis. Function and activity of Clp1 are thought to be regulated, at least in part by its localization. However, the regulatory mechanism that promotes early release of Clp1 from the nucleolus is still not understood. In budding yeast, early release of Cdc14 is controlled by a group of proteins called the FEAR network. In Chapter II, we have demonstrated that *S. pombe* homologous components of the FEAR network are not required to promote Clp1 release from the nucleolus (Chen et al., 2006). Moreover, the nucleolar anchor or inhibitor of Clp1 is not yet identified. A nucleolar protein Dnt1 showed sequence homology to Net1/Cfi1, which anchors and sequesters Cdc14 in the nucleolus. However, Dnt1 showed weak interaction with Clp1, and the absence of *dnt1* does not cause premature release of Clp1 from the nucleolus (Jin

et al., 2007), suggesting that the sequestration of Clp1 may be regulated by other mechanisms. The finding of Clp1 being hyperphosphorylated upon entry into mitosis (Cueille et al., 2001) suggests that phosphorylation of Clp1 itself could promote its release from the nucleolus. However, the molecular mechanism remains to be clarified.

The SIN pathway is fully activated during late anaphase, and is required to promote actomyosin ring assembly, constriction, and septum formation. However, its targets during late mitosis remain unclear. One function of the SIN pathway is to retain the Cdc14 homolog Clp1 in the cytoplasm until cytokinesis is completed. In Chapter III, we identified six major Sid2 phosphorylation sites on Clp1 that are required for Rad24 binding and responsible for cytoplasmic retention of Clp1. Losing these sites (Clp1-6A) resulted in premature return of Clp1 to the nucleolus after anaphase, and caused cytokinetic defects when the actomyosin ring was perturbed. It has been proposed that only cytoplasmic Clp1 is able to maintain the cytokinesis checkpoint, whereas nucleolar Clp1 (Clp1 in *rad24*Δ and Clp1-GFP-NLS) failed to inhibit further rounds of the nuclear cycle when cytokinesis is perturbed (Mishra et al., 2005; Trautmann et al., 2005). To our surprise, Clp1-6A, which showed premature return to the nucleolus, was still able to activate the cytokinesis checkpoint, which stops further rounds of the nuclear division cycle until cytokinesis is complete (Figure S3-3B). One explanation could be that Clp1-6A can still reach its substrates by shuttling between the nucleolus/nucleus and cytoplasm, but the steady state localization of Clp1-6A is in the nucleolus. Conversely, it is also possible that the substrate of Clp1 that helps to maintain the checkpoint reaches the

phosphatase by shuttling between the nucleolus/nucleus and cytoplasm. A Clp1-GFP-NLS mutant was created in our lab (Trautmann and McCollum, 2005) showed similar but not identical localization pattern to Clp1-6A. Both Clp1-GFP-NLS and Clp1-6A were released from the nucleolus upon entry into mitosis, localized to the kinetochores in prophase-metaphase, and the mitotic spindle during anaphase (Trautmann and McCollum, 2005; AppendixA, figure 3-S2A). Clp1-6A localized to the actomyosin ring throughout early mitosis to anaphase, and returned to the nucleolus prematurely in late mitosis, whereas Clp1-GFP-NLS was never found on the actomyosin ring. Interestingly, while Clp1-6A and Clp1-GFP-NLS were sequestered in the nucleolus during interphase and prolonged cytokinesis, Clp1-6A could localize to the spindle pole body (SPB) whereas Clp1-GFP-NLS could not. Neither Clp1-6A nor Clp1-GFP-NLS affects cell cycle progression as judged by the cell size when the cell divides (Trautmann and McCollum, 2005; Appendix A, figure 3-S6B-C). Moreover, the *clp1-6A* and *clp1-GFP-NLS* mutants do not have negative interactions with mutations in genes involved in chromosome segregation such as *dis1* (Trautmann and McCollum, 2005; AppendixA, figure 3-S6A), suggesting that the phosphatase activity is still intact in these mutants. Even though Clp1-GFP-NLS showed similar localization pattern to Clp1-6A, Clp1-GFP-NLS failed to halt further rounds of nuclear division cycle when the cytokinesis checkpoint is activated by the actin inhibitor Latrunculin B, whereas Clp1-6A was able to arrest the nuclear cycle (Appendix A, figure 3-S3B). The major difference observed between Clp1-GFP-NLS and Clp1-6A is that Clp1-6A still localizes strongly to the SPB during the prolonged cytokinesis (Figure 3-2B). One of our speculations is that Clp1-6A localization to the

SPB during mitosis may function to promote the cytokinesis checkpoint, maybe through interacting with the SIN components that are recruited to the SPB, hence retaining SIN activity during the cytokinesis delay. The biological significance of SPB localization of Clp1 is not well understood, since Clp1 still localizes to the SPB in interphase when the SIN is not active. It is possible that SPB-localized Clp1 is subject to interphase/mitotic specific modification, such as phosphorylation, resulting in the same localization but different function. It would be interesting to target Clp1, Clp1-6A, or Clp1-GFP-NLS to the SPB to see if it rescues the checkpoint defect in *clp1*Δ cells, and gain better understanding of the function of SPB-localized Clp1.

The 14-3-3 protein Rad24 plays an important role in retaining Clp1 in the cytoplasm (Chapter III). The 14-3-3 proteins are known for their ability to bind phosphoproteins and cytoplasmic sequestration of their binding partners. However, the molecular consequence of 14-3-3 binding could be diverse. For example, binding to 14-3-3 may result in inhibition or activation of the partner proteins, and may be accompanied by a conformational change (Obsil et al., 2001; Yaffe 2002); 14-3-3 binding may stimulate protein-protein interaction (Braselmann and McCormick, 1995); 14-3-3 binding may regulate the subcellular localization of the binding proteins; 14-3-3 can mask binding sites for other regulatory proteins (Van Heusden and Steensma, 2006). The functions of the 14-3-3 family proteins are extensively studied, as they are involved in many cell cycle regulations. The regulation of 14-3-3 protein Rad24 in cytoplasmic retention of Clp1 is very similar to the regulation of 14-3-3 protein in cytoplasmic

sequestration of the mitotic activator, Cdc25. Cdc25 is a conserved phosphatase that functions to dephosphorylate the inhibitory tyrosine phosphorylation of Cdc2 in *S. pombe* resulting in subsequent mitotic entry (MacNeill and Nurse, 1997). When the DNA damage checkpoint is activated, Cdc25 is phosphorylated the checkpoint kinase Chk1, leading to Rad24 binding and nuclear exclusion of Cdc25 (Peng et al., 1997; Lopez-Girona et al., 1999; Zen and Piwnica-Worms, 1999). This regulation is observed in other organisms, like Xenopus and human (Dalal et al., 1999; Kumagai, A. and Dunphy, 1999; Kumagai et al., 1998; Yang et al., 1999). Even though Chk1 phosphorylation on Cdc25 creates a 14-3-3 binding site, it may also play an inhibitory role in regulating phosphatase activity of Cdc25, since nuclear exclusion of Cdc25 in not required for the DNA damage checkpoint (Lopez-Girona et al., 2001). It is not clear whether Sid2 phosphorylation on Clp1 and Rad24 binding would affect Clp1 phosphatase activity. However, it is less likely to be the case, because Clp1-6A mutant does not show reduction of its catalytic activity compared to wild-type Clp1 in vivo and in vitro (Figure 3-1G, and Figure 3-S6). It also would be interesting to tag Clp1-6A with a nuclear export signal (NES) to see if Clp1-6A-NES would rescue the negative interaction of Clp1-6A with the actomyosin ring mutants (*mid1-18*, *myo2-E1*, *and cdc15-140*). This experiment may determine whether Sid2 phosphorylation and/or Rad24 binding affect phosphatase activity of Clp1.

The molecular function of Clp1 at the actomyosin ring is still not clear. We found that Clp1-6A is sensitive to perturbation of the actomyosin ring by low doses of Latrunculin B, and negatively interacts with several actomyosin ring mutants (*mid1-18*,

myo2-E1, *and cdc15-140*) (Figure 3-3). However, the actomyosin ring is still intact in *clp1-6A* cells (Figure 3-S4) when cytokinesis has been delayed, and an abnormal but complete septum is still formed in *clp1-6A myo2-E1* cells (Figure 3-S8). It has been reported that Clp1 is recruited to the actomyosin ring by interacting with the anillin-related protein Mid1. The physical tethering of Clp1 to the actomyosin ring promotes dephosphorylation of an actomyosin ring component Cdc15 and reduces the mobility of actomyosin ring proteins (Cdc15, and Myo2) (Clifford et al., 2008), suggesting that even though Clp1 is not essential under normal conditions, it contributes to the robustness of cytokinesis when the process is challenged.

A previous report showed that the serine residues on Clp1 that are phosphorylated by Sid2 can also be phosphorylated by Cds1 to release Clp1 to the nucleus in response to DNA replication blocks (Diaz-Cuervo and Bueno, 2008). In the budding yeast, it has also been shown that the Sid2 homolog Dbf2 kinase is required to drive cytoplasmic release of Cdc14 during exit from mitosis (Mohl et al., 2009). Interestingly, the Lats1/2 kinase, which is the Sid2 homolog in mammalian cells, also retains the oncogene YAP1 in the cytoplasm through phosphorylation and causing its binding to a 14-3-3 protein (Hao et al, 2007; Lei et al., 2008; Zhao et al., 2007; Oh and Irvine, 2008; Dong et al., 2007). Collectively, these results suggested that this regulatory strategy might be conserved from lower to higher eukaryotic cells.

In Chapter IV, we have identified Sid2 substrates by performing a Rad24-TAP purification in different SIN mutant backgrounds. This method was based on our observation that when Sid2 phosphorylates its one known substrate Clp1, it creates binding sites for Rad24 because of their overlapping recognition sequence (RXXS). The rationale is that if Sid2 phosphorylation promotes Rad24 binding, then some of the Rad24 binding partners may be Sid2 substrates. Rad24 binding partners whose level of binding change with changes in SIN activity were considered as potential Sid2 targets. Several proteins showed more binding to Rad24 in hyperactivated SIN, and less binding in SIN defective mutants, including Clp1, which has been shown to be one of the Sid2 kinase substrates (Chapter III). There are still some proteins whose level of Rad24 binding change in SIN activity, but showed lower coverage rate and/or peptide count from the mass spectrometry analysis, making it very hard to determine whether these were real substrates or not. One of the disadvantages of this method is that we were not able to equalize the protein level subjected to mass spectrometry analysis. Even though we started with the same amount of yeast cells, the cell breaking efficiency and the efficiency of TAP purification may vary between experiments. This issue can be resolved, or at least improved, by repeating the purification to see if the patterns of interacting proteins with Rad24 remain similar. Another disadvantage is that this method could not identify Sid2 substrates that do not interact with Rad24; for example, Klp2. Klp2 showed no interaction with Rad24 in asynchronous culture, but can be phosphorylated by Sid2 kinase in vitro. Even though Klp2 localization in late mitosis

responds to SIN activity, probably through Sid2 phosphorylation, we cannot exclude the possibility that Klp2 is a substrate for other SIN kinases, including Cdc7, or Sid1.

Identification of Ase1 and Klp2 as Sid2 candidate substrates suggested that the SIN pathway is not only required for maintaining the eMTOC stability (Pardo and Nurse, 2003) but also functions to regulate nuclear positioning in late mitosis. The significance of Sid2 phosphorylation on Ase1 is still unclear. Further study will be required to understand the role it plays in preventing nuclear congression under regulation of the SIN pathway. It is tempting to speculate that Sid2 phosphorylation of Ase1 affects it ability to load Klp2 on the microtubules but not its localization (Figure 4-4), since His-ase1 and Klp2-GFP bind to each other in vitro (Figure 4-8). However, there is no evidence showing Ase1 interacts with Klp2 in vivo. Moreover, localization of Klp2-GFP to the microtubules does not require Ase1 (Figure 4-9) suggesting that it is less likely the SIN activity prevent nuclear congression by disrupting Ase1 and Klp2 interaction.

The findings of His-klp2 being phosphorylated in vitro and the manner in which localization of Klp2-GFP responds to active SIN suggest that the Sid2 phosphorylation of Klp2 affects its ability to interact with microtubules. Sid2 phosphorylates Klp2 in vitro, and specifically to the tail domain of Klp2 (Figure 4-3). Like Clp1 and other Sid2 candidate substrates, the Klp2 tail-domain contains a cluster of RXXS sites, which are putative Sid2 phosphorylation sites. We hypothesize that Sid2 phosphorylation of Klp2 on the tail-domain may affect its ability to interact to the microtubules, since it has been

reported that the tail region of Klp2 is required to bind and bundle microtubules (Braun et al., 2009). To test the effect of Sid2 phosphorylation on Klp2, it would be interesting to design a phosphomimetic and a non-phosphorylatable version of Klp2 to analyze their ability to interact and organize microtubules in vitro.

However, we still cannot exclude the possibility that the Sid2 phosphorylation of Klp2 affects its ability to interact with other microtubule-associating proteins. Results from Sebastían Mana-Capelli in our lab showed that Klp2 localization on microtubules depended on an EB1 protein Mal3, and *mal3*Δ also rescued the *sid2-250* nuclear clustering phenotype (data not shown). It has been reported that Mal3 binds and stabilizes the microtubule lattice seam (Sandblad et al., 2006), and functions to recruit the +TIP proteins to the growing microtubule plus end, such as Tea2 (a kinesin), and Tip1 (a CLIP-170 homolog) (Busch and Brunner, 2004; Brusch et al., 2004). It is not known whether Klp2 interaction with Mal3 depends on phosphorylation, or there are other microtubule-associating proteins are required to load Klp2 on the microtubules. Performing a co-immunoprecipitation between Klp2 and Mal3 and the Klp2-TAP purification may answer these questions. Moreover, analyzing mutant versions of Klp2 in vivo is required to test how phosphorylation affects Klp2 localization and/or activity. Overall, these findings suggest that the SIN may regulate nuclear positioning and microtubule dynamics through these microtubule-binding proteins.

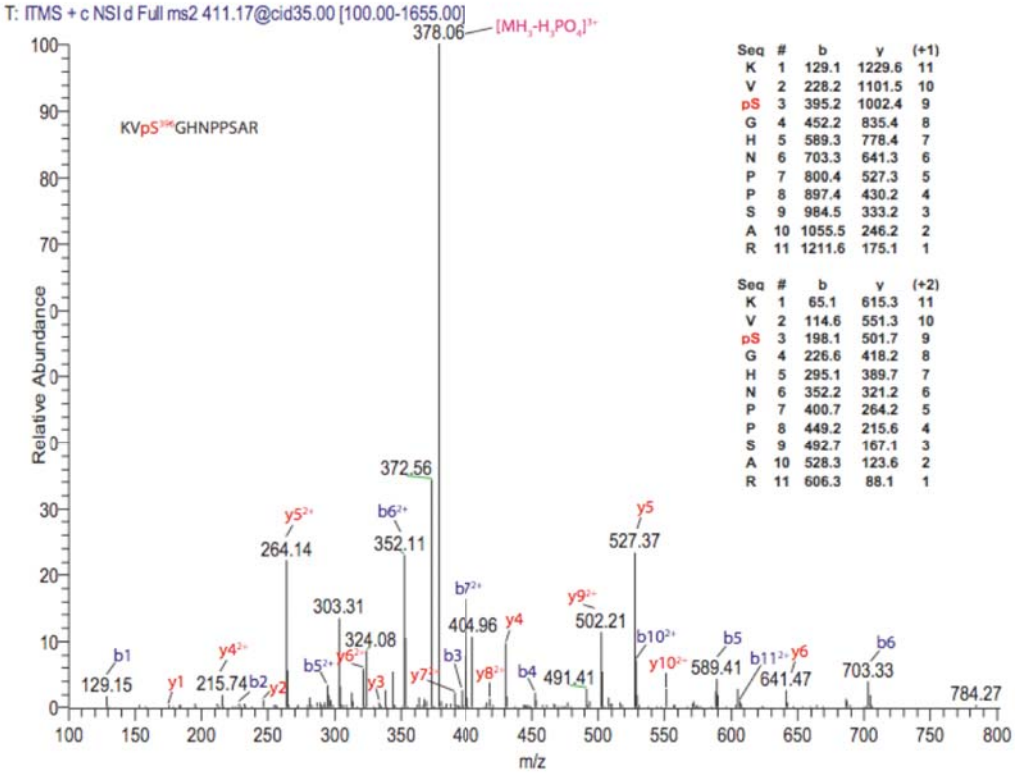
In conclusion, these results suggested that this method of identifying Sid2 substrates not only provides several potential candidates but also give us several hints about the function of the SIN pathway during late mitosis.

Appendix A

Supplemental Figures

Figure 3-S1

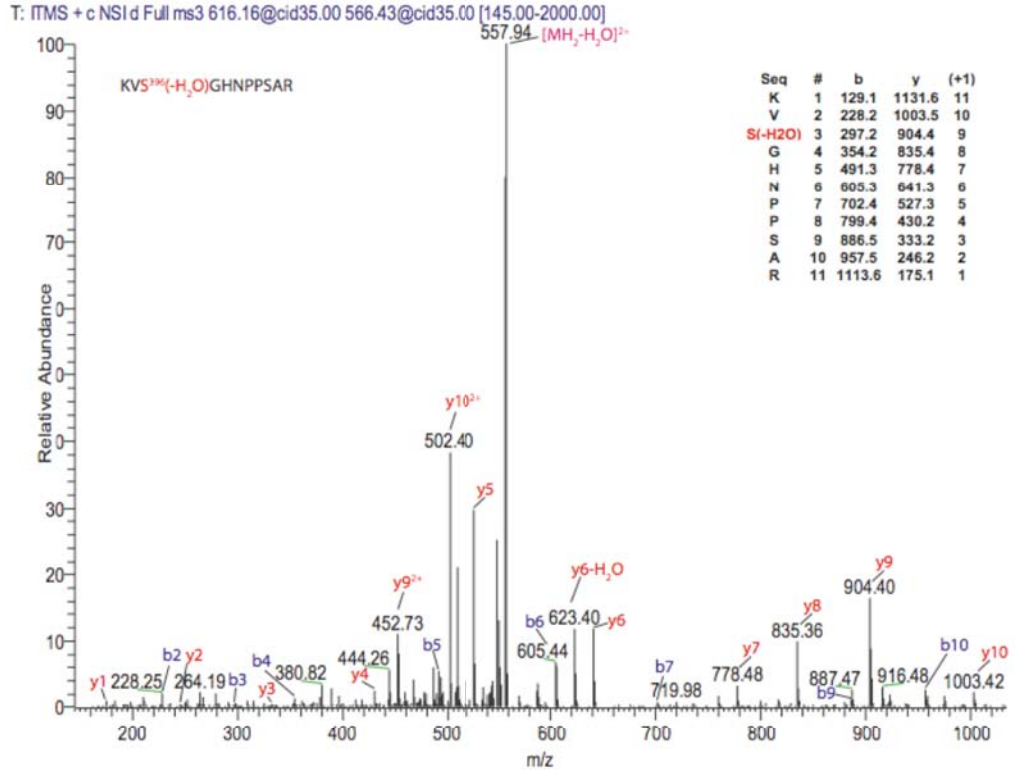
A



Seq	#	b	y	(+1)
K	1	129.1	1229.6	11
V	2	228.2	1101.5	10
pS	3	395.2	1002.4	9
G	4	452.2	835.4	8
H	5	589.3	778.4	7
N	6	703.3	641.3	6
P	7	800.4	527.3	5
P	8	897.4	430.2	4
S	9	984.5	333.2	3
A	10	1055.5	246.2	2
R	11	1211.6	175.1	1

Seq	#	b	y	(+2)
K	1	65.1	615.3	11
V	2	114.6	551.3	10
pS	3	198.1	501.7	9
G	4	226.6	418.2	8
H	5	295.1	389.7	7
N	6	352.2	321.2	6
P	7	400.7	264.2	5
P	8	449.2	215.6	4
S	9	492.7	167.1	3
A	10	528.3	123.6	2
R	11	606.3	88.1	1

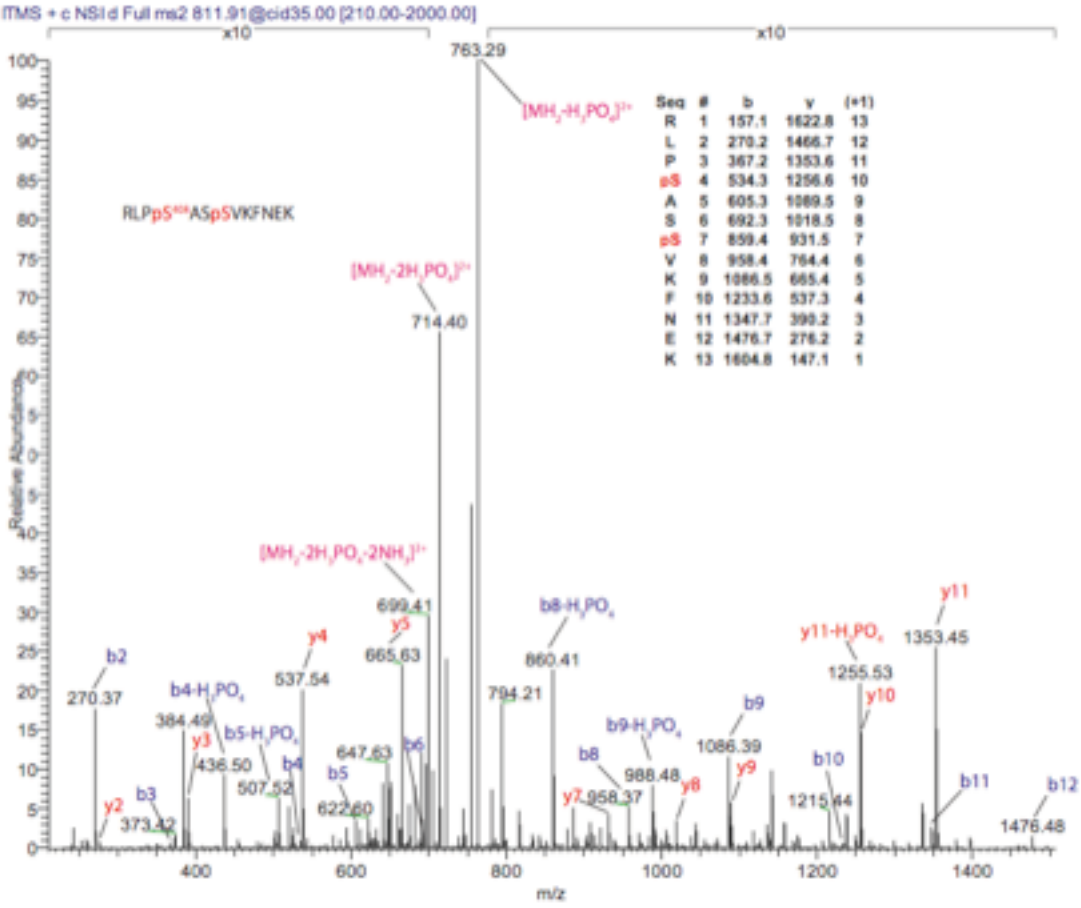
B



Seq	#	b	y	(+1)
K	1	129.1	1131.6	11
V	2	228.2	1003.5	10
S(-H2O)	3	297.2	904.4	9
G	4	354.2	835.4	8
H	5	491.3	778.4	7
N	6	605.3	641.3	6
P	7	702.4	527.3	5
P	8	799.4	430.2	4
S	9	886.5	333.2	3
A	10	957.5	246.2	2
R	11	1113.6	175.1	1

Figure 3-S1

C



D

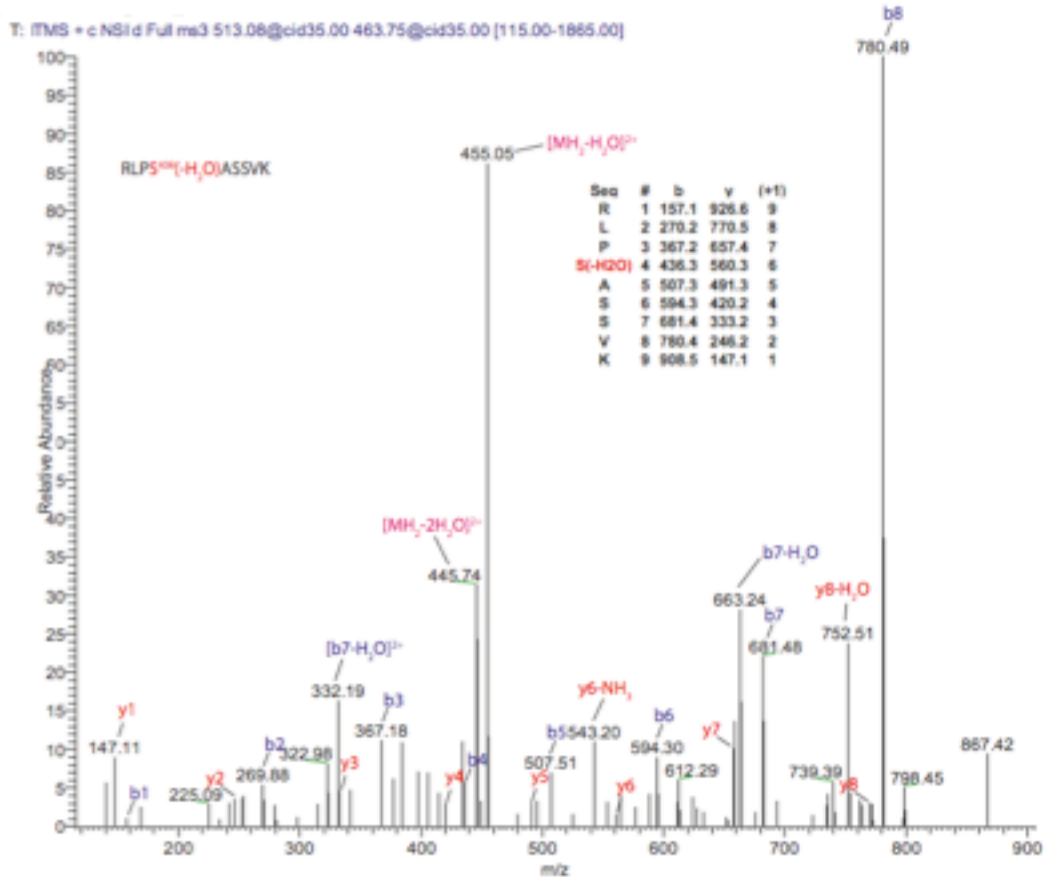
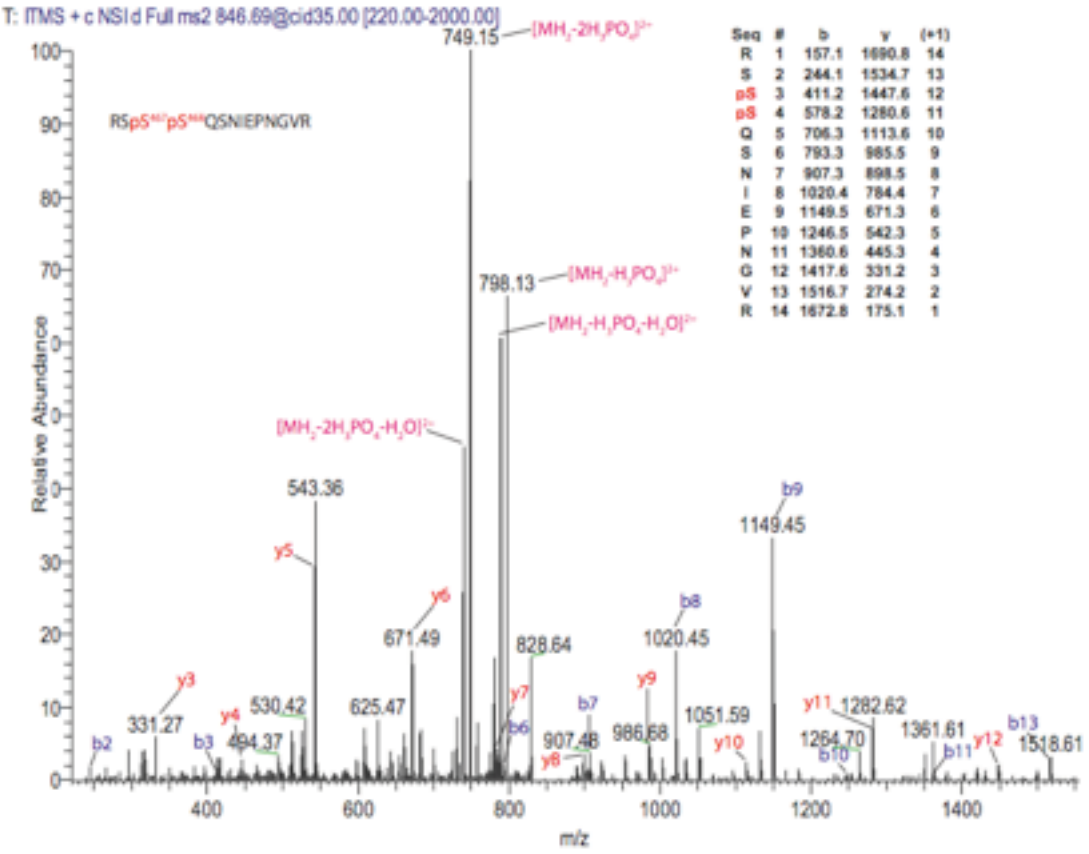


Figure 3-S1

E



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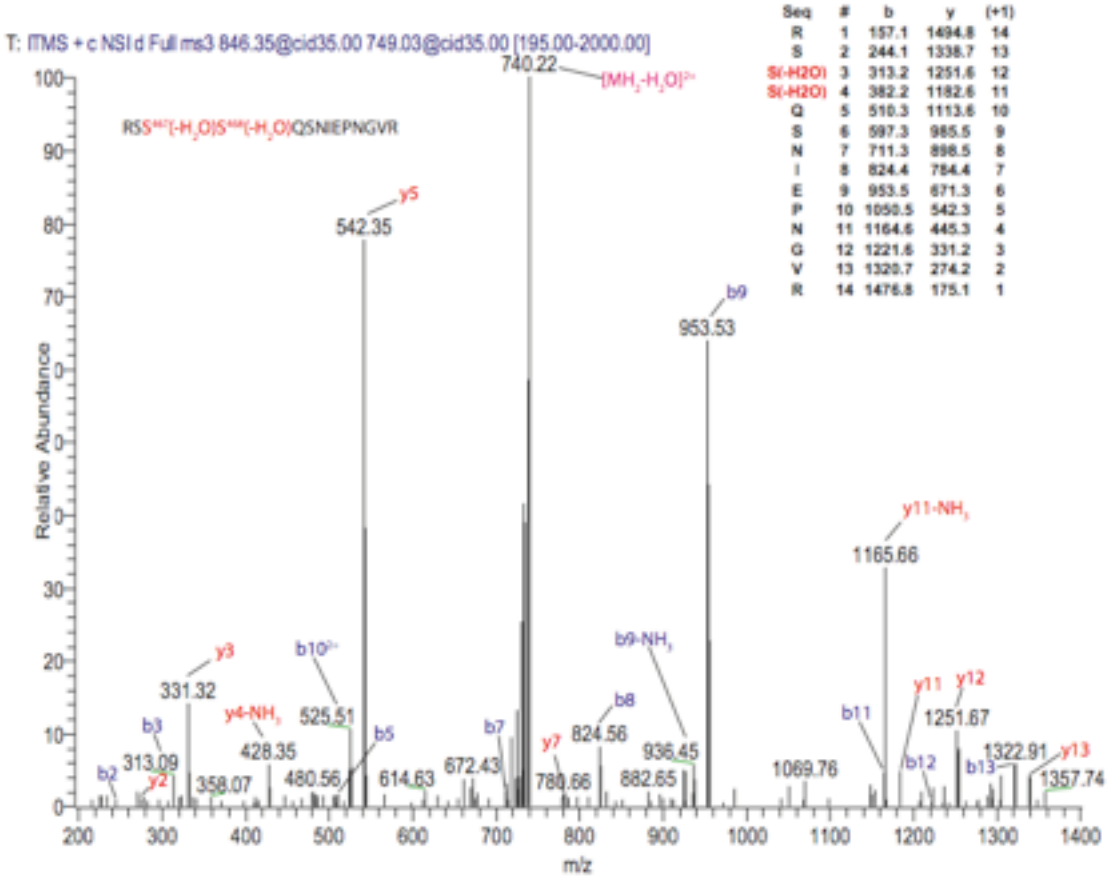
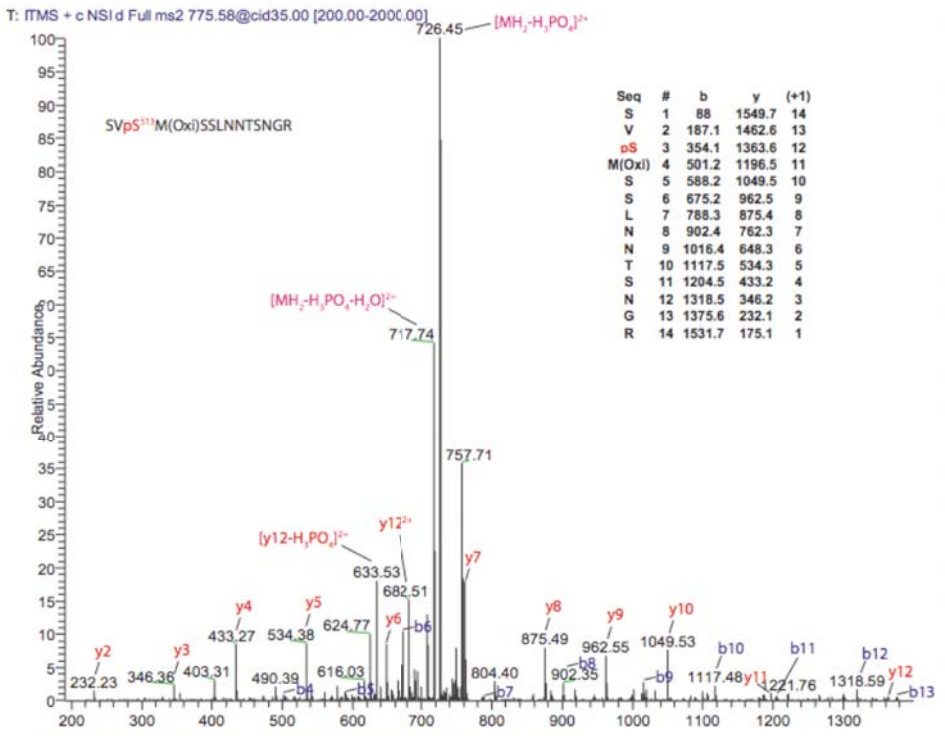


Figure 3-S1

G



H

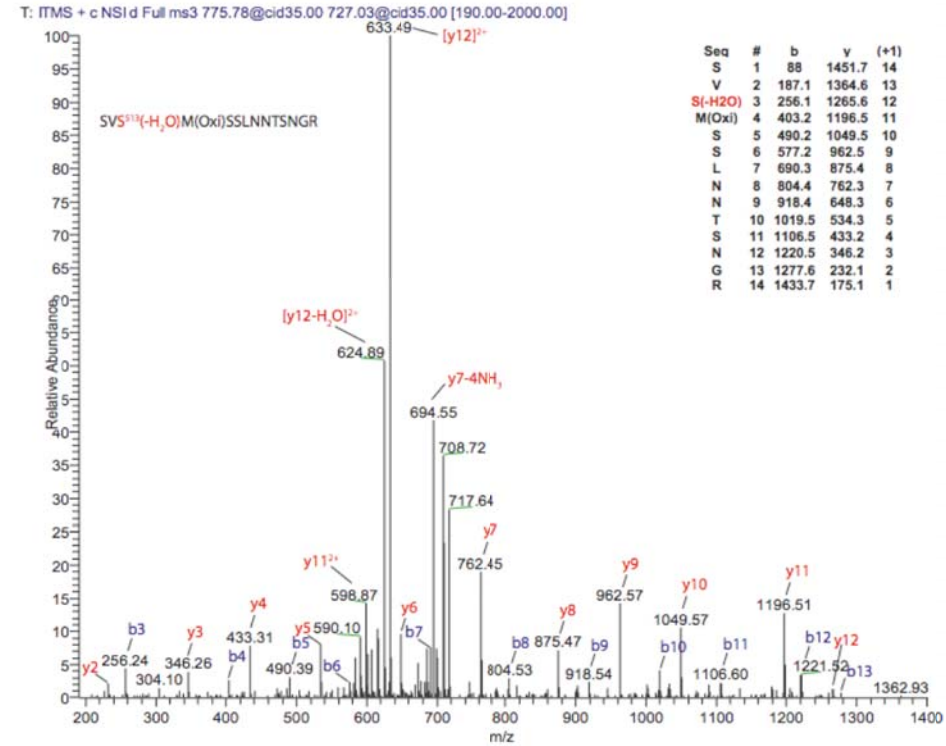


Figure S1. MS2 and MS3 spectra of Clp1 peptides containing the identified phosphorylation sites by Sid2.

Clp1 tandem affinity purification followed by MudPIT mass spectrometric analysis was performed from lysates made from *clp1-TAP nda3-km311* or *clp1-C286S-TAP nda3-km311* cells 30 minutes after release from a prometaphase arrest. The MS2 spectra (Fig. S1A, C, E, and G) of Clp1 phosphopeptides identified as well as the MS3 spectra (Fig. S1B, D, F, and H) of peptides resulting from the neutral loss of phosphoric acid are shown. b- and y-type ions are labeled.

Figure 3-S2

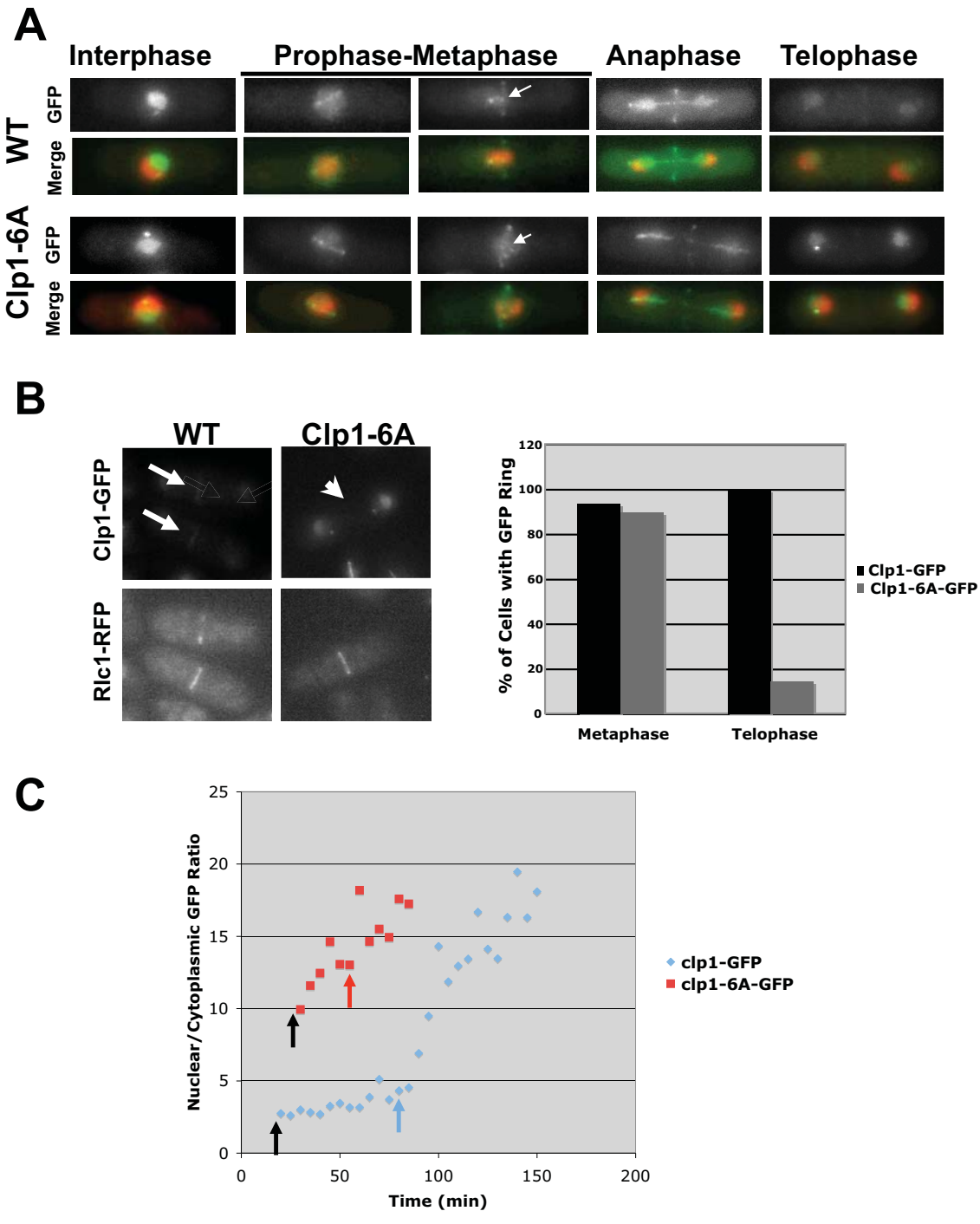

A
Interphase
Prophase-Metaphase
Anaphase
Telophase
WT
GFP
Merge
Clp1-6A
GFP
Merge
B
WT
Clp1-6A
Clp1-GFP
Rlc1-RFP
% of Cells with GFP Ring
120
100
80
60
40
20
0
Metaphase
Telophase
Clp1-GFP
Clp1-6A-GFP
C
Nuclear/Cytoplasmic GFP Ratio
25
20
15
10
5
0
0
50
100
150
200
Time (min)
clp1-GFP
clp1-6A-GFP

Figure S2. Clp1-6A-GFP localization during the cell cycle.
(A) *clp1-GFP* or *clp1-6A-GFP* cells were grown to mid-log phase at 25°C, fixed with methanol, and stained with DAPI. The GFP localization at different stages of cell cycle is shown in the top panels, and GFP images (green) merged with DAPI (red) are in the bottom panels. β-tubulin mutant cells, *nda3-KM311*, (third panel with arrows) expressing Clp1-GFP or Clp1-6A-GFP were arrested at 19°C prior to fixation and DAPI staining. The arrows indicate the localization of Clp1-GFP and Clp1-6A-GFP to nuclear puncta presumed to be kinetochores. (B) *clp1-GFP* and *clp1-6A-GFP* both expressing Rlc1-RFP were cultured to mid-log phase at 30°C. The GFP and RFP signals in live cells are shown. The localization of Clp1-GFP on the actomyosin ring is indicated by arrows, and the absence of Clp1-6A-GFP from the ring is indicated by an arrowhead. Cells with GFP signal at the actomyosin ring were quantified in different mitotic stages (metaphase and telophase) using Rlc1-RFP as a marker for the actomyosin ring. At least 100 cells were scored for each strain. (C) Nucleolar/cytoplasmic GFP ratios of cells shown in the Figure 2A time-lapse analysis were measured using IPLab Spectrum software. Measurements were taken from the point that the mitotic spindle disassembled (20 minutes for *clp1-GFP*, and 30 minutes for *clp1-6A-GFP*). The background signal was subtracted from the average nucleolar and cytoplasmic GFP intensity, and the ratio of nucleolar to cytoplasmic GFP intensity is shown. The black arrows indicate the time when the mitotic spindle breaks down, and the colored arrows indicate the time when the actomyosin ring finished constriction. We realize that cytoplasmic Rlc1-GFP contributes slightly to the overall cytoplasmic signal, however its contribution should be the same in both *clp1-GFP* and *clp1-6A-GFP* cells and not effect overall conclusions about the relative timing of return of Clp1 and Clp1-6A to the nucleolus.

Figure 3-S3

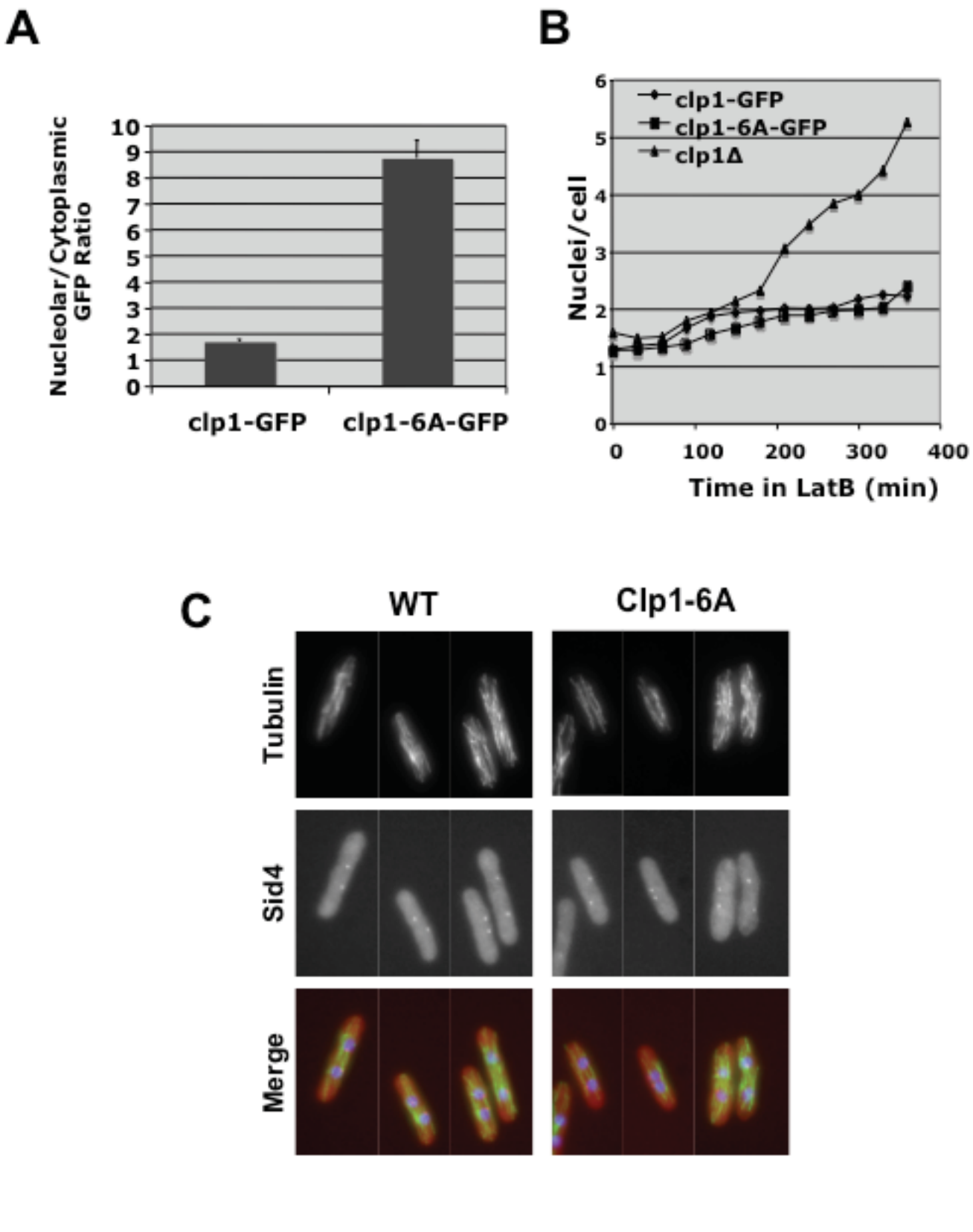
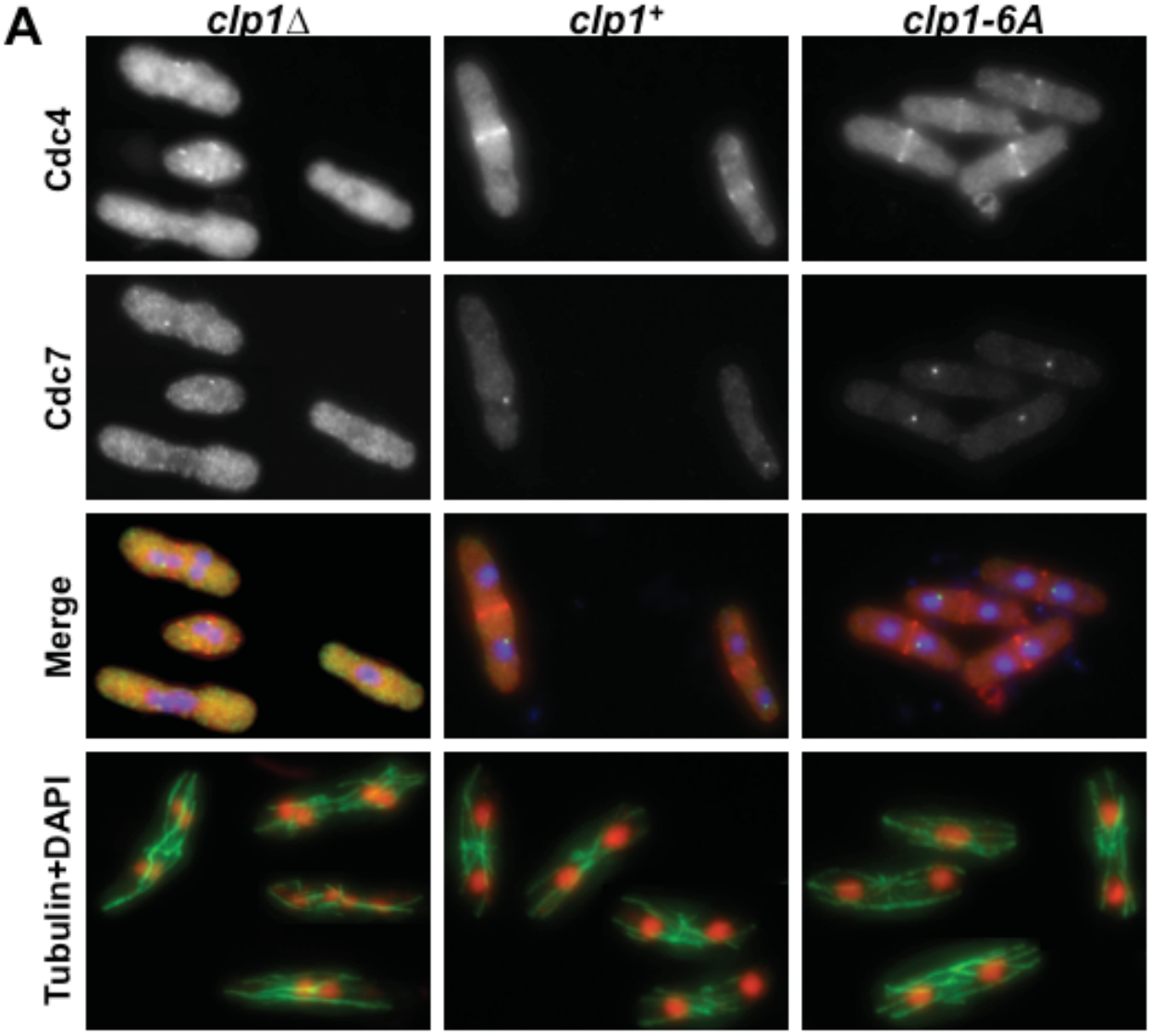

A
Nucleolar/Cytoplasmic GFP Ratio
0
1
2
3
4
5
6
7
8
9
10
clp1-GFP
clp1-6A-GFP
B
Nuclei/cell
0
1
2
3
4
5
6
clp1-GFP
clp1-6A-GFP
clp1Δ
0
100
200
300
400
Time in LatB (min)
C
WT
Clp1-6A
Tubulin
Sid4
Merge

Figure S3. Clp1-6A-GFP function in the cytokinesis checkpoint.
(A) *clp1-GFP*, and *clp1-6A-GFP* cells were grown to mid-log phase, treated with 4μM Latrunculin B (Sigma) for 3.5 hours, and then fixed with methanol and DAPI stained. The nucleolar/cytoplasmic GFP ratio was measured as described in Figure S2C. (B) *clp1-GFP*, *clp1-6A-GFP*, and *clp1Δ* cells were treated as described in Figure 2B. The number of nuclei per cell in *clp1-GFP*, *clp1-6A-GFP*, and *clp1Δ* were scored at each time point. (C) *clp1-GFP*, and *clp1-6A-GFP* cells expressing Sid4-RFP (as an SPB marker) were treated with 4μM Latrunculin B as described in Figure S3A. Cells were methanol fixed, and stained with α-TAT1 antibodies, and DAPI to visualize tubulin, and DNA (Appendix C). Tubulin was shown in green, Sid4 in red, and DNA in blue.

Figure 3-S4


A
clp1Δ
clp1+
clp1-6A
Cdc4
Cdc7
Merge
Tubulin+DAPI

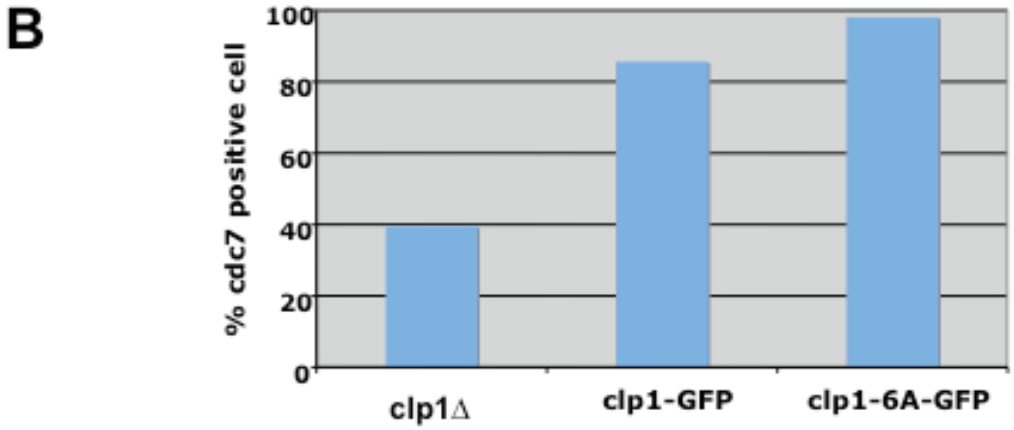

B
% cdc7 positive cell
100
80
60
40
20
0
clp1Δ
clp1-GFP
clp1-6A-GFP

Figure S4. Clp1-6A-GFP function in the cytokinesis checkpoint.
(A) *clp1Δ cdc7-HA cps1-191*, *clp1-GFP cdc7-HA cps1-191*, and *clp1-6A-GFP cdc7-HA cps1-191* cells were grown to mid-log phase at 25°C, and shifted to 36°C for 4 hours. Cells were methanol fixed, and stained with α-Cdc4 (red) α-HA antibodies (green), and, and DAPI (blue) to visualize actomyosin rings, Cdc7-3HA, and DNA, respectively in the merged image (Merge). The bottom panel shows tubulin (green) and DNA (red) stained with α-TAT1 antibodies and DAPI respectively. (B) Percentage of binucleate cells with Cdc7 signal at one SPB was scored. At least 100 cells were counted for each strain.

Figure 3-S5

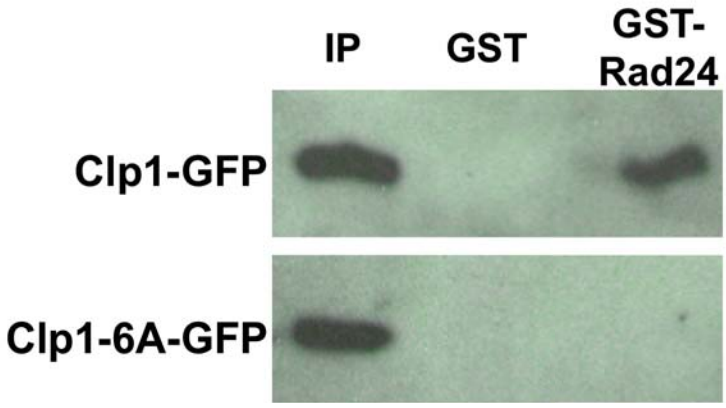
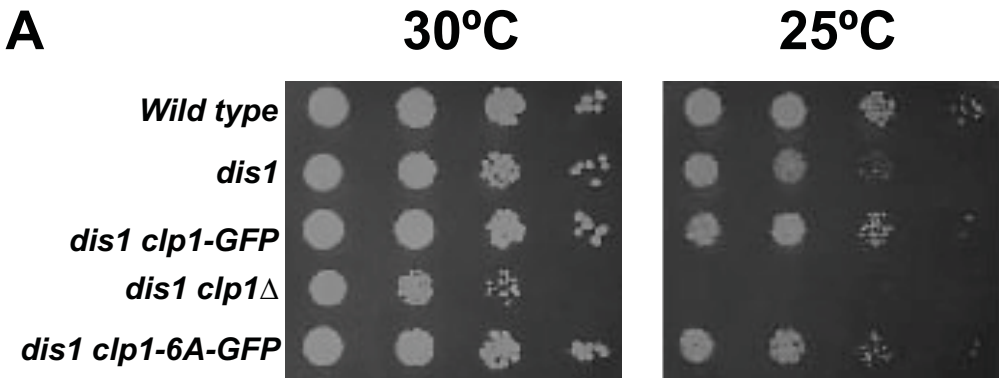
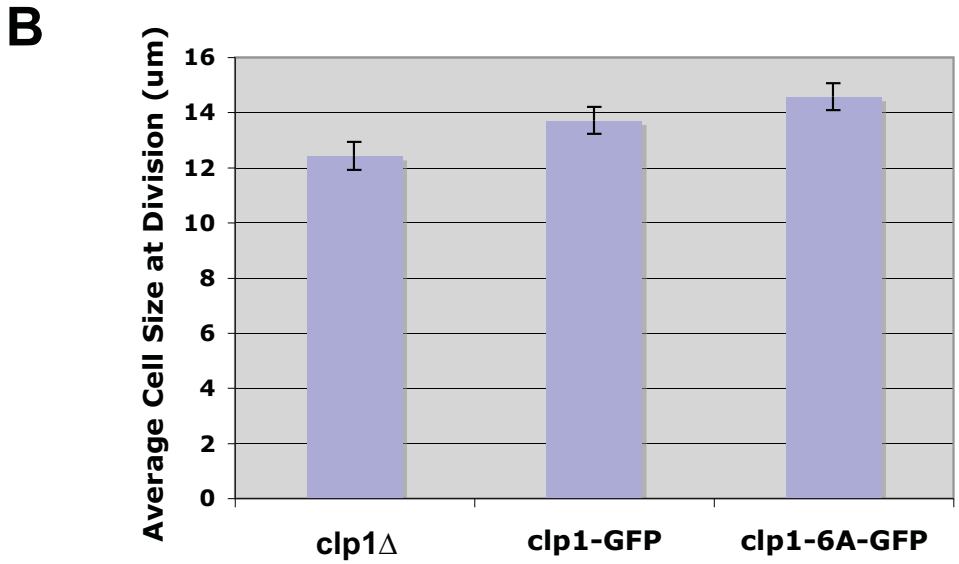


Figure S5. Clp1-6A-GFP failed to bind to GST-Rad24.
clp1-GFP and *clp1-6A-GFP* cells in an *nda3-KM311* cold sensitive background were grown at 30°C, shifted to 19°C for 6 hours, then released to 30°C for 20 minutes. In vitro binding assay was performed as described in Figure 2D.

Figure 3-S6


A
30ºC
25ºC
Wild type
dis1
dis1 clp1-GFP
dis1 clp1Δ
dis1 clp1-6A-GFP


B
Average Cell Size at Division (um)
16
14
12
10
8
6
4
2
0
clp1Δ
clp1-GFP
clp1-6A-GFP

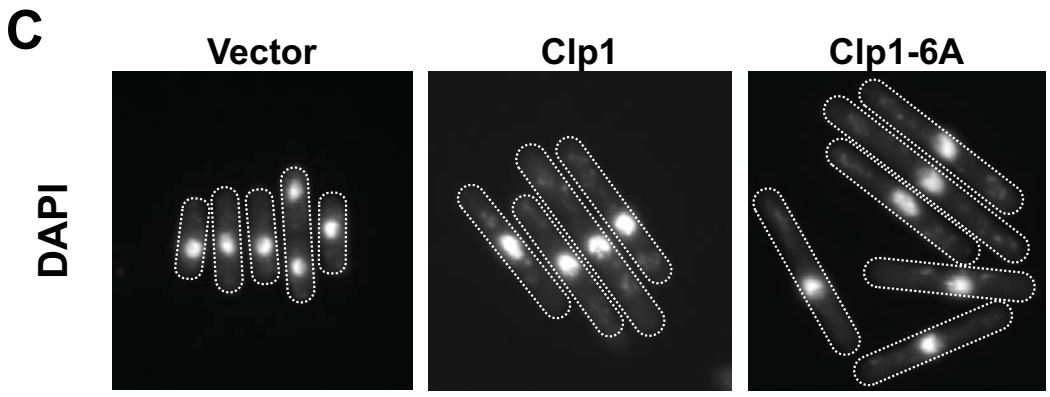

C
Vector
Clp1
Clp1-6A
DAPI

Figure S6. *clp1-6A-GFP* function in cell cycle regulation.
(A) *dis1* in different *clp1* mutant backgrounds (*clp1-GFP*, *clp1-6A-GFP*, and *clp1Δ*) and wild-type cells were grown to mid-log phase, spotted on YE plates in 10-fold serial dilutions, and kept at 25°C and 30°C. (B) Cell size at division was examined in asynchronous cultures of *clp1Δ*, *clp1-GFP*, and *clp1-6A-GFP* at 36°C. Lengths are shown in μm. 100 cells were scored per genotype. (C) *clp1*$^{+}$ and *clp1-6A* were cloned into the pRep3X plasmid, and the plasmids were transformed into wild-type cells. Protein expression was induced in media lacking thiamine for 16 hours at 30°C. Cells were methanol fixed and DAPI stained. Cell edges (dotted lines) were outlined manually.

Figure 3-S7

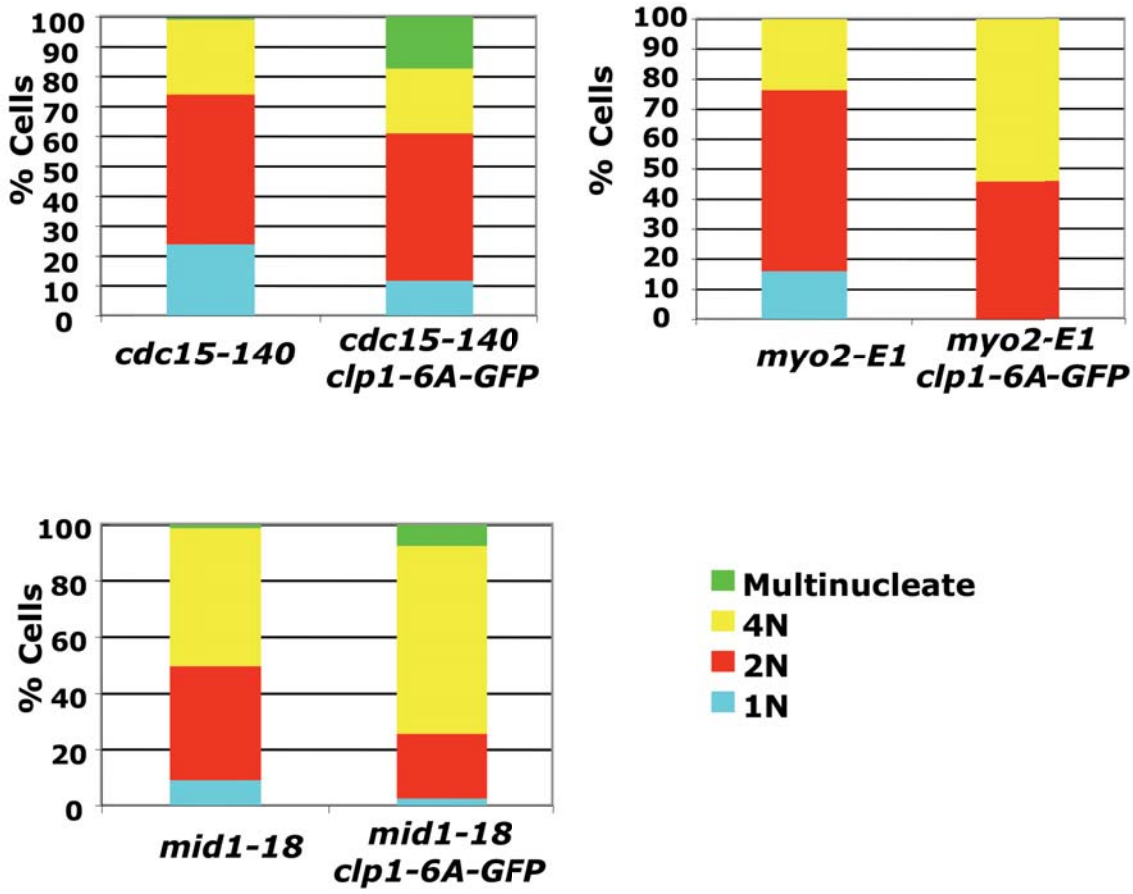


Figure S7. Cytokinetic defects of *clp1-6A-GFP* after actomyosin ring perturbation. Cells with the indicated genotypes were grown to mid-log phase at 25°C. *cdc15-140*, *cdc15-140 clp1-6A-GFP*, *myo2-E1*, and *myo2-E1 clp1-6A-GFP* were shifted to 30°C for 8 hours, and *mid1-18*, *mid1-18 clp1-6A-GFP* were shifted to 36°C for 8 hours. The cells were subjected to methanol fixation, DAPI and calcofluor white staining. Quantification of mononucleate (1N), binucleate (2N), tetranucleate (4N), and multinucleate (> 4 nuclei) cells were scored. At least 100 cells were counted per genotype.

Figure 3-S8

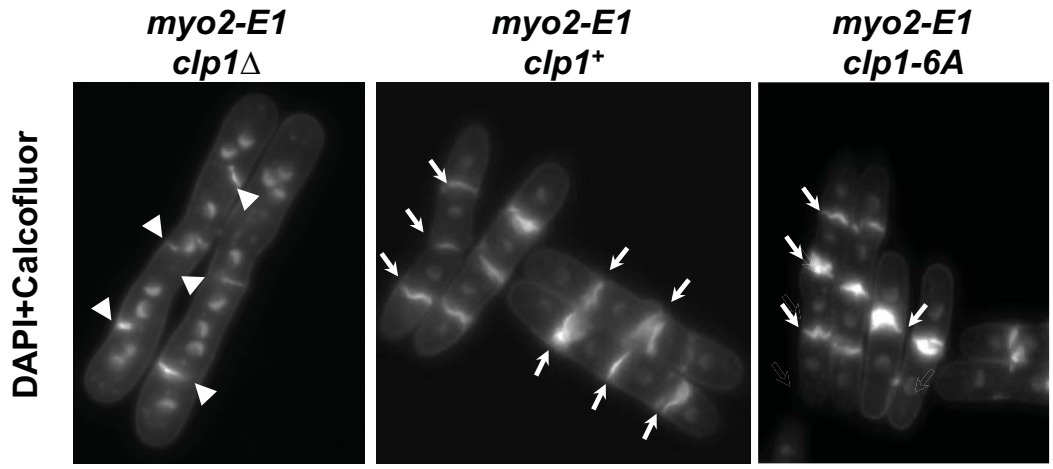


Figure S8. Role of Clp1 in ring formation.
clp1-GFP, *clp1-6A-GFP*, and *clp1Δ* cells in a *myo2-E1* temperature sensitive background were grown to mid-log phase at 25°C and shifted to 30°C for 8 hours. Cells were methanol fixed and stained with DAPI and calcofluor white (10 μg/μl) to visualize DNA and cell wall.

Figure 3-S9

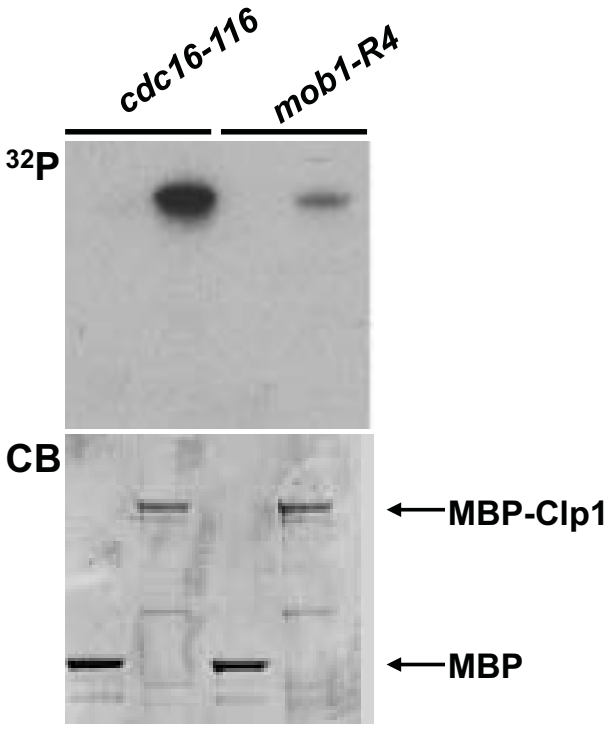


Figure S9. MBP-Clp1 phosphorylation depends on Sid2 kinase.
Sid2-13Myc was immunoprecipitated from a *cdc16-116* or *mob1-R4* background that had been shifted to 36°C for 4 hours, and the immunoprecipitates were incubated with purified MBP or MBP-Clp1 in the presence of labeled ATP. Following 30 minutes at 30°C, the reactions were resolved by SDS-PAGE. The gel was stained with Coomassie Blue (CB) (lower panel), dried, and then exposed to film (^{32}P) (top panel).

Appendix B

Candidate substrates of Sid2 kinase

- Summary of candidates whose Rad24 co-purification was altered by elevated and reduced SIN pathway activity.

Candidate Proteins	Peptide count		
Protein name	Active SIN (*cdc16-116*)	Asynchronous	Inactive SIN (*sid1-239*)
SPAC3G9.05	355.0625521	243.7395765	64.2061702
SPBC3B8.10c	276.0783001	223.6548709	147.240662
Clp1	166.7464334	124.6619753	6.447419591
Scw1	223.6548709	93.84025234	65.11365966
Ase1	181.4797995	64.2061702	20.59767144
Cdc11	65.11365966	51.89839971	47.93439979
SPAC16E8.08	98.82155623	50.12729307	4.704885081
SPAC31A2.14	50.12729307	47.93439979	42.33370822
mph1	54.01876276	32.78985216	18.80233852
SPAC12B10.03	42.77241121	30.9951568	19.82778848
ags1	28.54600188	24.23101796	14.17033544
cct6	28.87993002	20.17274892	9.374161888
SPCPB16A4.02c	25.45729895	20.59767144	17.31877577
rip1	21.18959382	19.82778848	15.04975193
rga6	21.87076656	17.31877577	3.464101615
SPAC17A5.10	20.59767144	17.98145283	14.38673902
SPAC23C11.05	75.26551823	18.2261547	12.56930154
rsp1	28.05785615	14.17033544	7.085167718
SPBC1703.07	41.10147477	14.58252197	2.783157684
cct8	17.31877577	12.94491717	9.374161888
SPBP4H10.11c	16.2889528	13.8019598	5.383563271
SPBC646.09c	17.31877577	11.91578427	5.82590126
nak1	142.6808726	10.88029143	10.41362251
arg11	10.88029143	9.188442941	3.080070288
puf3	16.08140784	9.374161888	7.672304127
rpn2	10.01994028	9.374161888	2.783157684
SPCC895.08c	15.45143125	8.323582901	3.600205744
Sog2	173.3425193	8.323582901	6.447419591
rpt1	13.8019598	7.085167718	3.600205744
rpt4	15.25455753	7.085167718	2.514866859
SPAC17G8.11c	22.42356059	7.085167718	6.447419591
cct3	6.900979901	5.383563271	3.464101615
cct4	17.31877577	5.383563271	4.704885081
SPAC22A12.16	42.33370822	5.634626495	2.942830956
SPAC1F12.07	9.374161888	6	2.514866859

rad24-HA-TAP sampleA		Coverage rate	Peptide count
|rad24||14-3-3 protein Rad24|Schizosaccharomyces pombe|chr	SPAC8E11.02c	96.70%	7576
|rad25||14-3-3 protein Rad25|Schizosaccharomyces pombe|chr	SPAC17A2.13c	98.50%	2026
|mug161||CwfJ family protein|Schizosaccharomyces pombe|chr	SPAC1F3.09	26.40%	278
|sec2||guanyl-nucleotide exchange factor Sec2 |Schizosaccha	SPAC23C4.10	49.70%	243
|||leucine-rich repeat protein, unknown|Schizosaccharomyces	SPAC926.06c	40.10%	226
|cdc15||cell division control protein Cdc15|Schizosaccharom	SPAC20G8.05c	52.20%	214
|zfs1|moc4|transcription factor Zfs1 |Schizosaccharomyces p	SPBC1718.07c	50.00%	186
|||GTPase activating protein |Schizosaccharomyces pombe|chr	SPAC3G9.05	57.20%	185
|||NLI interacting factor family|Schizosaccharomyces pombe|	SPBC3B8.10c	45.20%	177
|mac1||membrane anchored protein Mac1 |Schizosaccharomyces	SPAC13G7.04c	50.00%	155
|ppk36|atg1|serine/threonine protein kinase Ppk36|Schizosac	SPCC63.08c	29.80%	119
|cyk3||cytokinesis protein Cyk3|Schizosaccharomyces pombe|c	SPAC9G1.06c	37.20%	118
|||ketopantoate reductase |Schizosaccharomyces pombe|chr 1|	SPAC24B11.07c	41.00%	116
||SPCC1906.05|zf-CCCH type zinc finger protein|Schizosaccha	SPCC1739.01	50.80%	111
|pik1||phosphatidylinositol kinase Pik1|Schizosaccharomyces	SPAC22E12.16c	29.50%	98
|||arrestin/PY protein 2|Schizosaccharomyces pombe|chr 3|||	SPCC584.15c	43.10%	96
|||diacylglycerol binding protein |Schizosaccharomyces pomb	SPCC297.05	37.30%	95
|rga4||GTPase activating protein Rga4|Schizosaccharomyces p	SPBC28E12.03	35.80%	94
|cdc25|sal2|serine/threonine protein phosphatase Cdc25|Schi	SPAC24H6.05	37.10%	93
|dfr1||dihydrofolate reductase Dfr1|Schizosaccharomyces pom	SPCC1223.08c	43.20%	86

|clp1|flp1|Cdc14-related protein phosphatase Clp1/Flp1|Schi	SPAC1782.09c	42.50%	82
|pom1||DYRK family protein kinase Pom1|Schizosaccharomyces	SPAC2F7.03c	27.10%	80
	SPBP35G2.14	24.40%	76
|ppk15||serine/threonine protein kinase Ppk15 |Schizosaccha	SPAC823.03	41.40%	75
|whi5|mug54|cell cycle transcriptional repressor Whi5|Schiz	SPBC800.02	52.40%	74
|||inositol polyphosphate kinase |Schizosaccharomyces pombe	SPCC970.08	24.90%	74
|gaf1|SPCC417.01c|transcription factor Gaf1 |Schizosaccharo	SPCC1902.01	18.40%	72
|scw1||RNA-binding protein Scw1|Schizosaccharomyces pombe|c	SPCC16C4.07	41.00%	69
|nte1||lysophospholipase|Schizosaccharomyces pombe|chr 3|||	SPCC4B3.04c	16.60%	62
|||Usp |Schizosaccharomyces pombe|chr 2|||Manual	SPBC25B2.10	35.80%	62
|dna2||DNA replication endonuclease-helicase Dna2|Schizosac	SPBC16D10.04c	26.30%	61
|chr4|cfh3, SPBC1539.11c|chitin synthase regulatory factor	SPBC1289.01c	43.00%	58
|||protein disulfide isomerase |Schizosaccharomyces pombe|c	SPBC3D6.13c	21.90%	58
|mei2||RNA-binding protein involved in meiosis Mei2|Schizos	SPAC27D7.03c	27.30%	57
|hem14||protoporphyrinogen oxidase|Schizosaccharomyces pomb	SPAC1F5.07c	36.70%	52
|oac1||anion transporter|Schizosaccharomyces pombe|chr 1|||	SPAC139.02c	34.10%	52
|rgf2||RhoGEF Rgf2|Schizosaccharomyces pombe|chr 1|||Manual	SPAC1006.06	15.80%	52
|||BAR adaptor protein|Schizosaccharomyces pombe|chr 2|||Ma	SPBC19C2.10	27.70%	52
	SPAPB17E12.14c	43.70%	51
|||RNA-binding protein|Schizosaccharomyces pombe|chr 1|||Ma	SPAC17H9.04c	34.60%	51
|ase1||microtubule-associated protein Ase1 |Schizosaccharom	SPAPB1A10.09	38.60%	47
|||Spo7 homolog|Schizosaccharomyces pombe|chr 2|||Manual	SPBC902.03	41.70%	47

|chk1|rad27|Chk1 protein kinase|Schizosaccharomyces pombe|c	SPCC1259.13	30.80%	46
|rad22||DNA repair protein Rad22|Schizosaccharomyces pombe|	SPAC30D11.10	45.20%	46
|ppk38||Ark1/Prk1 family protein kinase Ppk38|Schizosacchar	SPCP1E11.02	23.10%	43
	SPAC18G6.09c	25.00%	42
|its3||1-phosphatidylinositol-4-phosphate 5-kinase Its3|Sch	SPAC19G12.14	20.10%	41
|||inorganic phosphate transporter |Schizosaccharomyces pom	SPBC1703.13c	32.20%	41
|cdc11||SIN component scaffold protein Cdc11|Schizosaccharo	SPCC1739.11c	19.10%	40
|||sequence orphan|Schizosaccharomyces pombe|chr 1|||Manual	SPAC16E8.08	51.70%	38
|cam1||calmodulin Cam1 |Schizosaccharomyces pombe|chr 1|||M	SPAC3A12.14	92.00%	37
|wis1|spc2, smf2|MAP kinase kinase Wis1|Schizosaccharomyces	SPBC409.07c	21.50%	37
|||AAA family ATPase, unknown biological role|Schizosacchar	SPBC947.01	18.80%	37
|||WD repeat protein, human WRDR48 family|Schizosaccharomyc	SPAC31A2.14	17.90%	37
|mod5||Tea1 anchoring protein Mod5|Schizosaccharomyces pomb	SPBC530.04	19.20%	34
|scr1||transcription factor Scr1|Schizosaccharomyces pombe|	SPBC1D7.02c	18.10%	34
|wis4|wak1, wik1|MAP kinase kinase kinase Wis4|Schizosaccha	SPAC9G1.02	14.10%	34
|rap1||telomere binding protein Rap1|Schizosaccharomyces po	SPBC1778.02	23.80%	33
|bud6|aip3, fat1, SPAC15E1.01|actin interacting protein 3 h	SPAC15A10.16	15.90%	31
|||NAD/NADH kinase |Schizosaccharomyces pombe|chr 1|||Manua	SPAC1B1.02c	22.30%	31
|rga3||GTPase activating protein Rga3|Schizosaccharomyces p	SPAC29A4.11	19.50%	29
|||WD repeat protein, human WDR68 family|Schizosaccharomyce	SPBC17D11.08	36.30%	29
|ptc1||protein phosphatase 2C Ptc1|Schizosaccharomyces pomb	SPCC4F11.02	40.60%	28
|arf1||ADP-ribosylation factor Arf1|Schizosaccharomyces pom	SPBC4F6.18c	51.70%	27

	SPAPB1A10.13	16.80%	27
|taf1||Taz1 interacting factor 1|Schizosaccharomyces pombe|	SPAC7D4.04	12.20%	26
|mph1|SPBC1271.16c, SPBC243.01|dual specificity protein kin	SPBC106.01	22.40%	25
|nrm1||negative regulator of MBF|Schizosaccharomyces pombe|	SPBC16A3.07c	21.90%	25
|||zf-C3HC4 type zinc finger|Schizosaccharomyces pombe|chr	SPBC25B2.03	25.50%	25
|uve1|uvde|endonuclease Uve1 |Schizosaccharomyces pombe|chr	SPBC19C7.09c	5.00%	24
||SPCC1753.06c|sequence orphan|Schizosaccharomyces pombe|ch	SPCC162.12	18.80%	24
|gef1||RhoGEF Gef1|Schizosaccharomyces pombe|chr 1|||Manual	SPAC24H6.09	15.90%	23
|||WD repeat protein, human WDR20 family|Schizosaccharomyce	SPAC12B10.03	15.70%	23
|cdc22||ribonucleoside reductase large subunit Cdc22|Schizo	SPAC1F7.05	12.50%	22
|ppk22||serine/threonine protein kinase Ppk22 |Schizosaccha	SPBC1861.09	18.10%	22
||SPCC285.18|ubiquitin-protein ligase E3 |Schizosaccharomyc	SPCC1223.01	11.30%	22
|||1-acylglycerol-3-phosphate O-acyltransferase|Schizosacch	SPAC1851.02	20.10%	22
|cek1||serine/threonine protein kinase Cek1|Schizosaccharom	SPCC1450.11c	14.60%	21
|exo1|mut2|exonuclease I Exo1|Schizosaccharomyces pombe|chr	SPBC29A10.05	18.60%	21
|tea3||cell end marker Tea3|Schizosaccharomyces pombe|chr 1	SPAC6G10.02c	7.90%	21
|vps901|vps9a|guanyl-nucleotide exchange factor Vps901 |Sch	SPBC4F6.10	18.40%	21
||SPCC736.16|DUF1769 family protein|Schizosaccharomyces pom	SPCC594.01	12.60%	21
|fba1||fructose-bisphosphate aldolase Fba1|Schizosaccharomy	SPBC19C2.07	26.30%	20
|map1||MADS-box transcription factor Map1|Schizosaccharomyc	SPAC11E3.06	25.90%	20
|pal1||membrane associated protein Pal1 |Schizosaccharomyce	SPCP1E11.04c	24.50%	20
|||CTP synthase |Schizosaccharomyces pombe|chr 1|||Manual	SPAC10F6.03c	17.80%	20

|||MTC tricarboxylate transporter|Schizosaccharomyces pombe	SPAC17G6.15c	24.90%	20
|ags1|mok1, SPCC338.01c, SPCC17A7.01|alpha-1,4-glucan synth	SPCC1281.01	4.70%	19
|cdr2||GIN4 family protein kinase Cdr2|Schizosaccharomyces	SPAC57A10.02	11.00%	19
|vps1|SPAC9G1.14c|dynamin family protein Vps1|Schizosacchar	SPAC767.01c	6.90%	18
|mbx1||MADS-box transcription factor Mbx1|Schizosaccharomyc	SPBC19G7.06	14.40%	17
|||NADH dehydrogenase|Schizosaccharomyces pombe|chr 2|||Man	SPBC947.15c	12.30%	17
|alg2|SPBC32H8.14|mannosyltransferase complex subunit Alg2|	SPBC11B10.01	15.30%	16
|cdc24||DNA replication protein Cdc24|Schizosaccharomyces p	SPAC8F11.07c	11.20%	16
|mug190||C2 domain protein Tcb3 |Schizosaccharomyces pombe|	SPCP31B10.06	1.30%	16
|ppk2||serine/threonine protein kinase Ppk2 |Schizosaccharo	SPAC12B10.14c	9.20%	16
|tps1||alpha,alpha-trehalose-phosphate synthase [UDP-formin	SPAC328.03	18.70%	16
|||sequence orphan|Schizosaccharomyces pombe|chr 2|||Manual	SPBC17D1.05	13.90%	16
|cct6||chaperonin-containing T-complex zeta subunit Cct6|Sc	SPBC646.11	8.60%	15
|cps3|mug188|zinc finger protein Cps3|Schizosaccharomyces p	SPAC3A11.02	7.70%	15
|elf1||AAA family ATPase ELf1|Schizosaccharomyces pombe|chr	SPAC3C7.08c	11.50%	15
	SPCPB16A4.02c	17.40%	15
|chr3|cfh1|chitin synthase regulatory factor Chr3 |Schizosa	SPAC24B11.10c	8.00%	14
|gua1||IMP dehydrogenase Gua1 |Schizosaccharomyces pombe|ch	SPBC2F12.14c	11.50%	14
|rip1||ubiquinol-cytochrome-c reductase complex subunit 5|S	SPBC16H5.06	12.30%	14
|msa1|SPAC6C3.01c|RNA-binding protein Msa1|Schizosaccharomy	SPAC13G7.13c	10.50%	13
	SPAC1687.09	6.30%	13
|mcs4||two-component response regulator |Schizosaccharomyce	SPBC887.10	19.00%	12

|rad50|SPAP4C9.01c|DNA repair protein Rad50|Schizosaccharom	SPAC1556.01c	3.80%	12
|rga6||GTPase activating protein Rga6|Schizosaccharomyces p	SPBC354.13	10.10%	12
|tuf1||mitochondrial translation elongation factor EF-Tu Tu	SPBC9B6.04c	15.50%	12
|||NADPH dehydrogenase |Schizosaccharomyces pombe|chr 1|||M	SPAC5H10.10	16.80%	12
|||acyl-coA desaturase |Schizosaccharomyces pombe|chr 3|||M	SPCC1281.06c	19.00%	12
	SPAC17A5.10	16.10%	12
|||hypothetical protein|Schizosaccharomyces pombe|chr 1|||M	SPAC18G6.12c	14.60%	12
|||inorganic pyrophosphatase |Schizosaccharomyces pombe|chr	SPAC23C11.05	37.40%	12
|mit1||SHREC complex subunit Mit1|Schizosaccharomyces pombe	SPBP35G2.10	2.80%	11
|ned1||lipin|Schizosaccharomyces pombe|chr 1|||Manual	SPAC1952.13	8.80%	11
|tea4|wsh3|tip elongation aberrant protein Tea4|Schizosacch	SPBC1706.01	8.50%	11
||SPBC29A3.20c|serine palmitoyltransferase complex subunit	SPBC18E5.02c	13.00%	11
|ef1a-a||translation elongation factor EF-1 alpha Ef1a-a |S	SPCC794.09c	7.40%	10
|rsp1||random septum position protein Rsp1|Schizosaccharomy	SPBC11B10.05c	12.30%	10
|sar1||ADP-ribosylation factor Sar1|Schizosaccharomyces pom	SPBC31F10.06c	29.50%	10
|smc6|rad18|Smc5-6 complex SMC subunit Smc6|Schizosaccharom	SPCC5E4.06	2.50%	10
||SPAC824.01|phosphatidylinositol 4-kinase Lsb6 |Schizosacc	SPAC343.19	6.40%	10
|||ATP citrate synthase subunit 1 |Schizosaccharomyces pomb	SPBC1703.07	10.60%	10
	SPBC56F2.08c	5.60%	10
|||mitochondrial citrate transporter|Schizosaccharomyces po	SPAC19G12.05	14.80%	10
|||threonine ammonia-lyase|Schizosaccharomyces pombe|chr 2|	SPBC1677.03c	7.50%	10
|||transcription factor |Schizosaccharomyces pombe|chr 2|||	SPBC27B12.11c	14.50%	10

|||triglyceride lipase-cholesterol esterase |Schizosaccharo	SPCC1672.09	6.90%	10
|cct8||chaperonin-containing T-complex theta subunit Cct8 |	SPBC337.05c	8.60%	9
|hem1||5-aminolevulinate synthase|Schizosaccharomyces pombe	SPAC2F3.09	18.60%	9
|mkh1||MEK kinase |Schizosaccharomyces pombe|chr 1|||Manual	SPAC1F3.02c	9.30%	9
|shy1||SURF-family protein Shy1|Schizosaccharomyces pombe|c	SPBC1215.01	23.80%	9
|||cargo receptor for soluble proteins |Schizosaccharomyces	SPCC970.06	9.60%	9
|||long-chain-fatty-acid-CoA ligase |Schizosaccharomyces po	SPBP4H10.11c	11.90%	9
|||phospholipase|Schizosaccharomyces pombe|chr 1|||Manual	SPAC20G8.02	6.50%	9
|alo1||D-arabinono-1,4-lactone oxidase|Schizosaccharomyces	SPAPB1A10.12c	7.60%	8
|arg5||arginine specific carbamoyl-phosphate synthase Arg5	SPBC56F2.09c	15.70%	8
|int6|yin6|translation initiation factor eIF3e|Schizosaccha	SPBC646.09c	12.60%	8
|ntp1||alpha,alpha-trehalase Ntp1|Schizosaccharomyces pombe	SPBC660.07	10.90%	8
|ppk25||serine/threonine protein kinase Ppk25 |Schizosaccha	SPBC32C12.03c	10.60%	8
|||EST1 family protein|Schizosaccharomyces pombe|chr 2|||Ma	SPBC2F12.03c	10.80%	8
|||arrestin Aly1 related|Schizosaccharomyces pombe|chr 2|||	SPBC2D10.04	12.00%	8
|||asparagine-tRNA ligase Ded81 |Schizosaccharomyces pombe|	SPBC1773.10c	15.70%	8
|||inositol polyphosphate phosphatase |Schizosaccharomyces	SPBC19F5.03	5.00%	8
|||ribose-phosphate pyrophosphokinase |Schizosaccharomyces	SPCC1620.06c	8.40%	8
|||serine-tRNA ligase|Schizosaccharomyces pombe|chr 1|||Man	SPAC29A4.15	14.20%	8
|ccr1|SPBC365.17|NADPH-cytochrome p450 reductase |Schizosac	SPBC29A10.01	6.20%	7
|lcb2|SPAC2C4.02|serine palmitoyltransferase |Schizosacchar	SPAC21E11.08	9.30%	7
|mex67||mRNA export receptor	SPBC1921.03c	7.20%	7

Mex67|Schizosaccharomyces pomb			
|mrpl4||mitochondrial ribosomal protein subunit L4|Schizosa	SPCC4G3.06c	18.10%	7
|nak1|orb3, mor4|PAK-related kinase Nak1|Schizosaccharomyce	SPBC17F3.02	5.10%	7
|phx1||homeobox transcription factor Phx1|Schizosaccharomyc	SPAC32A11.03c	3.10%	7
|pub1||ubiquitin-protein ligase E3|Schizosaccharomyces pomb	SPAC11G7.02	10.70%	7
|sec16||multidomain vesicle coat component Sec16|Schizosacc	SPAC29B12.07	2.70%	7
|ssb1|rpa1, rad11|DNA replication factor A subunit Ssb1 |Sc	SPBC660.13c	6.60%	7
|trp2||tryptophan synthase|Schizosaccharomyces pombe|chr 1|	SPAC19A8.15	9.50%	7
|||alpha-1,2-galactosyltransferase|Schizosaccharomyces pomb	SPBC8D2.17	6.80%	7
|||conserved fungal protein|Schizosaccharomyces pombe|chr 1	SPAC1565.01	8.70%	7
|||conserved fungal protein|Schizosaccharomyces pombe|chr 2	SPBC26H8.11c	13.70%	7
|||enoyl reductase|Schizosaccharomyces pombe|chr 2|||Manual	SPBC646.07c	11.90%	7
|||guanine nucleotide transporter|Schizosaccharomyces pombe	SPCC1682.09c	16.70%	7
|||ribomal-ubiquitin fusion protein Ubi5|Schizosaccharomyce	SPAC589.10c	22.70%	7
|arg11||N-acetyl-gamma-glutamyl-phosphate reductase/acetylg	SPAC4G9.09c	6.80%	6
|ght5||hexose transporter Ght5 |Schizosaccharomyces pombe|c	SPCC1235.14	9.90%	6
|ppk6|SPAPJ736.02c|serine/threonine protein kinase Ppk6|Sch	SPAC1805.01c	5.00%	6
|puf3|SPAC222.02c|RNA-binding protein Puf3 |Schizosaccharom	SPAC1687.22c	7.20%	6
|rgf1||RhoGEF for Rho1, Rgf1|Schizosaccharomyces pombe|chr	SPCC645.07	6.20%	6
|rim1||mitochondrial single-stranded DNA binding protein Ri	SPAC2F3.04c	26.00%	6
|rpn2||19S proteasome regulatory subunit Rpn2|Schizosacchar	SPBC17D11.07c	2.30%	6
|shk1|pak1, orb2|PAK-related kinase Shk1|Schizosaccharomyce	SPBC1604.14c	15.00%	6

|tom70||mitochondrial TOM complex subunit Tom70|Schizosacch	SPAC6B12.12	5.30%	6
||SPBC1861.10|sequence orphan|Schizosaccharomyces pombe|chr	SPBC14F5.01	11.50%	6
||SPBC4C3.01|sequence orphan|Schizosaccharomyces pombe|chr	SPBC405.02c	10.30%	6
|||6-phosphofructo-2-kinase |Schizosaccharomyces pombe|chr	SPAC222.13c	11.10%	6
|||Haemolysin-III family protein|Schizosaccharomyces pombe|	SPAC30D11.11	11.30%	6
|||amino acid transporter |Schizosaccharomyces pombe|chr 1|	SPAC3H1.09c	8.70%	6
|||phosphomethylpyrimidine kinase|Schizosaccharomyces pombe	SPBP8B7.17c	4.70%	6
	SPBC557.02c	6.40%	6
|cki3||serine/threonine protein kinase Cki3|Schizosaccharom	SPAC1805.05	11.60%	5
|cpc2|rkp1|RACK1 homologue Cpc2|Schizosaccharomyces pombe|c	SPAC6B12.15	16.20%	5
|grx4||glutaredoxin Grx4|Schizosaccharomyces pombe|chr 2|||	SPBC26H8.06	15.60%	5
|kin1||microtubule affinity-regulating kinase Kin1 |Schizos	SPBC4F6.06	3.00%	5
|med15|SPBP35G2.15|mediator complex subunit Med15 |Schizosa	SPBC146.01	6.30%	5
|pro1||gamma-glutamyl phosphate reductase Pro1 |Schizosacch	SPAC821.11	10.40%	5
|rpn3|SPBPJ4664.07|19S proteasome regulatory subunit Rpn3|S	SPBC119.01	6.20%	5
|tom40|SPBC8D2.22|mitochondrial TOM complex subunit Tom40|S	SPBC27B12.13	27.60%	5
|trp3||anthranilate synthase component I|Schizosaccharomyce	SPCC1442.09	7.20%	5
|win1|SPAC1250.06c, SPAPJ730.01|MAP kinase kinase kinase Wi	SPAC1006.09	3.60%	5
||SPAC30D11.15c|Moeb/ThiF domain|Schizosaccharomyces pombe|	SPAC1A6.10	14.00%	5
||SPBP4G3.01|inorganic phosphate transporter |Schizosacchar	SPBC8E4.01c	7.50%	5
|||DUF1776 family protein|Schizosaccharomyces pombe|chr 2||	SPBC106.03	17.10%	5
|||Mvp17/PMP22 family|Schizosaccharomyces pombe|chr 1|||Man	SPAC3G6.05	13.10%	5

|||alpha-1,2-galactosyltransferase |Schizosaccharomyces pom	SPAC637.06	6.90%	5
|||conserved eukaryotic protein|Schizosaccharomyces pombe|c	SPBC1539.04	10.80%	5
|||conserved fungal protein|Schizosaccharomyces pombe|chr 3	SPCC895.08c	5.10%	5
|||leucine-rich repeat protein Sog2 |Schizosaccharomyces po	SPBC887.09c	7.10%	5
|||lipoate-protein ligase |Schizosaccharomyces pombe|chr 1|	SPAC4F10.05c	21.00%	5
|||nucleoporin Nup60 |Schizosaccharomyces pombe|chr 3|||Man	SPCC285.13c	4.80%	5
|||translation initiation factor |Schizosaccharomyces pombe	SPBC16C6.05	11.10%	5
|ams2|SPCC4F11.01|cell cycle regulated GATA-type transcript	SPCC290.04	4.90%	4
|ccs1|pccs, pccs|metallochaperone Ccs1 |Schizosaccharomyces	SPAC22E12.04	11.80%	4
|cox5||cytochrome c oxidase subunit V|Schizosaccharomyces p	SPCC338.10c	8.00%	4
|csx1||RNA-binding protein Csx1|Schizosaccharomyces pombe|c	SPAC17A2.09c	5.20%	4
|etp1|cox15|mitochondrial type I [2Fe-2S] ferredoxin Etp1/	SPAC22E12.10c	10.30%	4
|hhp2||serine/threonine protein kinase Hhp2 |Schizosaccharo	SPAC23C4.12	11.50%	4
|kap123||karyopherin Kap123|Schizosaccharomyces pombe|chr 2	SPBC14F5.03c	4.40%	4
|ncs1||related to neuronal calcium sensor Ncs1|Schizosaccha	SPAC18B11.04	15.80%	4
|oca2||serine/threonine protein kinase Oca2 |Schizosaccharo	SPCC1020.10	4.50%	4
|pef1||Pho85/PhoA-like cyclin-dependent kinase Pef1|Schizos	SPCC16C4.11	14.90%	4
|peg1|cls1|CLASP family microtubule-associated protein|Schi	SPAC3G9.12	1.20%	4
|rpb2|SPAC521.06|DNA-directed RNA polymerase II complex sub	SPAC23G3.01	2.00%	4
|rpt1||19S proteasome regulatory subunit Rpt1|Schizosacchar	SPBC16C6.07c	7.80%	4
|rpt4||19S proteasome regulatory subunit Rpt4|Schizosacchar	SPCC1682.16	7.00%	4
|sdh1||succinate dehydrogenase	SPAC1556.02c	4.50%	4

Sdh1|Schizosaccharomyces pom			
|sec26|SPBC337.01c|coatomer beta subunit |Schizosaccharomyc	SPBC146.14c	2.80%	4
|sep1||fork head transcription factor Sep1|Schizosaccharomy	SPBC4C3.12	4.80%	4
|vma1||V-type ATPase subunit A|Schizosaccharomyces pombe|ch	SPAC343.05	7.40%	4
|||NAD dependent epimerase/dehydratase family protein|Schiz	SPCC1840.09	11.60%	4
|||cytochrome b5 reductase |Schizosaccharomyces pombe|chr 3	SPCC970.03	9.60%	4
|||mannosyltransferase complex subunit |Schizosaccharomyces	SPAC17G8.11c	6.50%	4
|||phospholipase |Schizosaccharomyces pombe|chr 1|||Manual	SPAC1786.02	3.40%	4
	SPBC1A4.05	3.10%	4
|arg1||acetylornithine aminotransferase|Schizosaccharomyces	SPCC777.09c	10.90%	3
|ayr1||1-acyldihydroxyacetone phosphate reductase |Schizosa	SPAC23D3.11	7.10%	3
|cct3||chaperonin-containing T-complex gamma subunit Cct3|S	SPBC1A4.08c	6.10%	3
|cct4||chaperonin-containing T-complex delta subunit Cct4|S	SPBC106.06	5.90%	3
|gly1||threonine aldolase |Schizosaccharomyces pombe|chr 1|	SPAC23H3.09c	13.30%	3
|imp2||contractile ring protein Imp2|Schizosaccharomyces po	SPBC11C11.02	6.30%	3
|lid2|SPBP4H10.01|Lid2 complex subunit Lid2 |Schizosaccharo	SPBP19A11.06	1.60%	3
|mae2||malic enzyme|Schizosaccharomyces pombe|chr 3|||Manua	SPCC794.12c	3.50%	3
|met6||homoserine O-acetyltransferase|Schizosaccharomyces p	SPBC56F2.11	9.00%	3
|pdr1||ABC transporter Pdr1|Schizosaccharomyces pombe|chr 1	SPAPB24D3.09c	3.90%	3
|ppk30||Ark1/Prk1 family protein kinase Ppk30|Schizosacchar	SPBC6B1.02	5.00%	3
|rgf3|lad1|RhoGEF Rgf3|Schizosaccharomyces pombe|chr 3|||Ma	SPCC645.06c	2.00%	3
|sre2||membrane-tethered transcription factor |Schizosaccha	SPBC354.05c	6.60%	3

|vht1||vitamin H transporter Vth1|Schizosaccharomyces pombe	SPAC1B3.16c	6.70%	3
||SPBC17D11.09|sequence orphan|Schizosaccharomyces pombe|ch	SPBC17D1.01	5.30%	3
|||ATP-citrate synthase subunit 2 |Schizosaccharomyces pomb	SPAC22A12.16	7.30%	3
|||RNA-binding protein|Schizosaccharomyces pombe|chr 2|||Ma	SPBC4F6.14	5.90%	3
|||WD repeat protein Wdr44 family, WD repeat protein|Schizo	SPBC18H10.05	7.20%	3
|||aldehyde dehydrogenase |Schizosaccharomyces pombe|chr 1|	SPAC922.07c	9.30%	3
|||alpha,alpha-trehalose-phosphate synthase |Schizosaccharo	SPACUNK4.16c	5.80%	3
|||mitochondrial tricarboxylic acid transporter|Schizosacch	SPBC83.13	14.70%	3
|||nucleotide sugar transporter |Schizosaccharomyces pombe|	SPAC144.18	11.00%	3
|||phosphoserine aminotransferase |Schizosaccharomyces pomb	SPAC1F12.07	5.90%	3
|cut11|SPAC24C9.01|integral membrane nucleoporin|Schizosacc	SPAC1786.03	5.70%	2
|fab1|ste12, SPBC6B1.11c|1-phosphatidylinositol-3-phosphate	SPBC3E7.01	0.60%	2
|gad8||serine/threonine protein kinase Gad8 |Schizosaccharo	SPCC24B10.07	9.00%	2
|hrp3||ATP-dependent DNA helicase Hrp3|Schizosaccharomyces	SPAC3G6.01	1.20%	2
|mtr4||ATP-dependent RNA helicase, TRAMP complex subunit Mt	SPAC6F12.16c	1.00%	2
|mug99||meiotically upregulated gene Mug99|Schizosaccharomy	SPAC1610.04	8.40%	2
|pyr1||pyruvate carboxylase|Schizosaccharomyces pombe|chr 2	SPBC17G9.11c	2.90%	2
|rpt6|let1|19S proteasome regulatory subunit Rpt6|Schizosac	SPBC23G7.12c	9.70%	2
|sec74|SPAPJ691.01c|guanyl-nucleotide exchange factor Sec74	SPAC26F1.01	2.60%	2
|set6||histone lysine methyltransferase Set6 |Schizosacchar	SPBP8B7.07c	6.40%	2
|ssn6||transcriptional corepressor Ssn6|Schizosaccharomyces	SPBC23E6.09	3.10%	2
|vph1||V-type ATPase subunit a|Schizosaccharomyces	SPAC16E8.07c	2.70%	2

pombe|ch			
|||CCR4-Not complex subunit Not1 |Schizosaccharomyces pombe	SPAC20G8.06	1.20%	2
|||CGR1 family|Schizosaccharomyces pombe|chr 1|||Manual	SPAC1556.05c	27.90%	2
|||COPII-coated vesicle component Erv46|Schizosaccharomyces	SPAC24B11.08c	2.80%	2
|||FAD-dependent oxidoreductase |Schizosaccharomyces pombe|	SPAC1F5.03c	5.20%	2

|ptc1||protein phosphatase 2C Ptc1|Schizosaccharomyces pomb	SPCC4F11.02	59.90%	152
|pom1||DYRK family protein kinase Pom1|Schizosaccharomyces	SPAC2F7.03c	36.80%	148
|tea3||cell end marker Tea3|Schizosaccharomyces pombe|chr 1	SPAC6G10.02c	39.70%	137
|||WD repeat protein, human WDR68 family|Schizosaccharomyce	SPBC17D11.08	70.30%	137
|rga4||GTPase activating protein Rga4|Schizosaccharomyces p	SPBC28E12.03	49.20%	133
|||sequence orphan|Schizosaccharomyces pombe|chr 1|||Manual	SPAC16E8.08	74.30%	124
|mug161||CwfJ family protein|Schizosaccharomyces pombe|chr	SPAC1F3.09	27.60%	121
|gaf1|SPCC417.01c|transcription factor Gaf1 |Schizosaccharo	SPCC1902.01	34.70%	116
	SPBP35G2.14	31.00%	111
	SPBC1A4.05	45.40%	111
||SPCC1906.05|zf-CCCH type zinc finger protein|Schizosaccha	SPCC1739.01	41.90%	110
|pal1||membrane associated protein Pal1 |Schizosaccharomyce	SPCP1E11.04c	71.10%	106
|||Spo7 homolog|Schizosaccharomyces pombe|chr 2|||Manual	SPBC902.03	41.70%	102
|||inorganic pyrophosphatase |Schizosaccharomyces pombe|chr	SPAC23C11.05	58.50%	92
|ppk25||serine/threonine protein kinase Ppk25 |Schizosaccha	SPBC32C12.03c	51.30%	89
|ppk38||Ark1/Prk1 family protein kinase Ppk38|Schizosacchar	SPCP1E11.02	40.90%	89
|sec16||multidomain vesicle coat component Sec16|Schizosacc	SPAC29B12.07	16.70%	87
|||protein disulfide isomerase |Schizosaccharomyces pombe|c	SPBC3D6.13c	26.70%	84
|cdc11||SIN component scaffold protein Cdc11|Schizosaccharo	SPCC1739.11c	26.90%	83
|||diacylglycerol binding protein |Schizosaccharomyces pomb	SPCC297.05	41.40%	83
|kin1||microtubule affinity-regulating kinase Kin1 |Schizos	SPBC4F6.06	26.30%	81
|mod5||Tea1 anchoring protein Mod5|Schizosaccharomyces pomb	SPBC530.04	29.10%	66
9 ft	SPAC19A10.10	39.10%	155

|tps1||alpha,alpha-trehalose-phosphate synthase [UDP-	SPAC328.03	22.00%	37
formin			
|dfr1||dihydrofolate reductase Dfr1|Schizosaccharomyces pom	SPCC1223.08c	18.20%	36
|ef1a-a||translation elongation factor EF-1 alpha Ef1a-a |S	SPCC794.09c	6.30%	34
|tif51||translation initiation factor eIF5A|Schizosaccharom	SPAC26H5.10c	45.20%	34
|wis4|wak1, wik1|MAP kinase kinase kinase Wis4|Schizosaccha	SPAC9G1.02	11.80%	34
|cam1||calmodulin Cam1 |Schizosaccharomyces pombe|chr 1|||M	SPAC3A12.14	58.70%	33
|hem14||protoporphyrinogen oxidase|Schizosaccharomyces pomb	SPAC1F5.07c	26.90%	33
|arg5||arginine specific carbamoyl-phosphate synthase Arg5	SPBC56F2.09c	34.70%	32
|mts4|rpn1|19S proteasome regulatory subunit Mts4|Schizosac	SPBP19A11.03c	10.10%	32
|||DUF1776 family protein|Schizosaccharomyces pombe|chr 2||	SPBC106.03	27.50%	32
|||NAD/NADH kinase |Schizosaccharomyces pombe|chr 1|||Manua	SPAC1B1.02c	23.80%	32
||SPAC17G6.01|CorA family magnesium ion transporter|Schizos	SPAC17A2.14	18.80%	31
|ags1|mok1, SPCC338.01c, SPCC17A7.01|alpha-1,4-glucan synth	SPCC1281.01	8.20%	30
|cct6||chaperonin-containing T-complex zeta subunit Cct6|Sc	SPBC646.11	23.00%	30
|rsp1||random septum position protein Rsp1|Schizosaccharomy	SPBC11B10.05c	26.10%	28
|||zf-C3HC4 type zinc finger|Schizosaccharomyces pombe|chr	SPBC25B2.03	18.80%	28
|sar1||ADP-ribosylation factor Sar1|Schizosaccharomyces pom	SPBC31F10.06c	29.50%	27
	SPAC1687.09	7.40%	27
	SPBC17D1.05	37.50%	27
|gef1||RhoGEF Gef1|Schizosaccharomyces pombe|chr 1|||Manual	SPAC24H6.09	15.00%	26
|rad22||DNA repair protein Rad22|Schizosaccharomyces pombe|	SPAC30D11.10	28.10%	26
|mcs4||two-component response regulator |Schizosaccharomyce	SPBC887.10	26.10%	25
formin	SPAC328.03	22.00%	37

|||glycine tRNA-ligase|Schizosaccharomyces pombe|chr 1|||Ma	SPAC3F10.03	15.80%	19
|ppk2||serine/threonine protein kinase Ppk2 |Schizosaccharo	SPAC12B10.14c	16.70%	18
|cct4||chaperonin-containing T-complex delta subunit Cct4|S	SPBC106.06	17.50%	17
|cct8||chaperonin-containing T-complex theta subunit Cct8 |	SPBC337.05c	22.30%	17
|int6|yin6|translation initiation factor eIF3e|Schizosaccha	SPBC646.09c	16.60%	17
|ssa1||heat shock protein Ssa1|Schizosaccharomyces pombe|ch	SPAC13G7.02c	18.00%	17
|mbx1||MADS-box transcription factor Mbx1|Schizosaccharomyc	SPBC19G7.06	15.60%	16
|puf3|SPAC222.02c|RNA-binding protein Puf3 |Schizosaccharom	SPAC1687.22c	13.90%	16
|sec13||COPII-coated vesicle component Sec13|Schizosaccharo	SPBC215.15	28.30%	16
|||long-chain-fatty-acid-CoA ligase |Schizosaccharomyces po	SPBP4H10.11c	16.30%	16
	SPAPB1A10.13	9.60%	16
	SPBC1289.06c	22.90%	16
|pda1||pyruvate dehydrogenase e1 component alpha subunit Pd	SPAC26F1.03	21.50%	15
|rpl3001|rpl30-1, rpl30|60S ribosomal protein L30|Schizosac	SPAC9G1.03c	29.40%	15
|rpt4||19S proteasome regulatory subunit Rpt4|Schizosacchar	SPCC1682.16	13.40%	15
|||alpha-1,2-galactosyltransferase|Schizosaccharomyces pomb	SPBC8D2.17	8.30%	15
	SPCC895.08c	13.50%	15
|cek1||serine/threonine protein kinase Cek1|Schizosaccharom	SPCC1450.11c	5.80%	14
|its3||1-phosphatidylinositol-4-phosphate 5-kinase Its3|Sch	SPAC19G12.14	11.90%	14
|rpt1||19S proteasome regulatory subunit Rpt1|Schizosacchar	SPBC16C6.07c	8.70%	14
|taf1||Taz1 interacting factor 1|Schizosaccharomyces pombe|	SPAC7D4.04	9.30%	14
||SPAC824.01|phosphatidylinositol 4-kinase Lsb6 |Schizosacc	SPAC343.19	10.60%	14
|||glycine tRNA-ligase|Schizosaccharomyces pombe|chr 1|||Ma	SPAC3F10.03	15.80%	19

|ned1||lipin|Schizosaccharomyces pombe|chr 1|||Manual	SPAC1952.13	6.10%	11
|ppk22||serine/threonine protein kinase Ppk22 |Schizosaccha	SPBC1861.09	12.40%	11
|pro1||gamma-glutamyl phosphate reductase Pro1 |Schizosacch	SPAC821.11	14.60%	11
|rpn6||19S proteasome regulatory subunit Rpn6|Schizosacchar	SPAC23G3.11	6.20%	11
|sec26|SPBC337.01c|coatomer beta subunit |Schizosaccharomyc	SPBC146.14c	6.20%	11
|sec63||ER protein translocation subcomplex subunit Sec63 |	SPBC36B7.03	11.10%	11
|||3 beta-hydroxysteroid dehydrogenase/delta 5-->4-isomeras	SPBC3F6.02c	7.90%	11
|||Arf GAP protein|Schizosaccharomyces pombe|chr 1|||Manual	SPAC26A3.10	7.50%	11
|||cytochrome b5 reductase |Schizosaccharomyces pombe|chr 3	SPCC970.03	17.90%	11
|||dolichyl-diphospho-oligosaccharide-protein glycosyltrans	SPAC27F1.07	10.90%	11
	SPAC688.07c	9.60%	11
|||serine-tRNA ligase|Schizosaccharomyces pombe|chr 1|||Man	SPAC29A4.15	22.70%	11
|oac1||anion transporter|Schizosaccharomyces pombe|chr 1|||	SPAC139.02c	17.50%	10
|ogm2|oma2|protein O-mannosyltransferase Ogm2|Schizosacchar	SPAPB1E7.09	7.40%	10
|rpn2||19S proteasome regulatory subunit Rpn2|Schizosacchar	SPBC17D11.07c	3.90%	10
|rps2602|rps26-2|40S ribosomal protein S26|Schizosaccharomy	SPAC1805.11c	45.40%	10
|rpt3||19S proteasome regulatory subunit Rpt3|Schizosacchar	SPCC576.10c	14.40%	10
|tpx1||thioredoxin peroxidase Tpx1|Schizosaccharomyces pomb	SPCC576.03c	25.00%	10
|||1-acylglycerol-3-phosphate O-acyltransferase|Schizosacch	SPAC1851.02	28.70%	10
|||delta-1-pyrroline-5-carboxylate dehydrogenase|Schizosacc	SPBC24C6.04	9.50%	10
|his7||phosphoribosyl-AMP cyclohydrolase/phosphoribosyl-ATP	SPBC29A3.02c	26.40%	9
|mug164||microtubule-associated protein|Schizosaccharomyces	SPBC25B2.07c	7.80%	9
Bgs4|Sch	SPCC1840.02c	3.70%	11

||SPBC4C3.01|sequence orphan|Schizosaccharomyces pombe|chr	SPBC405.02c	6.90%	8
|||DUF747 family protein|Schizosaccharomyces pombe|chr 2|||	SPBC13G1.05	10.30%	8
|||phospholipase|Schizosaccharomyces pombe|chr 1|||Manual	SPAC20G8.02	4.80%	8
|ade6|min1|phosphoribosylaminoimidazole carboxylase Ade6|Sc	SPCC1322.13	6.90%	7
|aro1||pentafunctional aromatic polypeptide Aro1 |Schizosac	SPAC1834.02	4.80%	7
|atg13|apg13, mug78|autophagy associated protein Atg13 |Sch	SPAC4F10.07c	6.60%	7
|bfr1|hba2, SPCPJ732.04c|brefeldin A efflux transporter Bfr	SPCC18B5.01c	2.40%	7
|cct3||chaperonin-containing T-complex gamma subunit Cct3|S	SPBC1A4.08c	12.30%	7
|crm1|caf2, SPAC1B2.01|nuclear export receptor Crm1|Schizos	SPAC1805.17	10.60%	7
|gpd1||glycerol-3-phosphate dehydrogenase Gpd1|Schizosaccha	SPBC215.05	13.00%	7
|grx4||glutaredoxin Grx4|Schizosaccharomyces pombe|chr 2|||	SPBC26H8.06	15.20%	7
|mug81||ATP-dependent RNA helicase Slh1|Schizosaccharomyces	SPBC13G1.10c	1.60%	7
|plb1||phospholipase B homolog Plb1|Schizosaccharomyces pom	SPAC1A6.04c	8.50%	7
|rga7||GTPase activating protein Rga7|Schizosaccharomyces p	SPBC23G7.08c	11.40%	7
|rpn9||19S proteasome regulatory subunit Rpn9|Schizosacchar	SPAC607.05	16.80%	7
|trp2||tryptophan synthase|Schizosaccharomyces pombe|chr 1|	SPAC19A8.15	7.90%	7
|||aldehyde dehydrogenase |Schizosaccharomyces pombe|chr 2|	SPBC21C3.15c	10.30%	7
|||amino acid transporter |Schizosaccharomyces pombe|chr 1|	SPAC3H1.09c	10.10%	7
|||asparagine-tRNA ligase Ded81 |Schizosaccharomyces pombe|	SPBC1773.10c	14.60%	7
|||dolichyl-di-phosphooligosaccharide-protein glycotransfer	SPCC338.15	22.70%	7
|||esterase/lipase|Schizosaccharomyces pombe|chr	SPAC8F11.08c	7.70%	7

|||sequence orphan|Schizosaccharomyces pombe|chr 2|||Manual	SPBC11C11.06c	42.70%	6
|||transcription factor |Schizosaccharomyces pombe|chr 2|||	SPBC27B12.11c	10.20%	6
|||translation initiation factor eIF4A|Schizosaccharomyces	SPAC1006.07	7.70%	6
|atp5||F0-ATPase delta subunit|Schizosaccharomyces pombe|ch	SPCC1840.06	18.10%	5
|cap1|cap|adenylyl cyclase-associated protein Cap1|Schizosa	SPCC306.09c	5.40%	5
|elf1||AAA family ATPase ELf1|Schizosaccharomyces pombe|chr	SPAC3C7.08c	3.70%	5
|fab1|ste12, SPBC6B1.11c|1-phosphatidylinositol-3-phosphate	SPBC3E7.01	2.10%	5
|hmt1|SPCC74.08c|ATP-binding cassette-type vacuolar membran	SPCC737.09c	5.30%	5
|idh2|glu2|isocitrate dehydrogenase |Schizosaccharomyces po	SPBC902.05c	11.60%	5
|mip1||WD repeat protein Mip1|Schizosaccharomyces pombe|chr	SPAC57A7.11	2.00%	5
|mug190||C2 domain protein Tcb3 |Schizosaccharomyces pombe|	SPCP31B10.06	3.20%	5
|plc1||phosphoinositide phospholipase C Plc1|Schizosaccharo	SPAC22F8.11	6.20%	5
|prp10|sap155|U2 snRNP-associated protein Sap155|Schizosacc	SPAC27F1.09c	2.40%	5
|rga2||GTPase activating protein Rga2|Schizosaccharomyces p	SPAC26A3.09c	2.00%	5
|tub1|atb2, alp2, ban5|tubulin alpha 2|Schizosaccharomyces	SPBC800.05c	11.80%	5
|ubp9||ubiquitin C-terminal hydrolase Ubp9|Schizosaccharomy	SPBC1703.12	7.50%	5
||SPCC63.01c|sequence orphan|Schizosaccharomyces pombe|chr	SPCC2H8.05c	7.40%	5
|||MTC tricarboxylate transporter|Schizosaccharomyces pombe	SPAC17G6.15c	9.50%	5
|||conserved fungal protein|Schizosaccharomyces pombe|chr 1	SPAC1565.01	20.20%	5
|||cytochrome b5 |Schizosaccharomyces pombe|chr 2|||Manual	SPBC29A10.16c	35.50%	5
|||folylpolyglutamate synthase|Schizosaccharomyces pombe|ch	SPBC1709.17	4.00%	5
|||threonine ammonia-lyase|Schizosaccharomyces	SPBC1677.03c	8.30%	5

|||conserved fungal protein|Schizosaccharomyces pombe|chr 3	SPCC1450.12	3.30%	4
|||fasciclin domain protein |Schizosaccharomyces pombe|chr	SPAC22H12.05c	3.30%	4
|||metaxin 1|Schizosaccharomyces pombe|chr 1|||Manual	SPAC589.04	9.20%	4
|||proline dehydrogenase|Schizosaccharomyces pombe|chr 3|||	SPCC70.03c	6.70%	4
|||pyruvate dehydrogenase protein x component|Schizosacchar	SPCC1259.09c	6.40%	4
|||ribonuclease II |Schizosaccharomyces pombe|chr 2|||Manua	SPBC609.01	2.20%	4
|||sequence orphan|Schizosaccharomyces pombe|chr 3|||Manual	SPCC777.12c	14.30%	4
|atp4||F0-ATPase subunit|Schizosaccharomyces pombe|chr 2|||	SPBC1604.07	10.70%	3
|cdc22||ribonucleoside reductase large subunit Cdc22|Schizo	SPAC1F7.05	3.80%	3
|dis2|sds1, bws1|serine/threonine protein phosphatase PP1|S	SPBC776.02c	9.20%	3
|gda1|gdp1|guanosine-diphosphatase Gda1|Schizosaccharomyces	SPAC824.08	6.30%	3
|idh1|glu3|isocitrate dehydrogenase |Schizosaccharomyces po	SPAC11G7.03	11.20%	3
|mcm3||MCM complex subunit Mcm3|Schizosaccharomyces pombe|c	SPCC1682.02c	3.00%	3
|ppk30||Ark1/Prk1 family protein kinase Ppk30|Schizosacchar	SPBC6B1.02	2.30%	3
|pyr1||pyruvate carboxylase|Schizosaccharomyces pombe|chr 2	SPBC17G9.11c	2.60%	3
|rad50|SPAP4C9.01c|DNA repair protein Rad50|Schizosaccharom	SPAC1556.01c	2.30%	3
|rpn11|pad1, sks1, bfr2, mts5|19S proteasome regulatory sub	SPAC31G5.13	10.40%	3
|rum1||CDK inhibitor Rum1|Schizosaccharomyces pombe|chr 2||	SPBC32F12.09	3.90%	3
|sak1||transcriptional repressor Sak1|Schizosaccharomyces p	SPAC3G9.14	5.10%	3
|scd1|ral1|RhoGEF Scd1|Schizosaccharomyces pombe|chr 1|||Ma	SPAC16E8.09	3.60%	3
|scr1||transcription factor Scr1|Schizosaccharomyces pombe|	SPBC1D7.02c	7.40%	3
|sec27||coatomer beta' subunit |Schizosaccharomyces pombe|c	SPBC16C6.13c	4.30%	3

|||protein kinase inhibitor |Schizosaccharomyces pombe|chr	SPCC736.15	10.30%	3
	SPBC36.11	14.00%	3
	SPBC365.16	10.10%	3
|||striatin homolog|Schizosaccharomyces pombe|chr 2|||Manua	SPBC1773.01	5.90%	3
|bsu1|SPAC1B1.05, bsu1|high-affinity import carrier for pyr	SPAC17A2.01	7.40%	2
|cdc8|fus4|tropomyosin|Schizosaccharomyces pombe|chr 1|||Ma	SPAC27F1.02c	13.00%	2
|ckb1||CK2 family regulatory subunit |Schizosaccharomyces p	SPAC1851.03	7.40%	2
|coq5||C-methytransferase |Schizosaccharomyces pombe|chr 3|	SPCC4G3.04c	13.40%	2
|cta4|sev4, SPAPYUK71.01|P-type ATPase, calcium transportin	SPACUNK4.07c	3.60%	2
|erg11||sterol 14-demethylase|Schizosaccharomyces pombe|chr	SPAC13A11.02c	5.10%	2
|ggt1||gamma-glutamyltranspeptidase Ggt1 |Schizosaccharomyc	SPAC664.09	4.30%	2
|mdm10||Mdm10/Mdm12/Mmm1 complex subunit Mdm10 |Schizosacch	SPAC17H9.17c	10.30%	2
|mob2||protein kinase activator Mob2|Schizosaccharomyces po	SPCC970.04c	3.70%	2
|sfc3||transcription factor TFIIIC complex subunit Sfc3|Sch	SPBC336.07	2.40%	2
|ssn6||transcriptional corepressor Ssn6|Schizosaccharomyces	SPBC23E6.09	1.80%	2
|zhf1|zhf, zhf|zinc ion transporter Zhf1|Schizosaccharomyce	SPAC23C11.14	6.50%	2
|||P-type ATPase |Schizosaccharomyces pombe|chr 1|||Manual	SPAC4F10.16c	0.90%	2
|||carbonic anhydrase |Schizosaccharomyces pombe|chr 2|||Ma	SPBP8B7.05c	12.30%	2
|||homoserine dehydrogenase |Schizosaccharomyces pombe|chr	SPBC776.03	8.50%	2
|||karyopherin|Schizosaccharomyces pombe|chr 3|||Manual	SPCC550.11	3.00%	2
|||phosphogluconate dehydrogenase, decarboxylating |Schizos	SPBC660.16	5.10%	2
|||ribokinase |Schizosaccharomyces pombe|chr 2|||Manual	SPBC16G5.02c	17.30%	2
[illegible]	[illegible]	[illegible]	[illegible]

sid1-239 Rad24TAP		Coverage rate	Peptide count
|rad24||14-3-3 protein Rad24|Schizosaccharomyces pombe|chr	SPAC8E11.02c	96.70%	8726
|rad25||14-3-3 protein Rad25|Schizosaccharomyces pombe|chr	SPAC17A2.13c	94.80%	1372
|ntp1||alpha,alpha-trehalase Ntp1|Schizosaccharomyces pombe	SPBC660.07	81.80%	3025
|cam1||calmodulin Cam1 |Schizosaccharomyces pombe|chr 1|||M	SPAC3A12.14	76.00%	26
|||Usp |Schizosaccharomyces pombe|chr 2|||Manual	SPBC25B2.10	74.90%	455
|ptc1||protein phosphatase 2C Ptc1|Schizosaccharomyces pomb	SPCC4F11.02	74.40%	370
|pal1||membrane associated protein Pal1 |Schizosaccharomyce	SPCP1E11.04c	60.50%	81
|||WD repeat protein, human WDR68 family|Schizosaccharomyce	SPBC17D11.08	58.90%	84
|ppk36|atg1|serine/threonine protein kinase Ppk36|Schizosac	SPCC63.08c	55.90%	342
|ppk15||serine/threonine protein kinase Ppk15 |Schizosaccha	SPAC823.03	54.70%	165
	SPBP35G2.14	54.30%	218
|||NLI interacting factor family|Schizosaccharomyces pombe|	SPBC3B8.10c	53.80%	183
|zfs1|moc4|transcription factor Zfs1 |Schizosaccharomyces p	SPBC1718.07c	53.00%	86
|whi5|mug54|cell cycle transcriptional repressor Whi5|Schiz	SPBC800.02	52.40%	202
|||inorganic pyrophosphatase |Schizosaccharomyces pombe|chr	SPAC23C11.05	52.20%	15
|sec2||guanyl-nucleotide exchange factor Sec2 |Schizosaccha	SPAC23C4.10	51.60%	323
|||leucine-rich repeat protein, unknown|Schizosaccharomyces	SPAC926.06c	51.40%	252
|rga4||GTPase activating protein Rga4|Schizosaccharomyces p	SPBC28E12.03	50.70%	182
|cdc15||cell division control protein Cdc15|Schizosaccharom	SPAC20G8.05c	50.50%	311
|hsp16||heat shock protein Hsp16|Schizosaccharomyces pombe|	SPBC3E7.02c	50.30%	8
|fba1||fructose-bisphosphate aldolase	SPBC19C2.07	49.20%	30

Fba1|Schizosaccharomy			
||SPCC1906.05|zf-CCCH type zinc finger protein|Schizosaccha	SPCC1739.01	47.90%	114
|nte1||lysophospholipase|Schizosaccharomyces pombe|chr 3|||	SPCC4B3.04c	47.30%	211
|taf1||Taz1 interacting factor 1|Schizosaccharomyces pombe|	SPAC7D4.04	47.30%	191
|||cytochrome b5 |Schizosaccharomyces pombe|chr 2|||Manual	SPBC29A10.16c	46.80%	3
|||BAR adaptor protein|Schizosaccharomyces pombe|chr 2|||Ma	SPBC19C2.10	45.70%	78
|pda1||pyruvate dehydrogenase e1 component alpha subunit Pd	SPAC26F1.03	44.30%	22
|dfr1||dihydrofolate reductase Dfr1|Schizosaccharomyces pom	SPCC1223.08c	44.00%	82
|scw1||RNA-binding protein Scw1|Schizosaccharomyces pombe|c	SPCC16C4.07	43.30%	64
|mac1||membrane anchored protein Mac1 |Schizosaccharomyces	SPAC13G7.04c	42.30%	83
|mod5||Tea1 anchoring protein Mod5|Schizosaccharomyces pomb	SPBC530.04	41.60%	98
|chr4|cfh3, SPBC1539.11c|chitin synthase regulatory factor	SPBC1289.01c	41.10%	53
|cyk3||cytokinesis protein Cyk3|Schizosaccharomyces pombe|c	SPAC9G1.06c	40.60%	147
|||zf-C3HC4 type zinc finger|Schizosaccharomyces pombe|chr	SPBC25B2.03	39.70%	47
|ppk38||Ark1/Prk1 family protein kinase Ppk38|Schizosacchar	SPCP1E11.02	39.50%	65
|rad22||DNA repair protein Rad22|Schizosaccharomyces pombe|	SPAC30D11.10	38.80%	45
|pom1||DYRK family protein kinase Pom1|Schizosaccharomyces	SPAC2F7.03c	38.50%	170
|||AAA family ATPase, unknown biological role|Schizosacchar	SPBC947.01	38.30%	114
|||ketopantoate reductase |Schizosaccharomyces pombe|chr 1|	SPAC24B11.07c	38.30%	105
|bud6|aip3, fat1, SPAC15E1.01|actin interacting protein 3 h	SPAC15A10.16	37.80%	154
|cki3||serine/threonine protein kinase Cki3|Schizosaccharom	SPAC1805.05	36.20%	50
|||NADPH-hemoprotein reductase |Schizosaccharomyces pombe|c	SPAC1F12.10c	36.10%	4

|||RNA-binding protein|Schizosaccharomyces pombe|chr 1|||Ma	SPAC17H9.04c	36.10%	156
|||cytochrome b5 reductase |Schizosaccharomyces pombe|chr 3	SPCC970.03	35.90%	21
|gaf1|SPCC417.01c|transcription factor Gaf1 |Schizosaccharo	SPCC1902.01	35.80%	197
|ned1||lipin|Schizosaccharomyces pombe|chr 1|||Manual	SPAC1952.13	35.80%	57
|||sequence orphan|Schizosaccharomyces pombe|chr 1|||Manual	SPAC16E8.08	34.20%	7
	SPBC17D1.05	34.00%	61
|hem14||protoporphyrinogen oxidase|Schizosaccharomyces pomb	SPAC1F5.07c	33.70%	49
	SPBC1A4.05	32.90%	53
|||ribomal-ubiquitin fusion protein Ubi5|Schizosaccharomyce	SPAC589.10c	32.70%	16
|||NAD/NADH kinase |Schizosaccharomyces pombe|chr 1|||Manua	SPAC1B1.02c	32.00%	45
|||arrestin/PY protein 2|Schizosaccharomyces pombe|chr 3|||	SPCC584.15c	31.80%	60
|gef1||RhoGEF Gef1|Schizosaccharomyces pombe|chr 1|||Manual	SPAC24H6.09	31.60%	65
|qcr10||ubiquinol-cytochrome-c reductase complex subunit Qc	SPBP4H10.08	31.60%	7
|||GTPase activating protein |Schizosaccharomyces pombe|chr	SPAC3G9.05	31.60%	62
|chk1|rad27|Chk1 protein kinase|Schizosaccharomyces pombe|c	SPCC1259.13	30.80%	34
|||arginine-tRNA protein transferase |Schizosaccharomyces p	SPAC3C7.07c	29.90%	24
|rpl3001|rpl30-1, rpl30|60S ribosomal protein L30|Schizosac	SPAC9G1.03c	29.40%	12
|ase1||microtubule-associated protein Ase1 |Schizosaccharom	SPAPB1A10.09	29.00%	25
|pik1||phosphatidylinositol kinase Pik1|Schizosaccharomyces	SPAC22E12.16c	28.80%	57
|||arrestin Aly1 related|Schizosaccharomyces pombe|chr 2|||	SPBC2D10.04	28.70%	40
|||DUF1776 family protein|Schizosaccharomyces pombe|chr 2||	SPBC106.03	28.60%	32
|arg5||arginine specific carbamoyl-phosphate synthase Arg5	SPBC56F2.09c	28.40%	15

|arf1||ADP-ribosylation factor Arf1|Schizosaccharomyces pom	SPBC4F6.18c	27.80%	12
|||Spo7 homolog|Schizosaccharomyces pombe|chr 2|||Manual	SPBC902.03	27.80%	73
||SPCC736.16|DUF1769 family protein|Schizosaccharomyces pom	SPCC594.01	27.40%	52
|hem15||ferrochelatase|Schizosaccharomyces pombe|chr 3|||Ma	SPCC320.09	27.30%	13
|mug161||CwfJ family protein|Schizosaccharomyces pombe|chr	SPAC1F3.09	27.10%	183
|rga3||GTPase activating protein Rga3|Schizosaccharomyces p	SPAC29A4.11	27.10%	53
||SPCC1753.06c|sequence orphan|Schizosaccharomyces pombe|ch	SPCC162.12	27.10%	44
	SPAC17A5.10	25.90%	17
|tom40|SPBC8D2.22|mitochondrial TOM complex subunit Tom40|S	SPBC27B12.13	25.60%	13
|||transcription factor |Schizosaccharomyces pombe|chr 2|||	SPBC27B12.11c	25.60%	48
|nhp2||RNA-binding protein Nhp2 |Schizosaccharomyces pombe|	SPAC1782.10c	25.30%	4
|ppi1|cyp2|cyclophilin family peptidyl-prolyl cis-trans iso	SPBC28F2.03	25.30%	9
|||CTP synthase |Schizosaccharomyces pombe|chr 1|||Manual	SPAC10F6.03c	25.30%	34
|||MSP domain|Schizosaccharomyces pombe|chr 1|||Manual	SPAC17C9.12	25.10%	7
|||lipoate-protein ligase |Schizosaccharomyces pombe|chr 1|	SPAC4F10.05c	25.10%	9
	SPBC1703.13c	24.80%	40
|set6||histone lysine methyltransferase Set6 |Schizosacchar	SPBP8B7.07c	24.60%	70
|||alpha,alpha-trehalose-phosphate synthase |Schizosaccharo	SPACUNK4.16c	24.60%	85
||SPAC824.01|phosphatidylinositol 4-kinase Lsb6 |Schizosacc	SPAC343.19	24.40%	55
|||MTC tricarboxylate transporter|Schizosaccharomyces pombe	SPAC17G6.15c	24.30%	26
|rip1||ubiquinol-cytochrome-c reductase complex subunit 5|S	SPBC16H5.06	24.10%	18
||SPAC955.02c|nuclease, XP-G family|Schizosaccharomyces pom	SPAC139.01c	23.30%	33

|||amino acid transporter |Schizosaccharomyces pombe|chr 1|	SPAC3H1.09c	22.70%	27
|cpc2|rkp1|RACK1 homologue Cpc2|Schizosaccharomyces pombe|c	SPAC6B12.15	22.60%	11
|tuf1||mitochondrial translation elongation factor EF-Tu Tu	SPBC9B6.04c	22.60%	19
|||serine-tRNA ligase|Schizosaccharomyces pombe|chr 1|||Man	SPAC29A4.15	22.20%	13
|cdc25|sal2|serine/threonine protein phosphatase Cdc25|Schi	SPAC24H6.05	22.00%	40
|mei2||RNA-binding protein involved in meiosis Mei2|Schizos	SPAC27D7.03c	21.70%	34
|shk1|pak1, orb2|PAK-related kinase Shk1|Schizosaccharomyce	SPBC1604.14c	21.70%	25
|chr3|cfh1|chitin synthase regulatory factor Chr3 |Schizosa	SPAC24B11.10c	21.50%	46
|mug35|SPAC23G3.13c|sequence orphan|Schizosaccharomyces pom	SPAC22H12.01c	21.40%	3
|||sequence orphan|Schizosaccharomyces pombe|chr 2|||Manual	SPBC1289.06c	21.20%	20
|||diacylglycerol binding protein |Schizosaccharomyces pomb	SPCC297.05	21.00%	118
|ppk22||serine/threonine protein kinase Ppk22 |Schizosaccha	SPBC1861.09	20.90%	38
|rap1||telomere binding protein Rap1|Schizosaccharomyces po	SPBC1778.02	20.90%	37
|sec13||COPII-coated vesicle component Sec13|Schizosaccharo	SPBC215.15	20.90%	6
|scr1||transcription factor Scr1|Schizosaccharomyces pombe|	SPBC1D7.02c	20.70%	49
|shy1||SURF-family protein Shy1|Schizosaccharomyces pombe|c	SPBC1215.01	20.70%	6
|||prohibitin Phb1|Schizosaccharomyces pombe|chr 1|||Manual	SPAC1782.06c	20.60%	3
|rgf2||RhoGEF Rgf2|Schizosaccharomyces pombe|chr 1|||Manual	SPAC1006.06	20.50%	81
|ssa1||heat shock protein Ssa1|Schizosaccharomyces pombe|ch	SPAC13G7.02c	20.30%	21
|mug33||conserved fungal protein|Schizosaccharomyces pombe|	SPCC1739.10	20.20%	7
	SPAC18G6.09c	20.20%	20
|taf50||histone H4-like TAF |Schizosaccharomyces pombe|chr	SPCC16C4.18c	20.10%	17

|cdr2||GIN4 family protein kinase Cdr2|Schizosaccharomyces	SPAC57A10.02	20.00%	28
|||yippee-like protein|Schizosaccharomyces pombe|chr 1|||Ma	SPAPJ691.02	19.80%	10
|tea3||cell end marker Tea3|Schizosaccharomyces pombe|chr 1	SPAC6G10.02c	19.70%	43
|taf9||transcription initiation factor Taf9|Schizosaccharom	SPAC12G12.05c	19.60%	19
|its3||1-phosphatidylinositol-4-phosphate 5-kinase Its3|Sch	SPAC19G12.14	19.50%	41
|kin1||microtubule affinity-regulating kinase Kin1 |Schizos	SPBC4F6.06	19.50%	76
|pro1||gamma-glutamyl phosphate reductase Pro1 |Schizosacch	SPAC821.11	19.50%	16
|cog2||Golgi transport complex subunit Cog2 |Schizosaccharo	SPBC36.08c	19.40%	6
|lcb2|SPAC2C4.02|serine palmitoyltransferase |Schizosacchar	SPAC21E11.08	19.40%	23
|rpl2102|rpl21-2, rpl21|60S ribosomal protein L21|Schizosac	SPAC959.08	19.40%	20
|nrm1||negative regulator of MBF|Schizosaccharomyces pombe|	SPBC16A3.07c	19.30%	21
|pmp1||dual-specificity MAP kinase phosphatase Pmp1|Schizos	SPBC1685.01	19.10%	5
|tif51||translation initiation factor eIF5A|Schizosaccharom	SPAC26H5.10c	19.10%	4
|prz1||transcription factor Prz1 |Schizosaccharomyces pombe	SPAC4G8.13c	18.80%	45
|rsp1||random septum position protein Rsp1|Schizosaccharomy	SPBC11B10.05c	18.80%	9
|||1-acylglycerol-3-phosphate O-acyltransferase|Schizosacch	SPAC1851.02	18.60%	29
|||alpha-1,2-galactosyltransferase|Schizosaccharomyces pomb	SPBC8D2.17	18.50%	41
|cox5||cytochrome c oxidase subunit V|Schizosaccharomyces p	SPCC338.10c	18.40%	3
|||aldehyde dehydrogenase|Schizosaccharomyces pombe|chr 1||	SPAC9E9.09c	18.30%	9
|mrpl4||mitochondrial ribosomal protein subunit L4|Schizosa	SPCC4G3.06c	18.10%	12
|cdr1|nim1|GIN4 family protein kinase Cdr1|Schizosaccharomy	SPAC644.06c	17.90%	17
|||glycerol-3-phosphate O-acyltransferase	SPBC1718.04	17.90%	26

|Schizosaccharomy			
|ppk2||serine/threonine protein kinase Ppk2 |Schizosaccharo	SPAC12B10.14c	17.70%	26
|alg2|SPBC32H8.14|mannosyltransferase complex subunit Alg2|	SPBC11B10.01	17.60%	25
|hhp1||serine/threonine protein kinase Hhp1|Schizosaccharom	SPBC3H7.15	17.50%	13
|||sphingosine hydroxylase |Schizosaccharomyces pombe|chr 2	SPBC887.15c	17.40%	13
|hhp2||serine/threonine protein kinase Hhp2 |Schizosaccharo	SPAC23C4.12	16.80%	13
|rgf1||RhoGEF for Rho1, Rgf1|Schizosaccharomyces pombe|chr	SPCC645.07	16.80%	34
|rpt6|let1|19S proteasome regulatory subunit Rpt6|Schizosac	SPBC23G7.12c	16.60%	13
|dna2||DNA replication endonuclease-helicase Dna2|Schizosac	SPBC16D10.04c	16.50%	37
|rga5|SPBC557.01|GTPase activating protein Rga5|Schizosacch	SPBC17F3.01c	16.30%	6
|||WD repeat protein, human WRDR48 family|Schizosaccharomyc	SPAC31A2.14	16.30%	48
|int6|yin6|translation initiation factor eIF3e|Schizosaccha	SPBC646.09c	16.00%	8
|||SAGA complex subunit Spt8 |Schizosaccharomyces pombe|chr	SPBC14C8.17c	16.00%	13
|med15|SPBP35G2.15|mediator complex subunit Med15 |Schizosa	SPBC146.01	15.70%	22
|||1-acylglycerol-3-phosphate acyltransferase |Schizosaccha	SPBC428.14	15.70%	5
|||metaxin 1|Schizosaccharomyces pombe|chr 1|||Manual	SPAC589.04	15.50%	4
|alo1||D-arabinono-1,4-lactone oxidase|Schizosaccharomyces	SPAPB1A10.12c	15.40%	5
|caf1|pop2|CCR4-Not complex subunit Caf1|Schizosaccharomyce	SPCC18.06c	15.40%	5
|arp9||SWI/SNF and RSC complex subunit Arp9|Schizosaccharom	SPAC1071.06	15.30%	18
|||WD repeat protein, human WDR20 family|Schizosaccharomyce	SPAC12B10.03	15.30%	24
|plc1||phosphoinositide phospholipase C Plc1|Schizosaccharo	SPAC22F8.11	15.10%	14
|nam9||mitochondrial ribosomal protein subunit S4|Schizosac	SPBC13G1.01c	15.00%	3

|||transcription adaptor protein |Schizosaccharomyces pombe	SPBC887.18c	15.00%	10
|||mitochondrial ribosomal protein subunit Yml6|Schizosacch	SPBC2D10.08c	14.90%	3
|||ATP-citrate synthase subunit 2 |Schizosaccharomyces pomb	SPAC22A12.16	14.80%	5
|||homoserine kinase |Schizosaccharomyces pombe|chr 2|||Man	SPBC4C3.03	14.80%	13
|||mitochondrial citrate transporter|Schizosaccharomyces po	SPAC19G12.05	14.80%	24
|mph1|SPBC1271.16c, SPBC243.01|dual specificity protein kin	SPBC106.01	14.60%	23
|ams2|SPCC4F11.01|cell cycle regulated GATA-type transcript	SPCC290.04	14.50%	19
||SPBC29A3.20c|serine palmitoyltransferase complex subunit	SPBC18E5.02c	14.50%	15
|||protein disulfide isomerase |Schizosaccharomyces pombe|c	SPBC3D6.13c	14.50%	46
|apt1||adenine phosphoribosyltransferase |Schizosaccharomyc	SPAC23A1.03	14.40%	4
|exo1|mut2|exonuclease I Exo1|Schizosaccharomyces pombe|chr	SPBC29A10.05	14.40%	14
|gcn5||histone acetyltransferase Gcn5|Schizosaccharomyces p	SPAC1952.05	14.30%	9
||SPAC17G6.01|CorA family magnesium ion transporter|Schizos	SPAC17A2.14	14.30%	38
	SPCPB16A4.02c	14.30%	21
|||methylthioribose-1-phosphate isomerase |Schizosaccharomy	SPBC23E6.10c	14.20%	2
|ppk6|SPAPJ736.02c|serine/threonine protein kinase Ppk6|Sch	SPAC1805.01c	14.10%	9
|rfc3|SPAPJ698.01c|DNA replication factor C complex subunit	SPAC27E2.10c	14.00%	10
|oac1||anion transporter|Schizosaccharomyces pombe|chr 1|||	SPAC139.02c	13.80%	19
|cps3|mug188|zinc finger protein Cps3|Schizosaccharomyces p	SPAC3A11.02	13.70%	35
|||translation initiation factor |Schizosaccharomyces pombe	SPBC16C6.05	13.70%	5
|cdc11||SIN component scaffold protein Cdc11|Schizosaccharo	SPCC1739.11c	13.60%	52
	SPAC1687.09	13.60%	34

|||alpha-1,2-galactosyltransferase |Schizosaccharomyces pom	SPAC637.06	13.50%	13
|coq5||C-methytransferase |Schizosaccharomyces pombe|chr 3|	SPCC4G3.04c	13.40%	2
|mge1||GrpE domain chaperone protein|Schizosaccharomyces po	SPBC3B9.19	13.00%	4
|mkh1||MEK kinase |Schizosaccharomyces pombe|chr 1|||Manual	SPAC1F3.02c	13.00%	30
|||conserved protein|Schizosaccharomyces pombe|chr 3|||Manu	SPCC736.12c	13.00%	11
|||serine/threonine protein kinase |Schizosaccharomyces pom	SPAP27G11.07c	13.00%	3
|tub1|atb2, alp2, ban5|tubulin alpha 2|Schizosaccharomyces	SPBC800.05c	12.90%	5
|ubp16||ubiquitin C-terminal hydrolase Ubp16|Schizosaccharo	SPCC1682.12c	12.90%	10
|||aldehyde dehydrogenase |Schizosaccharomyces pombe|chr 2|	SPBC21C3.15c	12.80%	8
|cct4||chaperonin-containing T-complex delta subunit Cct4|S	SPBC106.06	12.30%	7
|msa1|SPAC6C3.01c|RNA-binding protein Msa1|Schizosaccharomy	SPAC13G7.13c	12.20%	11
|||inositol polyphosphate kinase |Schizosaccharomyces pombe	SPCC970.08	12.20%	33
|arp42|arp4|SWI/SNF and RSC complex subunit Arp42|Schizosac	SPAC23D3.09	12.10%	4
|ppk8||serine/threonine protein kinase Ppk8 |Schizosaccharo	SPAC22G7.08	12.10%	25
|||enoyl reductase|Schizosaccharomyces pombe|chr 2|||Manual	SPBC646.07c	11.90%	4
||SPBP22H7.01c|membrane transporter |Schizosaccharomyces po	SPBC691.05c	11.80%	9
|ppk25||serine/threonine protein kinase Ppk25 |Schizosaccha	SPBC32C12.03c	11.60%	24
|||NAD dependent epimerase/dehydratase family protein|Schiz	SPCC1840.09	11.60%	5
|ubp9||ubiquitin C-terminal hydrolase Ubp9|Schizosaccharomy	SPBC1703.12	11.50%	11
|tea4|wsh3|tip elongation aberrant protein Tea4|Schizosacch	SPBC1706.01	11.40%	11
|vma1||V-type ATPase subunit A|Schizosaccharomyces pombe|ch	SPAC343.05	11.30%	7
|dma1||mitotic spindle checkpoint protein	SPAC17G8.10c	11.20%	3

Dma1|Schizosaccha			
|sak1||transcriptional repressor Sak1|Schizosaccharomyces p	SPAC3G9.14	11.00%	10
|||nucleotide sugar transporter |Schizosaccharomyces pombe|	SPAC144.18	11.00%	14
||SPAC30D11.15c|Moeb/ThiF domain|Schizosaccharomyces pombe|	SPAC1A6.10	10.90%	7
|||SWI/SNF complex subunit Snf59|Schizosaccharomyces pombe|	SPBC26H8.09c	10.90%	3
|mug154||conserved fungal protein|Schizosaccharomyces pombe	SPCC4G3.11	10.80%	5
|||mitochondrial NADH kinase |Schizosaccharomyces pombe|chr	SPAC323.01c	10.80%	3
|sre2||membrane-tethered transcription factor |Schizosaccha	SPBC354.05c	10.70%	11
|||conserved fungal protein|Schizosaccharomyces pombe|chr 1	SPAC1565.01	10.70%	3
|||hypothetical protein|Schizosaccharomyces pombe|chr 1|||M	SPAC18G6.12c	10.70%	17
|ade8||adenylosuccinate lyase Ade8|Schizosaccharomyces pomb	SPBC14F5.09c	10.60%	6
|taf72||transcription factor TFIID complex subunit 5 Taf72|	SPCC5E4.03c	10.60%	10
|||acyl-coA desaturase |Schizosaccharomyces pombe|chr 3|||M	SPCC1281.06c	10.60%	6
|||Golgi transport complex subunit Cog3|Schizosaccharomyces	SPBC1539.05	10.50%	9
|nfs1||iron-sulfur cluster assembly protein Nfs1|Schizosacc	SPBC21D10.11c	10.40%	3
|atg13|apg13, mug78|autophagy associated protein Atg13 |Sch	SPAC4F10.07c	10.30%	31
|snf5||chromatin remodeling complex subunit Snf5 |Schizosac	SPAC2F7.08c	10.30%	7
|trp2||tryptophan synthase|Schizosaccharomyces pombe|chr 1|	SPAC19A8.15	10.30%	8
	SPAPB1A10.13	10.20%	9
|pss1|ssp1, SPAP14E8.01c|heat shock protein Pss1|Schizosacc	SPAC110.04c	10.10%	7
|wis1|spc2, smf2|MAP kinase kinase Wis1|Schizosaccharomyces	SPBC409.07c	10.10%	16
|cct3||chaperonin-containing T-complex gamma subunit Cct3|S	SPBC1A4.08c	10.00%	6

|cdc17||ATP-dependent DNA ligase Cdc17|Schizosaccharomyces	SPAC20G8.01	10.00%	5
|cut11|SPAC24C9.01|integral membrane nucleoporin|Schizosacc	SPAC1786.03	10.00%	7
|cct8||chaperonin-containing T-complex theta subunit Cct8 |	SPBC337.05c	9.90%	11
|mex67||mRNA export receptor Mex67|Schizosaccharomyces pomb	SPBC1921.03c	9.90%	8
|gly1||threonine aldolase |Schizosaccharomyces pombe|chr 1|	SPAC23H3.09c	9.80%	3
|map1||MADS-box transcription factor Map1|Schizosaccharomyc	SPAC11E3.06	9.80%	4
|vps901|vps9a|guanyl-nucleotide exchange factor Vps901 |Sch	SPBC4F6.10	9.70%	15
|gpd1||glycerol-3-phosphate dehydrogenase Gpd1|Schizosaccha	SPBC215.05	9.40%	9
|ssr3||SWI/SNF and RSC complex subunit Ssr3|Schizosaccharom	SPAC23G3.10c	9.40%	4
|||triglyceride lipase-cholesterol esterase |Schizosaccharo	SPCC1672.09	9.40%	7
|ssr1||SWI/SNF and RSC complex subunit Ssr1|Schizosaccharom	SPAC17G6.10	9.30%	13
|taf10||transcription factor TFIID complex subunit Taf10 |S	SPBC21H7.02	9.30%	4
|vps1|SPAC9G1.14c|dynamin family protein Vps1|Schizosacchar	SPAC767.01c	9.30%	30
|cct6||chaperonin-containing T-complex zeta subunit Cct6|Sc	SPBC646.11	9.20%	11
|cek1||serine/threonine protein kinase Cek1|Schizosaccharom	SPCC1450.11c	9.10%	22
|ssb1|rpa1, rad11|DNA replication factor A subunit Ssb1 |Sc	SPBC660.13c	8.90%	9
|||UTP-glucose-1-phosphate uridylyltransferase |Schizosacch	SPCC1322.04	8.90%	13
|cdc24||DNA replication protein Cdc24|Schizosaccharomyces p	SPAC8F11.07c	8.80%	5
|||signal recognition particle receptor alpha subunit Srp10	SPBC3B9.03	8.80%	8
|pub3||ubiquitin-protein ligase E3|Schizosaccharomyces pomb	SPBC16E9.11c	8.70%	12
|ubp7||ubiquitin C-terminal hydrolase Ubp7|Schizosaccharomy	SPAC23G3.08c	8.70%	10
|||WD repeat protein Wdr44	SPAC3H5.08c	8.70%	23

family|Schizosaccharomyces pombe			
|||RNA-binding protein|Schizosaccharomyces pombe|chr 2|||Ma	SPBC56F2.08c	8.60%	7
|||asparagine-tRNA ligase Ded81 |Schizosaccharomyces pombe|	SPBC1773.10c	8.60%	6
|||cargo receptor for soluble proteins |Schizosaccharomyces	SPCC970.06	8.60%	10
	SPBC2G2.14	8.60%	4
|mbx1||MADS-box transcription factor Mbx1|Schizosaccharomyc	SPBC19G7.06	8.50%	7
|||phosphogluconate dehydrogenase, decarboxylating |Schizos	SPBC660.16	8.50%	5
|cdc22||ribonucleoside reductase large subunit Cdc22|Schizo	SPAC1F7.05	8.40%	8
|phx1||homeobox transcription factor Phx1|Schizosaccharomyc	SPAC32A11.03c	8.30%	21
|gua1||IMP dehydrogenase Gua1 |Schizosaccharomyces pombe|ch	SPBC2F12.14c	8.20%	9
|puf3|SPAC222.02c|RNA-binding protein Puf3 |Schizosaccharom	SPAC1687.22c	8.20%	9
|||CCR4/nocturin family endoribonuclease|Schizosaccharomyce	SPBC9B6.11c	8.20%	9
|||AAA family ATPase Rix7 |Schizosaccharomyces pombe|chr 2|	SPBC16E9.10c	8.10%	12
|||TRAPP complex subunit Trs120 |Schizosaccharomyces pombe|	SPAC6G10.05c	8.10%	16
|||hydroxyacid dehydrogenase |Schizosaccharomyces pombe|chr	SPACUNK4.10	8.10%	3
|||long-chain-fatty-acid-CoA ligase |Schizosaccharomyces po	SPBP4H10.11c	8.10%	7
|||uridine kinase |Schizosaccharomyces pombe|chr 3|||Manual	SPCC162.11c	8.10%	10
|ral2||Ras guanyl-nucleotide exchange factor Ral2 |Schizosa	SPBC21.05c	8.00%	4
|rga6||GTPase activating protein Rga6|Schizosaccharomyces p	SPBC354.13	8.00%	6
	SPAC4G8.04	8.00%	6
|||DUF887 family protein|Schizosaccharomyces pombe|chr 1|||	SPAC17A2.02c	7.90%	5
|||adenosylhomocysteinase |Schizosaccharomyces pombe|chr 2|	SPBC8D2.18c	7.90%	6

|mip1||WD repeat protein Mip1|Schizosaccharomyces pombe|chr	SPAC57A7.11	7.80%	15
|rpt1||19S proteasome regulatory subunit Rpt1|Schizosacchar	SPBC16C6.07c	7.80%	6
|||18S rRNA dimethylase|Schizosaccharomyces pombe|chr 2|||M	SPBC336.02	7.80%	7
|||striatin homolog|Schizosaccharomyces pombe|chr 2|||Manua	SPBC1773.01	7.80%	5
|itr2||MFS myo-inositol transporter|Schizosaccharomyces pom	SPAC20G8.03	7.70%	11
|rpt3||19S proteasome regulatory subunit Rpt3|Schizosacchar	SPCC576.10c	7.70%	8
|||translation initiation factor eIF4A|Schizosaccharomyces	SPAC1006.07	7.70%	5
|ade9||C-1-tetrahydrofolatesynthase/methylenetetrahydrofola	SPBC2G2.08	7.60%	7
|gef2||RhoGEF Gef2|Schizosaccharomyces pombe|chr 1|||Manual	SPAC31A2.16	7.60%	12
|vma2||V-type ATPase V1 subunit B |Schizosaccharomyces pomb	SPAC637.05c	7.60%	3
|||6-phosphofructo-2-kinase |Schizosaccharomyces pombe|chr	SPAPB17E12.14c	7.60%	13
|||Sad1-UNC-like C-terminal|Schizosaccharomyces pombe|chr 2	SPBC3E7.09	7.60%	6
	SPBC365.16	7.60%	5
|nak1|orb3, mor4|PAK-related kinase Nak1|Schizosaccharomyce	SPBC17F3.02	7.50%	12
|||conserved eukaryotic protein|Schizosaccharomyces pombe|c	SPBC1539.04	7.50%	13
||SPCC63.01c|sequence orphan|Schizosaccharomyces pombe|chr	SPCC2H8.05c	7.40%	10
|||EST1 family protein|Schizosaccharomyces pombe|chr 2|||Ma	SPBC2F12.03c	7.40%	9
|clp1|flp1|Cdc14-related protein phosphatase Clp1/Flp1|Schi	SPAC1782.09c	7.30%	8
|snf22|SPCC830.01c|ATP-dependent DNA helicase Snf22|Schizos	SPCC1620.14c	7.30%	9
|||inorganic phosphate transporter |Schizosaccharomyces pom	SPAC23D3.12	7.30%	16
|dsk1||SR protein-specific kinase Dsk1|Schizosaccharomyces	SPBC530.14c	7.20%	2
|klp2||kinesin-like protein Klp2|Schizosaccharomyces pombe|	SPAC664.10	7.20%	7

||SPBC17D11.09|sequence orphan|Schizosaccharomyces pombe|ch	SPBC17D1.01	7.20%	10
|||WD repeat protein Wdr44 family, WD repeat protein|Schizo	SPBC18H10.05	7.20%	7
|||fatty acid hydroxylase |Schizosaccharomyces pombe|chr 1|	SPAC19G12.08	7.20%	7
|cbp3||ubiquinol cytochrome-c reductase assembly protein Cb	SPCC4B3.17	7.10%	10
|||ornithine aminotransferase|Schizosaccharomyces pombe|chr	SPBC21C3.08c	7.10%	5
|alg11|gmd3|alpha-1,2-mannosyltransferase Alg11|Schizosacch	SPCC330.08	7.00%	6
|erg8||phosphomevalonate kinase |Schizosaccharomyces pombe|	SPAC343.01c	7.00%	5
|pub1||ubiquitin-protein ligase E3|Schizosaccharomyces pomb	SPAC11G7.02	7.00%	8
|rpt4||19S proteasome regulatory subunit Rpt4|Schizosacchar	SPCC1682.16	7.00%	4
|||argininosuccinate lyase|Schizosaccharomyces pombe|chr 2|	SPBC1539.03c	7.00%	3
|ppk30||Ark1/Prk1 family protein kinase Ppk30|Schizosacchar	SPBC6B1.02	6.90%	13
|rpn6||19S proteasome regulatory subunit Rpn6|Schizosacchar	SPAC23G3.11	6.90%	3
||SPBC4C3.01|sequence orphan|Schizosaccharomyces pombe|chr	SPBC405.02c	6.90%	9
|||leucine-rich repeat protein Sog2 |Schizosaccharomyces po	SPBC887.09c	6.80%	8
|||inositol polyphosphate phosphatase |Schizosaccharomyces	SPBC19F5.03	6.70%	5
|||nucleoporin Nup60 |Schizosaccharomyces pombe|chr 3|||Man	SPCC285.13c	6.70%	3
|hem1||5-aminolevulinate synthase|Schizosaccharomyces pombe	SPAC2F3.09	6.60%	7
|||Haemolysin-III family protein|Schizosaccharomyces pombe|	SPAC30D11.11	6.60%	14
|||histone acetyltransferase complex subunit Ada2 |Schizosa	SPCC24B10.08c	6.60%	5
|vht1||vitamin H transporter Vth1|Schizosaccharomyces pombe	SPAC1B3.16c	6.50%	3
|||mannosyltransferase complex subunit |Schizosaccharomyces	SPAC17G8.11c	6.50%	8
|pgi1||glucose-6-phosphate isomerase	SPBC1604.05	6.40%	2

|Schizosaccharomyces p			
|sts1|erg4|C-24|Schizosaccharomyces pombe|chr 1|||Manual	SPAC20G4.07c	6.40%	2
||SPCC285.18|ubiquitin-protein ligase E3 |Schizosaccharomyc	SPCC1223.01	6.40%	3
|||delta-1-pyrroline-5-carboxylate dehydrogenase|Schizosacc	SPBC24C6.04	6.40%	5
|||homoserine dehydrogenase |Schizosaccharomyces pombe|chr	SPBC776.03	6.40%	4
|||phosphoserine aminotransferase |Schizosaccharomyces pomb	SPAC1F12.07	6.40%	4
|||pyruvate dehydrogenase protein x component|Schizosacchar	SPCC1259.09c	6.40%	4
	SPBC557.02c	6.40%	2
|ef1a-a||translation elongation factor EF-1 alpha Ef1a-a |S	SPCC794.09c	6.30%	26
|rpn501|rpn5-a, rpn5|19S proteasome regulatory subunit Rpn5	SPAC1420.03	6.30%	6
|sec61||translocon alpha subunit Sec61|Schizosaccharomyces	SPBC354.02c	6.30%	25
|||HEAT repeat protein, unknown biological role|Schizosacch	SPCC794.08	6.30%	8
|scd1|ral1|RhoGEF Scd1|Schizosaccharomyces pombe|chr 1|||Ma	SPAC16E8.09	6.20%	6
||SPAC56E4.08c|DUF1752 family protein|Schizosaccharomyces p	SPAC1420.01c	6.20%	6
|dga1|SPCC548.01|diacylglycerol O-acyltransferase |Schizosa	SPCC1235.15	6.10%	4
|sec63||ER protein translocation subcomplex subunit Sec63 |	SPBC36B7.03	6.10%	3
||SPBP4G3.01|inorganic phosphate transporter |Schizosacchar	SPBC8E4.01c	6.10%	3
|sec21||coatomer gamma subunit Sec21 |Schizosaccharomyces p	SPAC57A7.10c	6.00%	5
|uap56||ATP-dependent RNA helicase Uap56|Schizosaccharomyce	SPAC17G6.14c	6.00%	9
	SPAC1A6.07	5.80%	6
|hop1||linear element associated protein Hop1|Schizosacchar	SPBC1718.02	5.70%	3
|rga1||GTPase activating protein Rga1|Schizosaccharomyces p	SPBC3F6.05	5.70%	9

|||conserved fungal protein|Schizosaccharomyces pombe|chr 2	SPBC26H8.11c	5.70%	4
|||electron transfer flavoprotein-ubiquinone oxidoreductase	SPAC20G8.04c	5.70%	6
|||protein phosphatase regulatory subunit Reg1 |Schizosacch	SPAC227.15	5.70%	5
|||NADPH dehydrogenase |Schizosaccharomyces pombe|chr 1|||M	SPAC5H10.10	5.60%	13
|||bromodomain protein|Schizosaccharomyces pombe|chr 2|||Ma	SPBC25H2.11c	5.60%	6
|cct5||chaperonin-containing T-complex epsilon subunit Cct5	SPAC1420.02c	5.50%	8
|rsd1||RNA-binding protein Rsd1|Schizosaccharomyces pombe|c	SPAC19G12.07c	5.50%	5
|seb1||RNA-binding protein Seb1 |Schizosaccharomyces pombe|	SPAC222.09	5.50%	3
|cdc48|SPAC6F12.01|AAA family ATPase Cdc48|Schizosaccharomy	SPAC1565.08	5.40%	3
|mug174||meiotically upregulated gene Mug174|Schizosaccharo	SPCC1682.03c	5.40%	2
|sol1||SWI/SNF complex subunit Sol1|Schizosaccharomyces pom	SPBC30B4.04c	5.40%	4
|ksp1|ppk20|serine/threonine protein kinase Ksp1 |Schizosac	SPBC16E9.13	5.30%	10
|kap123||karyopherin Kap123|Schizosaccharomyces pombe|chr 2	SPBC14F5.03c	5.20%	8
|||amino acid permease, unknown 8|Schizosaccharomyces pombe	SPBC359.03c	5.20%	6
|||human UVRAG |Schizosaccharomyces pombe|chr 2|||Manual	SPBC18H10.19	5.20%	3
|sec16||multidomain vesicle coat component Sec16|Schizosacc	SPAC29B12.07	5.10%	19
|||TRAPP complex subunit Trs130 |Schizosaccharomyces pombe|	SPCC285.14	5.10%	8
|ago1|csp9|argonaute|Schizosaccharomyces pombe|chr 3|||Manu	SPCC736.11	5.00%	2
|kap109||karyopherin Kap109|Schizosaccharomyces pombe|chr 2	SPBC30B4.05	5.00%	3
|tms1||hexitol dehydrogenase |Schizosaccharomyces pombe|chr	SPBC1773.05c	5.00%	3
||SPBC21D10.02|glutamine-fructose-6-phosphate transaminase	SPBC12C2.11	5.00%	10
|||sulfate transporter |Schizosaccharomyces pombe|chr	SPAC869.05c	5.00%	4

1|||M			
|elg1||DNA replication factor C complex subunit Elg1|Schizo	SPBC947.11c	4.90%	3
|ppk29||Ark1/Prk1 family protein kinase Ppk29|Schizosacchar	SPBC557.04	4.90%	4
	SPCC895.08c	4.90%	6
|||DUF1682 family protein|Schizosaccharomyces pombe|chr 2||	SPBC2G5.01	4.80%	7
|||PTR family peptide transporter|Schizosaccharomyces pombe	SPBC13A2.04c	4.70%	4
|rna14||mRNA cleavage and polyadenylation specificity facto	SPAC6F12.17	4.50%	6
|||ribosome biogenesis protein Rrp12|Schizosaccharomyces po	SPAPB8E5.07c	4.50%	2
|||amino acid permease, unknown 9|Schizosaccharomyces pombe	SPBC18H10.16	4.40%	8
|||membrane transporter|Schizosaccharomyces pombe|chr 2|||M	SPBC3B8.04c	4.40%	3
|||phospholipase|Schizosaccharomyces pombe|chr 1|||Manual	SPAC20G8.02	4.40%	13
|arg11||N-acetyl-gamma-glutamyl-phosphate reductase/acetylg	SPAC4G9.09c	4.30%	5
|gti1||gluconate transporter inducer Gti1|Schizosaccharomyc	SPAC1751.01c	4.30%	14
|hmt1|SPCC74.08c|ATP-binding cassette-type vacuolar membran	SPCC737.09c	4.30%	4
|||DUF1212 family protein|Schizosaccharomyces pombe|chr 1||	SPAC7D4.12c	4.10%	3
|||guanyl-nucleotide exchange factor |Schizosaccharomyces p	SPAC11E3.11c	4.10%	6
|hmg1||3-hydroxy-3-methylglutaryl-CoA reductase|Schizosacch	SPCC162.09c	3.90%	5
|||ATP citrate synthase subunit 1 |Schizosaccharomyces pomb	SPBC1703.07	3.90%	4
|prp11||ATP-dependent RNA helicase Prp11|Schizosaccharomyce	SPCC10H11.01	3.70%	2
|sec39||secretory pathway protein Sec39 |Schizosaccharomyce	SPAC7D4.11c	3.60%	3
|mts4|rpn1|19S proteasome regulatory subunit Mts4|Schizosac	SPBP19A11.03c	3.50%	4
|pik3|vps34|phosphatidylinositol 3-kinase Pik3|Schizosaccha	SPAC458.05	3.50%	3

|||tRNA uridine 5-carboxymethylaminomethyl modification enz	SPBC30B4.06c	3.50%	2
|||P-type ATPase |Schizosaccharomyces pombe|chr 1|||Manual	SPAC24B11.12c	3.40%	6
|||shuttle craft like transcriptional regulator|Schizosacch	SPCC18.03	3.30%	4
|||Arf GAP protein|Schizosaccharomyces pombe|chr 1|||Manual	SPAC26A3.10	3.10%	6
|||conserved fungal protein|Schizosaccharomyces pombe|chr 3	SPCC63.14	3.10%	4
|||ribonuclease II |Schizosaccharomyces pombe|chr 2|||Manua	SPBC609.01	3.10%	5
|elf1||AAA family ATPase ELf1|Schizosaccharomyces pombe|chr	SPAC3C7.08c	2.90%	5
|tea1|alp8|cell end marker Tea1|Schizosaccharomyces pombe|c	SPCC1223.06	2.90%	6
|||IPT/TIG ankyrin repeat protein|Schizosaccharomyces pombe	SPAC26H5.05	2.90%	3
|dcp2||mRNA decapping complex subunit Dcp2|Schizosaccharomy	SPAC19A8.12	2.80%	2
|||TFIIH regulator |Schizosaccharomyces pombe|chr 1|||Manua	SPAC1071.02	2.80%	3
|||lysine-tRNA ligase|Schizosaccharomyces pombe|chr 3|||Man	SPCC18.08	2.80%	14
|||translation elongation regulator Gcn1 |Schizosaccharomyc	SPAC18G6.05c	2.80%	16
|aro1||pentafunctional aromatic polypeptide Aro1 |Schizosac	SPAC1834.02	2.70%	3
|rpn2||19S proteasome regulatory subunit Rpn2|Schizosacchar	SPBC17D11.07c	2.70%	4
|wis4|wak1, wik1|MAP kinase kinase kinase Wis4|Schizosaccha	SPAC9G1.02	2.70%	7
|||karyopherin|Schizosaccharomyces pombe|chr 3|||Manual	SPCC550.11	2.70%	2
|pyr1||pyruvate carboxylase|Schizosaccharomyces pombe|chr 2	SPBC17G9.11c	2.60%	5
|||nuclear telomere cap complex subunit |Schizosaccharomyce	SPAC458.03	2.60%	5
||pmc1, pmc1|P-type ATPase, calcium transporting Pmc1 |Schi	SPAPB2B4.04c	2.50%	6
|||ubiquitin-protein ligase E3 |Schizosaccharomyces pombe|c	SPBC21D10.09c	2.50%	2
|efc25||exchange factor Cdc25p-like|Schizosaccharomyces pom	SPBC336.03	2.40%	7

|win1|SPAC1250.06c, SPAPJ730.01|MAP kinase kinase kinase Wi	SPAC1006.09	2.40%	6
|ags1|mok1, SPCC338.01c, SPCC17A7.01|alpha-1,4-glucan synth	SPCC1281.01	2.30%	16
|msh6||MutS protein homolog|Schizosaccharomyces pombe|chr 3	SPCC285.16c	2.30%	2
|pdr1||ABC transporter Pdr1|Schizosaccharomyces pombe|chr 1	SPAPB24D3.09c	2.30%	3
|rad50|SPAP4C9.01c|DNA repair protein Rad50|Schizosaccharom	SPAC1556.01c	2.30%	6
|||phosphatidylinositol kinase |Schizosaccharomyces pombe|c	SPBP16F5.03c	2.30%	11
|rpb2|SPAC521.06|DNA-directed RNA polymerase II complex sub	SPAC23G3.01	2.10%	3
|sal3|pse1|karyopherin Sal3|Schizosaccharomyces pombe|chr 3	SPCC1840.03	2.10%	4
||SPAC27F1.01c|actin cortical patch component, with EF hand	SPAC25G10.09c	2.00%	3
|vps1302|vps13b|chorein homolog|Schizosaccharomyces pombe|c	SPBC16C6.02c	1.90%	4
||SPAC31F12.02c, SPAC637.15c|ubiquitin-protein ligase E3 |S	SPAC12B10.01c	1.90%	5
|hrp3||ATP-dependent DNA helicase Hrp3|Schizosaccharomyces	SPAC3G6.01	1.80%	2
|chc1||clathrin heavy chain Chc1 |Schizosaccharomyces pombe	SPAC26A3.05	1.70%	4
|bgs1|cps1, drc1|1,3-beta-glucan synthase catalytic subunit	SPBC19G7.05c	1.60%	7
|ppk19||serine/threonine protein kinase Ppk19|Schizosacchar	SPBC119.07	1.60%	8
|||AMP binding enzyme |Schizosaccharomyces pombe|chr 1|||Ma	SPAC56F8.02	1.60%	3
|myo2|rng5|myosin II heavy chain|Schizosaccharomyces pombe|	SPCC645.05c	1.50%	3
|exo2||exonuclease II Exo2 |Schizosaccharomyces pombe|chr 1	SPAC17A5.14	1.40%	2
|||VIC sodium channel |Schizosaccharomyces pombe|chr 1|||Ma	SPAC6F6.01	1.40%	4
|bgs3||1,3-beta-glucan synthase subunit Bgs3|Schizosaccharo	SPAC19B12.03	1.30%	3
|cdc20|pol2|DNA polymerase epsilon catalytic subunit a Pol2	SPBC25H2.13c	1.20%	6

|lvs1|SPBC3H7.16|beige protein homolog|Schizosaccharomyces	SPBC28E12.06c	1.00%	4

Appendix C

Materials and Methods

Chapter II

Microscopy and data analysis

For time-lapse experiments, cells were pre-grown to early log phase in YE with supplements (YES) at 25°C. G2 cells obtained by centrifugal elutriation were concentrated and resuspended in 500 microliters of medium (approximately $5X10^6$ per ml). Cells were allowed to recover for 75 minutes at 25°C before recording. 2 microliters of concentrated cells were mounted on a thin layer of YES containing 1-2% agarose, and sealed under a coverslip with nail polish. Strains were imaged at 22°C-25°C using a Zeiss axiovert 200 microscope equipped with a confocal scanner unit model CSU10 (Yokogawa Electric Corporation), a coolSNAP HQ camera (Photometrics), and 63x 1.4 NA plan-apo or 100x 1.4NA plan-apo objective. Images were collected using Metamorph software (Universal Imaging, version 4.5) with 1x1 binning at intervals of 0.5-1 minute using exposures of 0.3 second for nuc1-GFP cdc11GFP and 1 second for sod2-LacO LacI-GFP cdc11GFP. The same software was used for image processing and quantifications. Analysis of meiosis in *clp1*Δ*/flp1*Δ cells was done as previously described (Krapp et al., 2006).

Chapter III

Strains construction

All yeast techniques and media were carried out as previously described (Moreno et al., 1991). The Clp1 mutant strains were constructed by integrating pJK210 based Clp1-GFP plasmids with various mutations, into the endogenous *clp1*$^{+}$ locus. The plasmid was linearized at the SnaB1 restriction site in *clp1*, transformed into the *clp1-C286S-13Myc* strain, and selected for *ura*$^{+}$ colonies. Correct clones were confirmed by PCR and direct sequencing of PCR products. The *clp1* copy expressed in the cell was controlled by its own endogenous promoter, and the second copy lacked a promoter and also contained the inactivating mutation cysteine 286 to serine.

In vitro kinase assays

The Sid2-Mob1 kinase complex was purified from *mob1-3HA-TAP::kanR* as described in (Gould et al., 2004) or by immunoprecipitation with anti-Myc antibodies from *sid2-13Myc cdc16-116* cells that had been shifted to 36°C for 4 hour. All the bacterially produced MBP-tagged Clp1 mutants were purified as described in (Tomlin et al., 2002). Kinase assays were performed as described previously (Sparks et al.,). Protein labeled by γ-^{32}P was imaged using a PhosphorImager (Molecular Dynamics).

Phosphatase assays

DiFMUP (6,8-difluoro-4-methylumbelliferyl phosphate; Invitrogen) continuous assays were performed on 75 ng of recombinant protein supplemented with bovine serum albumin (New England Biolabs) to 250 μg/ml in a 96 well plate. DiFMUP was added via

a FlexStation III (Molecular Devices) to a final concentration of 25 μM, and fluorescence was monitored at 30°C for 5 min every 1.5 s with excitation at 385 nm and emission measured at 455 nm (Wolfe et al., 2006). Fluorescent readings were plotted using Excel, and the rates of the reaction were determined using linear regression analysis. Protein quantifications for normalization were measured from Coomassie blue stained gels using Odyssey software.

Mass spectrometry

The bands containing MBP-Clp1 phosphorylated by Sid2 *in vitro* were excised and the samples were subjected to digestion with trypsin and chymotrypsin and then LC-MS/MS mass spectrometric analysis. The obtained mass spectra were filtered by Scandenser (Vanderbilt University Mass Spectrometry Research Center) and searched against the Sanger Institute *S. pombe* database using SEQUEST (Thermo Finnigan).

Microscopy

Cells were fixed by methanol as described in (Balasubramanian et al., 1997). All images were captured using the Nikon Eclipse E 600 microscope with a Hamamatsu ORCA-ER digital camera, and IPLab Spectrum software (Signal Analytics). Confocal microscopy was done by a Axiovert 200 microscope (Zeiss) with Argon Ion Laser System (Mellers Griot). Images were captured using IEEE 1394 digital CCD camera C4742-80-12AG (Hamamatsu) and UltraVIEW^{TM} RS confocal imaging system software (PerkinElmer).

In vitro binding assay

20 OD units at 595 nm of asynchronous *clp1-GFP* and *clp1-6A-GFP* cells were lysed by beating with glass beads in the presence of NP-40 buffers (6mM Na_2HPO_4, 4mM NaH_2PO_4, 1% NONIDET P-40, 150mM NaCl, 2mM EDTA, 50mM NaF, 0.1mM Na_3VO_4, 1μg/ml pepstatin, 10μg/ml leupeptin, 0.12 mg/ml AEBSF, 1mM PMSF, 1mM of Benzamidine). Lysate was divided into three portions. Bacterially produced GST, GST-Rad24, or the anti-GFP monoclonal antibodies (Molecular Probes) were added to each portion, and incubated at least for 1 hour at 4°C. 40 μl of glutathione sepharose resin (GE Healthcare) or protein G beads (Sigma) was added. After incubation for 1 hour at 4°C, the resin was collected, and the precipitated complex was subjected to SDS-PAGE, and detected by Western blot using anti-GFP (sc-9996) antibody (Santa Cruz Biotechnology).

Cytokinesis Checkpoint analysis

3ml overnight cultures of *clp1-GFP*, *clp1-6A-GFP*, and *clp1Δ* were diluted to OD 0.3 and treated with 4 μM Latrunculin B (Sigma). Samples were collected every 30 min, fixed with ice cold MeOH, and DAPI stained. The nuclear accumulation rate was scored by counting the total number of nuclei and dividing by the total cell number to get the average number of nuclei per cell (nuclei/cell).

Immunofluorescence

Immunostaining was performed as described in (Balasubramanian et al., 1997). Antibodies used included: monoclonal anti-HA (1:1000) from Covance), anti-tubulin TAT1 (1:30), a kind gift of Dr. Keith Gull, and anti-Cdc4 (1:100) (McCollum et al., 1995). Secondary antibodies were from Molecular Probes (1:400). The cells were mounted with media containing DAPI.

Chapter IV

In vitro kinase assays

The Sid2-Mob1 kinase complex was purified by immunoprecipitation with anti-Myc antibodies from *sid2*-13*Myc cdc16-116* cells that had been shifted to 36°C for 4 hour. All the bacterially produced His-ase1 and His-Klp2 were purified as described in (Janson et al., 2007). Both His-ase1 and His-Klp2 were dialysed using 20mM Tris-HCl (pH 7.4) overnight at 4°C before used as substrates in the in vitro kinase assay. Kinase assays were performed as described previously (Sparks et al. 1999). Protein labeled by γ-^{32}P was imaged using a PhosphorImager (Molecular Dynamics).

In vitro binding assay

20 OD units at 595 nm of asynchronous *Klp2-GFP* and wild type (no tag) cells were lysed by beating with glass beads in the presence of NP-40 buffers (6mM Na_2HPO_4, 4mM NaH_2PO_4, 1% NONIDET P-40, 150mM NaCl, 2mM EDTA, 50mM NaF, 0.1mM

Na_3VO_4, 1μg/ml pepstatin, 10μg/ml leupeptin, 0.12 mg/ml AEBSF, 1mM PMSF, 1mM of Benzamidine*).* *Klp2-GFP* lysate was divided into two portions and one adding bacterially produced His-ase1, whereas the other with buffer only. His-ase1 was also added to the lysate of wild type as a negative control. After at least 1 hour incubation at 4°C, 50 μl of Ni-NTA beads (Qiagen) was added. After another 1 hour incubation at 4°C, the resin was collected, and the precipitated complex was subjected to SDS-PAGE, and detected by Western blot using anti-GFP (sc-9996) antibody (Santa Cruz Biotechnology).

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