

## REGULATION OF CDC14: PATHWAYS AND CHECKPOINTS OF MITOTIC EXIT

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### 1. ABSTRACT

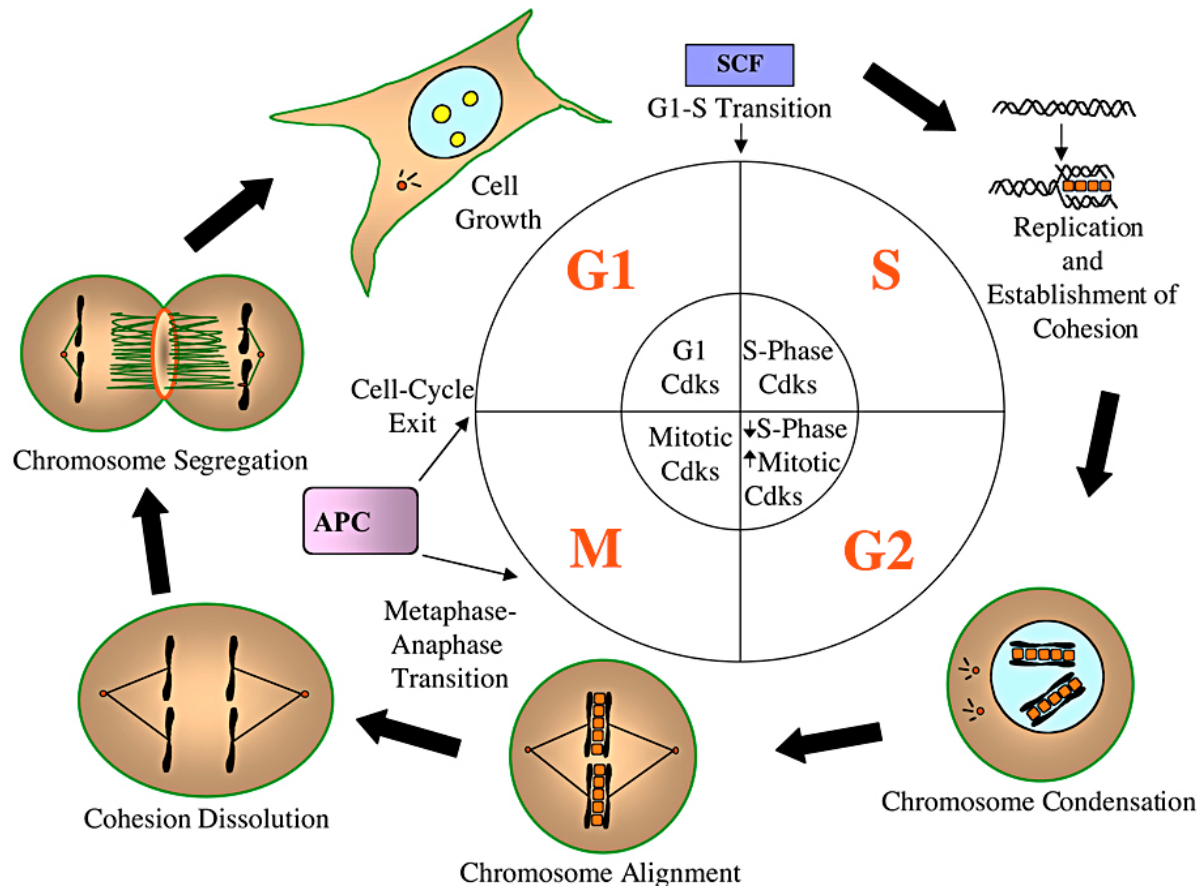
Progression of the mitotic cell cycle is driven by fluctuations of the cyclin-dependent kinase (Cdk) activities. Entry into mitosis is promoted by the elevated activity of Cdk1 associated with B-type cyclins. Conversely, exit from mitosis requires the inactivation of Cdk1 and the dephosphorylation of at least a subset of Cdk1 substrates. The Cdc14 family of phosphatases antagonizes the action of Cdk1, and is thus a major player in controlling the mitotic exit. We review recent discoveries in several model systems that have shed light on the function of Cdc14 and propose a general framework within which Cdc14 plays conserved roles in regulating the exit from mitosis and cytokinesis.

### 2. INTRODUCTION

During the eukaryotic cell cycle, the cell accomplishes the proper segregation of its genome and the equal partition of all cellular components into the two daughter cells. The cyclin-dependent kinases (Cdks) are the master regulators that orchestrate the various complicated cellular processes of cell division (Figure 1). The Cdks are generally heterodimers containing a kinase subunit and a cyclin (1). Combinations of the different

isoforms of kinase subunits and cyclins give rise to a large variety of functional Cdks that phosphorylate specific substrates and promote different stages of the cell cycle (2). The Cdc14 phosphatases appear to remove the Cdk-mediated phosphorylation of several key cell cycle regulators and antagonize the functions of Cdks. However, to understand how Cdc14 reverses the effect of Cdk1 and promotes mitotic exit, we first examine the cellular events leading to the exit of mitosis and the molecular players involved in these events.

Several key events serve as major landmarks during progression through mitosis and provide a roadmap for understanding how regulators of mitotic exit operate. The first such event is the separation of sister-chromatids. After all sister chromatids are attached to microtubules emanating from the two opposing centrosomes (known as spindle pole bodies (SPBs) in yeast), the cohesin protein complex that maintains the sister-chromatid cohesion is removed by proteolysis (3). This allows the mitotic spindle to move chromatids pole-ward during anaphase. In telophase, a mature actin-based contractile ring and the equatorial microtubule-organizing center (eMTOC) are formed. Once the chromosomes have been properly



**Figure 1.** Schematic representation of the eukaryotic cell cycle.

segregated toward the opposite poles, the cell is partitioned into two daughter cells through the process known as cytokinesis (4). To complete mitosis, cells then disassemble the mitotic spindle, decondense chromosomes, and reform the nuclear envelope (in mammals). In the end, each daughter cell acquires a complete set of genomic DNA, one centrosome, and approximately half of the cellular organelles. Errors during mitosis, most notably chromosome mis-segregation, lead to genetic instability, which may contribute to cancer progression. To avoid this dire consequence, cells employ strict regulatory mechanisms to ensure the orderly execution and thus the fidelity of various mitotic processes.

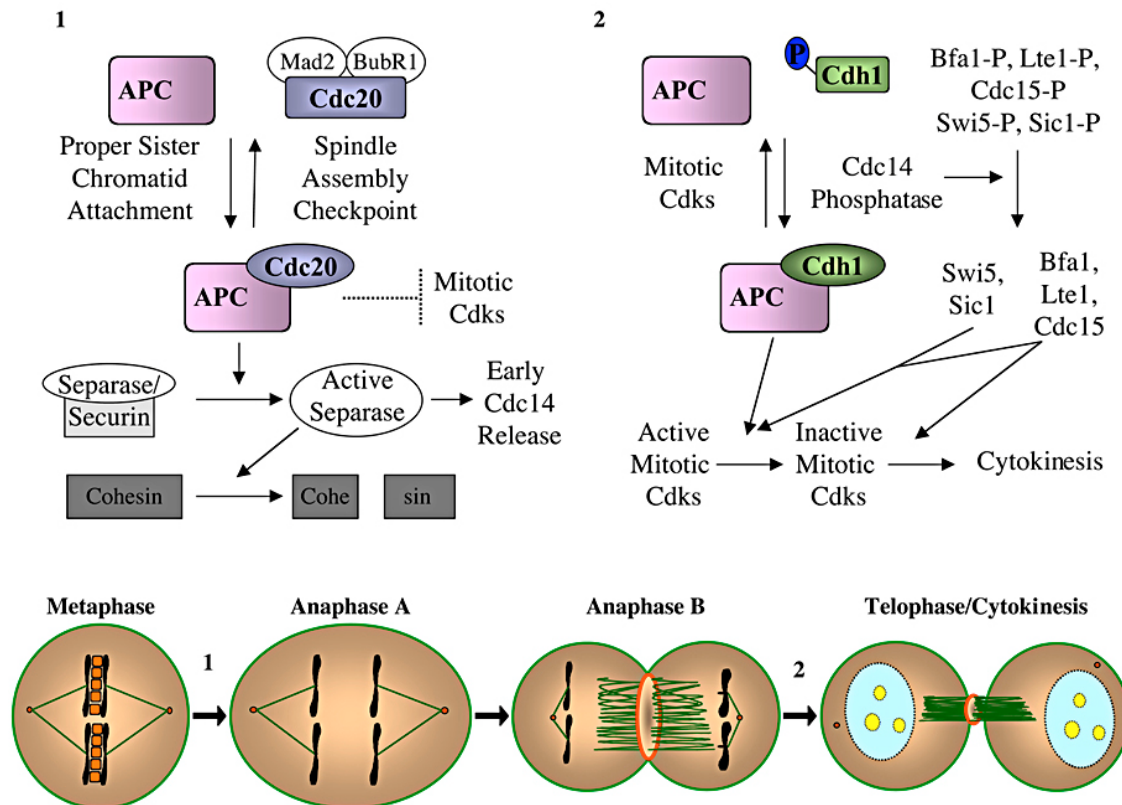
Significant advances have recently been made toward the understanding of the molecular basis of mitotic exit. In essence, exit from mitosis requires the inactivation of the Cdk activity, and equally importantly the reversal of some of the Cdk-mediated phosphorylation events. These dephosphorylation events in turn lead to changes in the localization, stability and activity of a large set of cellular proteins. In general, factors that act to promote mitosis are inactivated, and those that are required for the final stages of mitosis and subsequent G1 phase are activated. Results from the various model organisms used to study this problem suggest that the Cdc14 family of phosphatases may play critical roles in both aspects of mitotic exit, i.e.

inactivation of Cdks and reversal of Cdk-mediated phosphorylation. We review the evidence supporting the role of Cdc14 in various aspects of mitotic exit.

### 3. MOLECULAR BASIS OF MITOTIC EXIT

#### 3.1. Cyclin degradation and the Anaphase-Promoting Complex (APC)

The B-type cyclins in eukaryotes form complexes with Cdk1 to promote entry into mitosis. The activities of these mitotic Cdks must then be lowered for the cell to complete the final events of mitosis and exit into the following G1 phase. Ubiquitin-mediated protein degradation of the mitotic cyclins ensures the complete and irreversible inactivation of the mitotic Cdks. The anaphase-promoting complex (APC) or the cyclosome, a multi-subunit ubiquitin protein ligase (E3), is primarily responsible for the removal of B-type cyclins and other key mitotic regulators (5). The core APC has little intrinsic ubiquitin ligase activity and requires the association of either Cdc20 or Cdh1, two related WD40-repeat-containing proteins (6). Cdc20 and Cdh1 have recently been shown to recruit substrates to the APC and confer the substrate specificity of APC (7-14). Not surprisingly, binding of Cdc20 or Cdh1 to APC is controlled by elaborate signaling networks that act to prevent the degradation of the key mitotic regulators before the completion of certain cellular



**Figure 2.** Outline of the signaling pathways that regulate APC activity during mitosis.

events(15). The pathways that regulate Cdc20 and Cdh1 therefore indirectly control the timing of cyclin degradation, thus influencing the Cdk oscillator that dictates the progression of the cell cycle (Figure 2) (16,17).

### 3.2. APC<sup>Cdc20</sup> initiates chromosome segregation

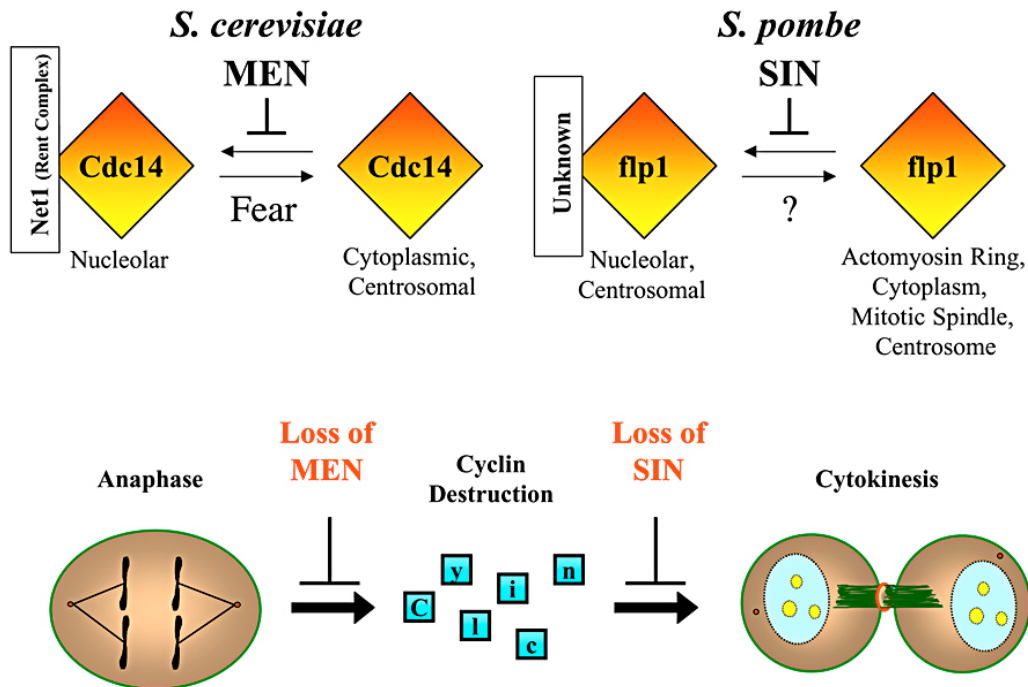
The Cdc20 protein (Fizzy in *Drosophila*, FZY-1 in *C. elegans*, p55Cdc or hCdc20 in humans, and Slp1 in fission yeast) binds to the APC and targets securin, an inhibitor of the separase, for ubiquitination (18-20). Separase released from securin then cleaves the Scc1 subunit of the cohesin complex that tethers sister-chromatids together during chromosome alignment on the metaphase plate(19). It is generally believed that cleavage of Scc1 by separase causes the loss of cohesion between sister-chromatids, which then migrate to opposing poles during anaphase. The spindle checkpoint senses tension and occupancy of kinetochores by microtubule attachments (21). Kinetochores without attachment and tension coalesce the spindle checkpoint proteins to transmit a diffusive signal that prevents Cdc20 from activating the APC (22,23). Once all kinetochores are correctly attached to microtubules, the spindle checkpoint is satisfied and Cdc20 is allowed to activate the APC, leading to ubiquitination of securin, activation of separase, cleavage of Scc1, and the onset of sister-chromatid separation (3,24). In addition to securin degradation, the APC<sup>Cdc20</sup> complex also begins the degradation of mitotic cyclins and possibly other important substrates (25).

### 3.3. Role of APC<sup>Cdh1</sup> and Cdc14 in mitotic exit

In certain systems, such as the early embryonic cell divisions of *Drosophila* and *Xenopus* that lack G1 or G2 phases, APC<sup>Cdc20</sup> is sufficient for mitotic exit by ubiquitinating enough cyclin to allow the inactivation of Cdk1. In fact, the Cdh1 protein is not even present in *Xenopus* egg extracts (26). However, during the somatic cell cycle that has a well-defined G1 phase, Cdh1 (also known Hct1 in *S. cerevisiae*, Fizzy-related in *Drosophila*, and Swi1/Ste9 in *S. pombe*) also mediates the degradation of mitotic cyclins and the inactivation of Cdk1, thus promoting mitotic exit (17,27). In addition to ubiquitinating mitotic cyclins, APC<sup>Cdh1</sup> is also known to degrade many important mitotic regulatory proteins, including Cdc20 and Cdc5 polo-like kinases, thus resetting the cell cycle and establishing G1 (28,29). To delay its own demise, the mitotic Cdk phosphorylates Cdh1 and block its association with the APC (30). It has been shown, in both budding yeast and mammalian cells, that the Cdc14 phosphatase dephosphorylates Cdh1 and promotes its stimulatory activity toward the APC (9,27,30-32).

### 3.4. Regulation of Cdc14 by MEN and SIN

Through genetic analysis, a complex network of genes in the budding yeast *Saccharomyces cerevisiae*, known as the Mitotic Exit Network (MEN), has been shown to control the Cdc14-dependent activation of APC<sup>Cdh1</sup>(33). Similarly, in the fission yeast *Schizosaccharomyces pombe*, Cdc14 is regulated by a



**Figure 3.** Comparisons of the MEN and SIN.

highly homologous signaling pathway, known as the Septation Initiation Network (SIN) (34,35). The SIN was identified by genetic analysis aimed at understanding the control of cytokinesis rather than cyclin degradation and mitotic exit. Surprisingly, SIN does not appear to be required for the activation of APC<sup>Cdh1</sup>. Thus, studies on the two genetic networks (MEN and SIN) operating in fission and budding yeast have led to the conclusion that, except the regulation of Cdc14, the MEN and SIN networks have been established to perform very different tasks (Figure 3).

The genetic dissection of MEN and SIN has been invaluable in identifying a large set of factors responsible for the inactivation of mitotic Cdks and/or for the completion of cytokinesis. However, the apparent discrepancy between the MEN and SIN outputs has caused some distraction in the studies of this important cell cycle circuitry. It is possible that the different findings between the budding and fission yeast may be caused by the specific features associated with the cell cycle of the two organisms. MEN and SIN could in fact have very similar functions in controlling both mitotic exit and cytokinesis in all organisms. We propose the following hypothesis to reconcile the difference between MEN and SIN in general and the difference between the roles of Cdc14 in MEN and SIN signaling in particular.

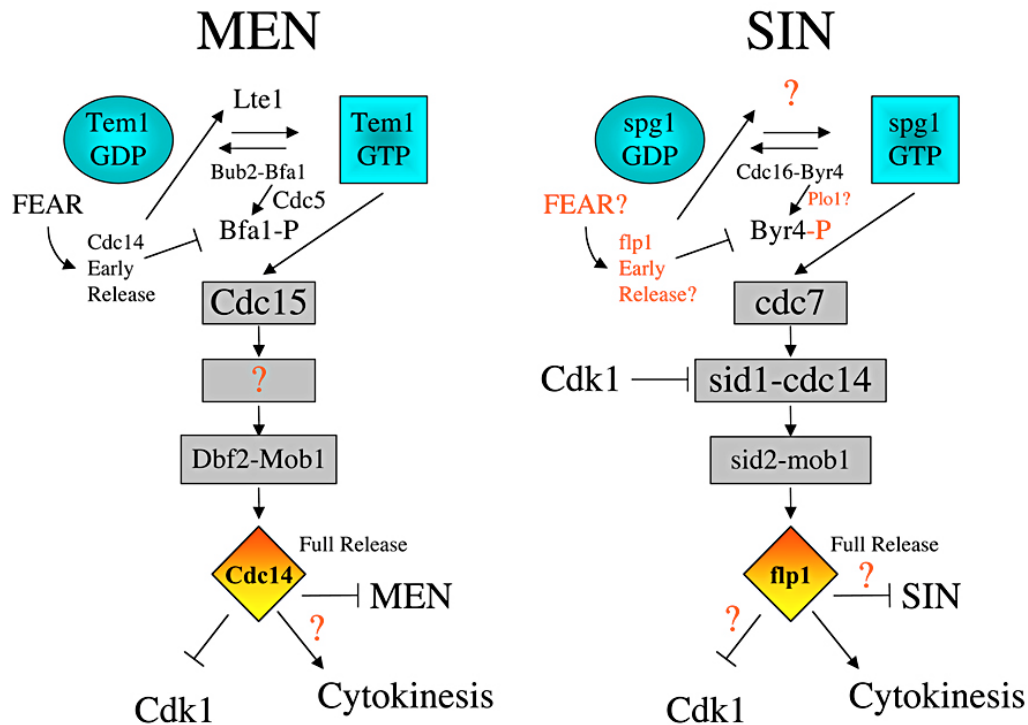
A fact common to all organisms studied to date is that cytokinesis cannot occur prior to the inactivation of Cdk1 (Figure 3). It is thus possible that factors required for cytokinesis are inhibited by mitotic Cdks. The general model derived from the evidence currently available in several model systems is that the Cdc14 phosphatase promotes both the exit from mitosis and cytokinesis in two

ways. First, Cdc14 dephosphorylates several substrates that cause the global inactivation of mitotic Cdk activity. Second, Cdc14 removes Cdk-mediated phosphorylations on specific substrates required during cytokinesis. Although the Cdc14 homologue in *S. pombe*, flp1/clp1, is not required for cyclin degradation during SIN signaling (34,35), flp1 is very likely to be involved in this process during the normal cell cycle. The flp1-null *S. pombe* mutants may degrade cyclins and inactivate Cdk1 through alternative mechanisms (possibly through the actions of the APC<sup>Cdc20</sup> complex), but display an elevated rate of cytokinesis defects compared to wild-type cells, highlighting the role of flp1 during cytokinesis (34,35). Unlike *S. pombe*, alternate mechanisms are unable to overcome the loss of Cdc14 function in *S. cerevisiae* for the degradation of mitotic cyclins. Inactivation of Cdc14 thus leads to cyclin stabilization, preventing both mitotic exit and cytokinesis. It is possible that the failure to degrade mitotic cyclins in the MEN mutants of *S. cerevisiae* masks any potential cytokinesis phenotypes. Extensive reviews are available on the MEN and SIN (36-40). We will briefly describe these networks with emphasis on the most recent findings and discuss how they relate to our general hypothesis of the function of Cdc14 in both mitotic exit and cytokinesis. We will also attempt to highlight important issues that may resolve the discrepancies between the MEN and SIN.

#### 4. THE MEN OF *S. CEREVISIAE*

##### 4.1. Molecular components of the MEN

Inactivation of the MEN in *S. cerevisiae* through temperature-sensitive mutants or deletion analysis causes cells to arrest in telophase with large buds and high mitotic



**Figure 4.** Schematic outline of the interactions between molecular players of the MEN and SIN.

Cdk activity. MEN activity is therefore required for proper cyclin degradation, and cyclin degradation is required for cytokinesis. MEN components include the Cdc14 dual-specificity phosphatase, the protein kinases Cdc5 (polo-like kinase family), Cdc15, and Dbf2 in complex with Mob1 (no known structural and functional motifs), the Tem1 GTPase, the Lte1 guanine nucleotide exchange factor (GEF), and Bub2 and Bfa1 that comprise a two-component GTPase-activating protein (GAP) (Figure 4).

#### 4.2. The TEM1 GTPase cycle

The small GTPase Tem1 is thought to act at or near the top of this signaling network. The two-component GAP Bub2-Bfa1 negatively regulates Tem1 (41). Deletion of Bub2, however, does not have the same effect as most of the other MEN components. Bub2 is a spindle checkpoint gene and was identified in a screen for genes required for maintaining the metaphase arrest in response to microtubule depolymerization by drug treatment (42). The majority of spindle checkpoint genes identified in this genetic screen regulate the ubiquitin ligase activity of APC<sup>Cdc20</sup> (43). On the other hand, Bub2 appears to be the only spindle checkpoint gene that negatively regulates the MEN through Tem1 inactivation (44). It should be pointed out that Bub2 is dispensable for the normal cell cycle in the absence of spindle damaging agents, largely due to additional regulatory mechanisms on Tem1 (see below) and the timing of cell cycle events (i.e. the rate of spindle positioning into the daughter cell occurs faster than cytokinesis after MEN activation). In the presence of a metaphase arrest generated through mutations or drug treatment, Bub2 is required to restrain the activity of MEN and stabilize the mitotic cyclins (44).

Mutations of Lte1 cause telophase arrest at low temperatures. Overexpression of Tem1 can bypass this arrest (45). This and other evidence support the role of Lte1 in activating Tem1 through exchange of GDP for GTP on Tem1. Presumably, the GEF activity of Lte1 is only required for Tem1 nucleotide exchange at lower temperatures, as Tem1 has high intrinsic exchange activity at higher temperatures (41). Interestingly, Tem1, Bub2 and Bfa1 localize preferentially to the SPB that repositions into the daughter cell (46). Lte1 is localized at the cortex of the daughter cell (46,47). This allows Tem1 to become activated when the leading spindle has properly moved into the daughter cell. For this reason, the Bub2 pathway is known as the spindle position checkpoint.

#### 4.3. Role of Cdc15, Dbf2, and Cdc5 during mitotic exit

Tem1 activation then leads to the recruitment the Cdc15 kinase to the SPB (48). Cdc15 is constitutively active and is thought to be regulated only by the availability of substrates, which are localized at the SPB (49). Cdc15 localization to the SPB leads to the activation of the kinase activity of Dbf2-Mob1 complex (50,51). In late mitosis, both Cdc15 and Dbf2-Mob1 relocate from the SPB to the mother-bud junction during cytokinesis (52,53). The exact role of these components at the bud neck is not clear.

Cdc5 plays a complex role during several stages of mitosis. First, during prometaphase, Cdc5 is involved in the activation of APC<sup>Cdc20</sup> through a yet unknown mechanism (29). Second, Cdc5 phosphorylates Scc1, which facilitates its cleavage by Esp1 (54). Third, Cdc5 phosphorylates Bfa1 and inhibits the GAP activity of Bub2-Bfa1 toward Tem1 (55). Fourth, Cdc5



phosphorylates Net1, which is thought to promote its dissociation from Cdc14 and the release of Cdc14 from sequestration (see below) (56,57). At the end of mitosis, Cdc5 also localizes to the bud neck, and plays a role in the completion of cytokinesis (58).

### 4.4. The release of Cdc14 from the nucleolus

The activation of this GTPase/kinase intracellular signaling cascade eventually leads to the activation of Cdc14 and mitotic exit. In interphase cells, Cdc14 is sequestered in the nucleolus where it remains inactive by association with the Net1/Cfi1 component of the RENT complex (59,60). An active MEN signal is required for the sustained release of Cdc14 from the nucleolus. Because mutations of Cdc5, Cdc15, Dbf2, and Tem1 all lead to a telophase arrest with Cdc14 sequestered in the nucleolus and because overexpression of Cdc14 bypasses the lethality of most MEN mutants, Cdc14 was thought to be the most downstream component of the MEN pathway (16).

Once Cdc14 is released from the nucleolus, it dephosphorylates many substrates, including Cdh1 (Hct1 in yeast), Swi5, Sic1, Cdc15, and Lte1 (49,61-63). Dephosphorylation of Swi5 leads to the transcriptional up-regulation of Sic1, a Cyclin-dependent Kinase Inhibitor (CKI) (61). Moreover, dephosphorylation of the Sic1 protein is required for its stabilization and accumulation. Finally, dephosphorylation of Cdh1/Hct1 activates the APC and leads to the degradation of Clb2 (31). Therefore, Cdc14 is a key component of the MEN and triggers the exit from mitosis through multiple inter-connected mechanisms.

### 4.5. The transient release of Cdc14 in early anaphase

Recently, several findings refine the aforementioned model of mitotic exit in budding yeast. Earlier evidence had suggested an additional mechanism for Cdc14 release from the nucleolus independent of MEN activity (59). For some time, it had been known that, in addition to controlling the cleavage of Sec1, Pds1 (securin) and Esp1 (separase) play a role in the proper execution of mitotic exit (64,65). The mitotic exit function of Pds1 and Esp1 is dependent on several MEN components, including Cdc5. Cdc5 overexpression results in stimulation of APC activity that does not affect the degradation of Pds1, implicating that it affects APC<sup>Cdh1</sup> (29). Additionally, Spo12, a protein involved in the regulation of meiosis and the proper timing of mitosis, was shown to be a high-copy suppressor of MEN components (33). Interestingly, Spo12 localized to the nucleolus, suggesting that a possible mechanism for suppression of MEN mutations by Spo12 might be through the delocalization of a small portion of Cdc14 from the nucleolus (66). These observations were tied together by several recent reports that made careful observations of the dynamics of Cdc14 localization throughout anaphase. It was demonstrated that a MEN-independent release of Cdc14 occurs in early anaphase, prior to MEN activation (67-69). Several genes, collectively referred to as the Cdc14 Early Anaphase Release (FEAR) network, were required for this early release of Cdc14, including Cdc5, Spo12, Esp1, and the Esp1 substrate, Slk19 (67).

Subsequently, Cdc5 was shown to affect Cdc14 release from the nucleolus in part by phosphorylating Net1,

weakening the affinity of Net1 for Cdc14 (56,57). In an elegant study, it was determined that Esp1 activation promotes Cdc14 release from the nucleolus independent of its role in chromosome segregation. The physical interaction between Esp1 and its substrate Slk19, rather than the protease activity of Esp1, is required for the transient release of Cdc14 (70). Thus, progression through mitosis requires two distinct functions of Esp1. These results strongly suggest that the events of mitotic exit are mechanistically coupled to the proper completion of metaphase and the release of inhibition of APC<sup>Cdc20</sup> by the spindle checkpoint.

### 4.6. The relationship between MEN and FEAR

Cdc5 is the only common component of the MEN and FEAR. It is required for Cdc14 release both in early and late anaphase, which is consistent with its pleiotropic roles in mitosis, including the phosphorylation of Bfa1, Net1, and Sec1. What is then the functional consequence of the early release of Cdc14? Not surprisingly, it is required for the full activation of the MEN. It turns out that the early released Cdc14 localizes to the SPB that moves into the daughter cell through the physical interaction with Bfa1 and Tem1 (68,69). This interaction may help relieve the GAP activity of Bfa1/Bub2 on Tem1 (in addition to the inhibitory phosphorylation of Bfa1 by Cdc5), thus allowing the activation of Tem1 by Lte1 (68).

In addition to binding to Bfa1 and Tem1 at the SPB, the transient release of Cdc14 in early anaphase may also stimulate the activity of the MEN through dephosphorylating Cdc15 and Lte1. The effect of Cdc15 dephosphorylation by Cdc14 is unknown, although it might affect the SPB localization of Cdc15, and thus Dbf2-Mob1 activation, or promote its relocation to the contractile ring. This will activate more players of MEN, and somehow enhance Cdc15 activity toward mitotic exit and/or cytokinesis. Lte1 has recently been shown to localize to the bud in a manner dependent on actin, Cdc42, and the mitotic Cdk (62), demonstrating a role for cell polarity proteins in mitotic exit. The Rho-like GTPase, Cdc42, activates the Cla4 kinase and leads to Lte1 phosphorylation and localization to the bud through binding to Kel1 (63,71). On the other hand, overexpression of Cdc14 in metaphase cells leads to the premature dephosphorylation and delocalization of Lte1 (62). The release of Cdc14 in early anaphase may positively influence the activity of Lte1 by releasing it from the cell cortex and allowing its access to Tem1 on the SPB. Thus, Cdc14 may potentiate the signaling of the MEN through several parallel pathways.

### 4.7. Role of MEN during cytokinesis

Several lines of evidence indicate that the MEN in budding yeast might also be important for cytokinesis, similar to the SIN in fission yeast and the Cdc14 proteins in mammals and *C. elegans*. For example, many MEN components localize to both the SPB and the actomyosin-based contractile ring. More interestingly, a Net1 mutant has been isolated that allows the release of Cdc14 in the absence of proper Tem1 function. This mutant degrades mitotic cyclins with the proper timing, but it exhibits severe

cytokinesis defects when the function of Tem1 is compromised. This strongly suggests that Tem1 (a MEN component) is required for certain aspects of cytokinesis. This function of Tem1 can only be revealed when the requirement for MEN in the inactivation of Cdk1 is bypassed, in this case, by suppressing the function of Net1. The Mob1 protein, which complexes with Dbf2, is required for actomyosin ring contraction and thus proper cytokinesis when its role in cyclin degradation is bypassed (72). Temperature sensitive mutations of Dbf2 cause the loss of the actomyosin ring and actin patches at the bud neck in late mitosis (52). Additionally, certain mutations in Cdc15 allow rebudding to occur, indicative of Cdk1 inactivation, and are defective for cytokinesis (73). Furthermore, Cdc15 has recently been shown to relocate from the mother SPB to the daughter SPB only after the activation of Cdc14 and inactivation of Cdk1 (53). Cells expressing Cdc15 fragments defective in SPB localization are capable of forming an actomyosin ring, but undergo several rounds of DNA replication and form chains of cells without completing cytokinesis. In addition, Cdc5 mutations also cause growth of chains of cells, disruption of septin formation, and cytokinesis defects, independently of the Bub2-Bfa1 pathway (74). Finally, overexpression of a C-terminal domain of Cdc5 inhibits cytokinesis (58).

### 4.8. Inactivation of MEN

All the available data now support the following order of events. First, Cdc14 dephosphorylates Lte1, both of which then associate with the SPB through interactions with Tem1 and Bfa1. This leads to the activation of the MEN. Cdc14 then dephosphorylates Cdc15, activating Dbf2-Mob1 and downstream MEN components. Cdc15 and Dbf2-Mob1 then relocate to the contractile ring and play an undetermined role in the execution of cytokinesis. Concurrent with these events, Cdc14 also promotes the destruction of mitotic cyclins through Cdh1. Cdc14 also dephosphorylates Swi5 and Sic1, leading to the accumulation of Sic1. This culminates in the global inactivation of Cdk1, allowing mitotic exit and cytokinesis. At telophase, Cdc14 inactivates the MEN signaling by reversing the Cdc5-dependent phosphorylation of Bfa1, which restores the GAP activity of Bub2-Bfa1 towards Tem1 (68).

Interestingly, despite their direct association, Cdc14 does not appear to dephosphorylate Bfa1 in early anaphase (68). The mechanism by which Cdc14 is prevented from dephosphorylating Bfa1 in early anaphase is unknown. One possibility is that the fully activated MEN causes a full release of Cdc14 from the nucleolus, increasing the concentration of Cdc14 in the cytoplasm and elevating Cdc14-binding to the SPB. Only high enough concentrations of Cdc14 at the SPB in telophase could then lead to the dephosphorylation of Bfa1 and the inactivation of MEN. Alternatively, a yet unidentified mechanism actively prevents the dephosphorylation of Bfa1 by Cdc14, but not its binding to Cdc14, until the MEN activity is no longer required.

## 5. THE SIN OF *S. POMBE*

In sharp contrast to the MEN defects in *S. cerevisiae*, most loss-of-function SIN mutants in *S. pombe*

properly degrade mitotic cyclins and exit mitosis. The primary defect of SIN mutants is the incomplete or improper execution of cytokinesis and septum formation, resulting in elongated and multinucleated cells with multiple septa that eventually undergo lysis. Despite these differences, the MEN and SIN are highly similar intracellular signaling networks with most MEN components having homologues in the SIN. In fact, it is highly likely that homologues of all known MEN components will eventually be found in the SIN, and vice versa (Figure 4).

### 5.1. Molecular components of the SIN

The SIN consists of spg1 (a Ras-like GTPase), cdc16-byr4 (a two-component GAP), plo1 (a polo-like kinase), cdc7 (a protein kinase), sid2 (a protein kinase), mob1, and flp1 (also known as clp1, a dual-specificity phosphatase; hereafter referred to as flp1 for clarity). These genes are homologues of Tem1, Bub2-Bfa1, Cdc5, Cdc15, Dbf2, Mob1, and Cdc14 of the MEN in *S. cerevisiae*, respectively. There is currently no known GEF for spg1 in the SIN that is homologous to Lte1 in the MEN, whereas the sid1-cdc14 (unrelated to the *S. cerevisiae* Cdc14) protein kinase complex has no known homologue in the MEN.

The spg1 GTPase is an activator of SIN signaling, and is inhibited by the two-component GAP, cdc16-byr4 (75,76). Overexpression of spg1 leads to multiple rounds of septum formation without cytokinesis (77). Likewise, deletion of either cdc16 or byr4 causes the same phenotype due to hyperactive SIN function (78,79). Additionally, cdc16-byr4 function is required, in a similar fashion to Bub2-Bfa1, to maintain high mitotic Cdk activity during a metaphase arrest caused by an active spindle checkpoint (78). The cdc7 kinase, the *S. cerevisiae* Cdc15 homologue, is recruited to the spindle in an spg1-GTP-dependent manner, and activates the sid2-mob1 kinase (Dbf2-Mob1 *S. cerevisiae* homologue) complex through the sid1-cdc14 kinase (80). The active sid1-cdc14 complex is recruited to the SPB only after the inactivation of Cdk1. This step may be one of the key links between the inactivation of mitotic Cdks and cytokinesis initiation.

The plo1 polo-like kinase, similar to Cdc5 of the MEN, has a complex role in SIN signaling. It is required for the formation of the equatorial microtubule organizing center, the medial ring and thus septation (81,82). Overexpression of plo1 causes the recruitment of cdc7 to the SPB and activation of the SIN (83). The plo1 kinase was also shown to activate spg1 and act at a step upstream of cdc16-byr4 (84). This indicates that plo1 may function in a similar fashion to the Cdc5 of the MEN network. It will be interesting to examine whether plo1 directly phosphorylates and inhibits byr4, causing the activation of spg1 and the recruitment of cdc7 to the SPB and if it influences the localization of flp1, the *S. pombe* Cdc14 homologue (see below). Likewise, there is currently no experimental evidence to support direct interactions between flp1 and spg1 or between flp1 and cdc16.

### 5.2. Differences between MEN and SIN

The SIN mutants are able to degrade mitotic cyclins and undergo mitotic exit. Consistent with this, flp1

## Cdc14 and Mitotic Exit

is not essential for viability in *S. pombe* (34,35). Instead of arresting in telophase with elevated cyclins, the *flp1* deletion mutants degrade mitotic cyclins normally (34,35). Dephosphorylation of the *rum1* and *ste9/srw1* proteins (homologues of *sic1* and *Hct1* of *S. cerevisiae* respectively) also occurs normally in these cells (34,35). Consequently, the *flp1* mutant cells exit mitosis, albeit with a slight increase (about 8% of the population) in cytokinesis failures (34,35). Unexpectedly, the *flp1* mutants exhibit a mild so-called *wee* phenotype first found in the *wee1* mutants, i.e. these cells are small in size due to premature entry into mitosis. Furthermore, although overexpression of *flp1* does not overcome the phenotypes of other SIN mutants, it does produce a dramatic delay in mitotic entry. This delay in mitotic entry correlates with the dephosphorylation of the *cdc25* phosphatase, an activator of *Cdk1* (34,35). These data are consistent with *flp1* playing a role in restraining mitotic entry by inhibiting *cdc25* and thus delaying the activation of *Cdk1*. Despite the failure to rescue the cytokinesis phenotype of several SIN mutants, there are strong genetic interactions between *flp1* and the SIN. Certain SIN mutant cells with *flp1* deleted lose viability and have a more pronounced septation defect (34,35). This strongly suggests that *flp1* might have a role in potentiating SIN signaling, similar to that of *Cdc14* in further activating other MEN components in the budding yeast. Interestingly, loss of *flp1* strongly attenuated the recruitment of the *sid1p* kinase to the SPB, a step that is dependent upon *Cdk1* inactivation. This indicates that *flp1* negatively regulates *Cdk1* during mitotic exit, despite not being strictly required for cyclin degradation and exit from mitosis (34,35).

Another apparent discrepancy between *flp1* and the budding yeast *Cdc14* lies in their localization patterns. In interphase cells, *flp1* localizes largely to the nucleolus, but a small portion of *flp1* is also found at the SPB (34,35). This observation casts doubt on the generality of the hypothesis that nucleolar sequestration by the RENT complex completely inactivates the *Cdc14* family of phosphatases, as first been suggested by results from *S. cerevisiae*. This localization pattern of *flp1* is also consistent with it playing a role during interphase, such as the regulation of *cdc25* and consequently *Cdk1*. During mitosis, *flp1* is released from the nucleolus into the cytoplasm and the nucleus. In addition to the diffuse localization, *flp1* released from the nucleolus also specifically localizes to the spindle, the eMTOC, and the central actin ring. Surprisingly, the SIN network is not required to release *flp1* from the nucleolus, but is necessary for maintaining *flp1* in a released state (34,35). These prominent differences between MEN and SIN led to the conclusion that, despite many homologous features, they function in very different fashions and control distinct cellular processes.

### 5.3. Requirement for *flp1* in mitotic exit under special circumstances

The *dma1* gene was identified as a multicopy suppressor of null phenotype of *cdc16*, a spindle checkpoint gene (85,86). The *dma1* protein contains an FHA domain (a putative phosphopeptide-binding module)

and a RING domain (a domain commonly found in E3 ubiquitin ligases) (85). Like *cdc16* and *byr4*, *dma1* is not an essential gene. However, similar to *cdc16* and *byr4*, deletion of *dma1* abrogates the ability of the spindle checkpoint to maintain a metaphase arrest in the presence of spindle damaging agents (85). In a study to further analyze the relationship of *dma1* with the SIN, Guertin *et al.* showed that *dma1* prevents *plp1* from localizing to the SPB. Although *dma1* has yet to be demonstrated to be an E3 ubiquitin ligase, its mammalian homologue, CHFR, has been shown to directly ubiquitinate *Plk1 in vitro* (87). It is thus possible that *dma1* directly ubiquitinates *plp1* and regulates its localization or turnover at the SPB, thus preventing the inactivation of *cdc16-byr4* by *plp1* and the untimely activation of *spg1* in the presence of an active spindle checkpoint (88).

As mentioned above, the *dma1* deletion mutant cells exhibit a defective spindle checkpoint and undergo mitotic exit in the presence of spindle damaging agents, such as nocodazole (88). Interestingly, *flp1* is required for the inactivation of mitotic Cdks and the mitotic exit of the *dma1*-null cells in the presence of an active spindle checkpoint (and thus an inactive APC<sup>Cdc20</sup> complex) (88). Normal mechanisms for the inactivation of active *Cdk1* in *S. pombe* again involve the stabilization and increased expression of the CKI *rum1* and the activation of the *Cdh1* homologue, *ste9/srw1* (89-93). Although the fission yeast cells can overcome the loss of *flp1* and exit mitosis, the failure of the *flp1* and *dma1* double mutants to undergo mitotic exit in the presence of spindle damage indicates that *flp1* is required for mitotic exit under certain adverse conditions (88). This strongly suggests that *flp1* might contribute to the activation of APC<sup>ste9/srw1</sup> and/or the stabilization of *rum1* during the normal cell cycle. It will be interesting to test whether *cdc16* mutants that have a defective spindle checkpoint also require the function of *flp1* for *Cdk1* inactivation and mitotic exit. Along this line, it will also be interesting to test the ability of *flp1* to directly dephosphorylate *ste9/srw1* and *rum1 in vitro*.

## 6. MITOTIC EXIT NETWORKS IN HIGHER EUKARYOTES

It is very likely that higher eukaryotes utilize signaling networks similar to MEN and SIN to monitor late mitotic events. Database searches have identified several homologues of the MEN and SIN networks in mammals and other higher eukaryotes. For example, GAPCENA has homology to *Bub2* and *cdc16*, and localizes to the centrosomes (94). The small GTPase *RhoA* has been shown to be required for the proper execution of cytokinesis in mammalian cells (95). Apparent homologues of *Dbf2/Sid2*, *Mob1*, *Cdc5/Plp1* have been identified in mammals (96-100). Likewise, two human homologues of *Cdc14*, *hCdc14A* and *hCdc14B*, have been identified (101). As expected, these proteins have been shown to play various roles in mitosis and some of them are shown to localize to mitotic structures including the centrosomes, similar to their yeast counterparts. However, these various factors have not yet been assembled into a signaling network that controls exit from mitosis in



mammals. We will focus our discussion on the role of Cdc14 in higher eukaryotes.

### 6.1. Mammalian Cdc14A and Cdc14B

The human genome encodes two homologues of Cdc14, hCdc14A and hCdc14B. The hCdc14A protein localizes to the centrosomes throughout the cell cycle whereas hCdc14B localizes to the nucleolus during interphase (9,102), our unpublished results. Both hCdc14A and hCdc14B can dephosphorylate hCdh1 and activate the activity of APC<sup>Cdh1</sup> *in vitro* (9), and our unpublished observations. Overexpression of hCdc14A promotes APC<sup>Cdh1</sup> formation even in the presence of non-degradable cyclin B in mammalian cells, leads to premature centriole splitting in S-phase, and aberrant, multi-polar mitotic spindles (103,104). Depletion of hCdc14A from U2OS cells by RNA interference (RNAi) also led to multiple defects in mitosis, including failure to undergo cytokinesis (104).

Several lines of evidence establish a link between the centrosomes and cytokinesis in mammals. First, micro-manipulation studies showed that anaphase was delayed in mammalian cells with mis-oriented spindles until spindle repositioning occurred (105). Microsurgical removal of the centrosomes in mammalian cells caused defects in cytokinesis, suggesting that proper cytokinesis in mammalian cells might be dependent upon the presence of intact centrosomes (106). Live imaging studies of cells stably expressing GFP-Centrin to label the centrosomes demonstrated that the mother centriole migrates to the midbody prior to abscission (106). Taken together, these data suggest that the centrosomal localization of hCdc14A might be connected to its function in cytokinesis, although the exact mechanism remains to be established. The essential role of centrosomes in mammalian cytokinesis is also consistent with the functions of the SPBs in yeast, which act as hubs for organizing and assembling active complexes of the MEN and SIN molecules involved with mitotic exit and cytokinesis.

### 6.2. The *C. elegans* Cdc14

In contrast to hCdc14A, the *in vivo* function of hCdc14B has not been carefully studied, but it is likely that both hCdc14A and hCdc14B contribute to various aspects of exit from mitosis in mammalian cells. Determining how these two Cdc14 homologues are regulated by other proteins homologous to MEN or SIN components will be among the next challenges for uncovering the mechanisms for mitotic exit in mammals. The Cdc14 homologue in *C. elegans* has also been identified. The CeCdc14 protein localizes to the eMTOC, similar to the *S. pombe* flp1, but it does not localize to the centrosomes (107). Removal of CeCdc14 by RNAi in *C. elegans* does not prevent mitotic cyclin degradation (107), consistent with the fact that the early embryos lack the expression of Cdh1 and that APC<sup>Cdc20</sup> is sufficient to complete mitotic cyclin degradation in early embryonic cell divisions. However, consistent with its role in cytokinesis, depletion of CeCdc14 does cause severe defects in eMTOC formation and cytokinesis (107). Interestingly, CeCdc14 requires the

mitotic kinesin ZEN-4 for localization to the eMTOC, and vice versa (107).

## 7. PERSPECTIVE

Lessons from cell cycle research in the past have led us to believe that the general principles for setting up the program of mitotic exit and cytokinesis are very likely to be conserved among species. A careful examination of the available data in the literature has already provided hints about how the apparent differences among various organisms might be eventually resolved. For example, an important unresolved question is why APC<sup>Cdc20</sup> is insufficient or prevented from completely degrading mitotic cyclins when the MEN pathway is compromised in budding yeast. In MEN mutants, cells arrest in telophase with high mitotic Cdk activity despite the completion of spindle assembly, sister-chromatid separation, and the loss of spindle checkpoint-mediated inhibition of APC<sup>Cdc20</sup>. In contrast, inactivation of the SIN in fission yeast does not appear to affect the ability of APC<sup>slp1</sup> (slp1 is the fission yeast homologue of Cdc20) to degrade mitotic cyclins. One possible mechanism may be that activation of the FEAR pathway that leads to early anaphase release of Cdc14 following inactivation of the spindle checkpoint might also somehow inactivate APC<sup>Cdc20</sup>. In this vein, it is interesting to note that the APC isolated from the Cdc5 mutants is less responsive to Cdh1 activation *in vitro* (30). Therefore, it is possible that certain MEN genes are required to maintain the integrity of the APC core. Alternatively, the MEN genes, such as Cdc5, actively promote the conversion from APC<sup>Cdc20</sup> to APC<sup>Cdh1</sup>. The loss of certain MEN genes would then trap APC in an inactive state that can not fully function with either Cdc20 or Cdh1, thus preventing the degradation of mitotic cyclins.

Why is this feature unique to the budding yeast? The answer might lie in the fact that the cell cycle defects caused by MEN mutations activate the Bub2 spindle positioning checkpoint. By coupling mitotic cyclin degradation and the status of spindle positioning, this checkpoint would ensure that the activity of the mitotic Cdk can persist until proper spindle positioning. In *S. pombe*, the spindle position checkpoint may have already been satisfied because both SPBs have to achieve the correct orientation to allow proper chromosome segregation. If the mechanism that senses spindle position is satisfied earlier in *S. pombe*, the APC<sup>Slp1</sup> complex is then sufficient to drive mitotic cyclin degradation, allowing exit from mitosis and establish G1, in the absence of flp1 function.

It is apparent that both MEN and SIN regulate cytokinesis. However, the mechanism by which these networks regulate cytokinesis remains to be established. Recent reports indicate that the SIN is required for the formation of the actomyosin ring and the eMTOC during late mitosis in *S. pombe* (81,82). Studies in *C. elegans* strongly suggest that Cdc14 might also be required for these processes. It will be interesting to determine whether this function of Cdc14 is conserved in other organisms including mammals. The exact role of Cdc14 homologues

in regulation of cytokinesis will undoubtedly be an exiting topic of future studies.

In conclusion, there are important unresolved differences with respect to the function of Cdc14 in various organisms. These differences might be due to our incomplete understanding of the signaling networks that control mitotic exit and cytokinesis. Future studies are needed to resolve the important differences between the MEN and SIN pathways in budding and fission yeast and to delineate similar mitotic exit and cytokinesis signaling networks in mammals and other higher eukaryotes.

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## 9. REFERENCES

1. Doree M, & T. Hunt: From Cdc2 to Cdk1: when did the cell cycle kinase join its cyclin partner? *J Cell Sci* 115, 2461-2464 (2002)
2. Miller M E, & F. R. Cross: Cyclin specificity: how many wheels do you need on a unicycle? *J Cell Sci* 114, 1811-1820 (2001)
3. Nasmyth K: Segregating sister genomes: the molecular biology of chromosome separation. *Science* 297, 559-565 (2002)
4. Guertin D A, S. Trautmann & D. Mccollum: Cytokinesis in eukaryotes. *Microbiol Mol Biol Rev* 66, 155-178 (2002)
5. Koepp D M, J. W. Harper & S. J. Elledge: How the cyclin became a cyclin: regulated proteolysis in the cell cycle. *Cell* 97, 431-434 (1999)
6. Fang G, Yu & M. W. Kirschner: Control of mitotic transitions by the anaphase-promoting complex. *Philos Trans R Soc Lond B Biol Sci* 354, 1583-1590 (1999)
7. Fang G, H. Yu & M. W. Kirschner: Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G1. *Mol Cell* 2, 163-171 (1998)
8. Hilioti Z, Y. S. Chung, Y. Mochizuki, C. F. Hardy & O. Cohen-Fix: The anaphase inhibitor Pds1 binds to the APC/C-associated protein Cdc20 in a destruction box-dependent manner. *Curr Biol* 11, 1347-1352 (2001)
9. Bembek J & H. Yu: Regulation of the anaphase-promoting complex by the dual specificity phosphatase human Cdc14a. *J Biol Chem* 276, 48237-48242 (2001)
10. Pflieger C M & M. W. Kirschner: The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1. *Genes Dev* 14, 655-665 (2000)
11. Pflieger C M, E. Lee & M. W. Kirschner: Substrate recognition by the Cdc20 and Cdh1 components of the anaphase-promoting complex. *Genes Dev* 15, 2396-2407 (2001)
12. Schwab M, M. Neutzner, D. Mocker & W. Seufert: Yeast Hct1 recognizes the mitotic cyclin Clb2 and other substrates of the ubiquitin ligase APC. *Embo J* 20, 5165-5175 (2001)
13. Visintin R, S. Prinz & A. Amon: CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. *Science* 278, 460-463 (1997)
14. Wan Y & M. W. Kirschner: Identification of multiple CDH1 homologues in vertebrates conferring different substrate specificities. *Proc Natl Acad Sci U S A* 98, 13066-13071 (2001)
15. Harper J W, J. L. Burton & M. J. Solomon: The anaphase-promoting complex: it's not just for mitosis any more. *Genes Dev* 16, 2179-2206 (2002)
16. Morgan D O: Regulation of the APC and the exit from mitosis. *Nat Cell Biol* 1, E47-53 (1999)
17. Peters J M: The anaphase-promoting complex: proteolysis in mitosis and beyond. *Mol Cell* 9, 931-943 (2002)
18. Shirayama M, A. Toth, M. Galova & K. Nasmyth: APC(Cdc20) promotes exit from mitosis by destroying the anaphase inhibitor Pds1 and cyclin Clb5. *Nature* 402, 203-207 (1999)
19. Uhlmann F, D. Wernic, M. A. Poupart, E. V. Koonin & K. Nasmyth: Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. *Cell* 103, 375-386 (2000)
20. Kitagawa R, E. Law, L. Tang & A. M. Rose: The Cdc20 Homolog, FZY-1, and Its Interacting Protein, IFY-1, Are Required for Proper Chromosome Segregation in *Caenorhabditis elegans*. *Curr Biol* 12, 2118-2123 (2002)
21. Yu H: Regulation of APC-Cdc20 by the spindle checkpoint. *Curr Opin Cell Biol* 14, 706-714 (2002)
22. Hoyt M A: A new view of the spindle checkpoint. *J Cell Biol* 154, 909-911 (2001)
23. Cleveland D W, Y. Mao & K. F. Sullivan: Centromeres and kinetochores. From epigenetics to mitotic checkpoint signaling. *Cell* 112, 407-421 (2003)
24. Shah J V & D. W. Cleveland: Waiting for anaphase: Mad2 and the spindle assembly checkpoint. *Cell* 103, 997-1000 (2000)
25. Imiger S: Cyclin destruction in mitosis: a crucial task of Cdc20. *FEBS Lett* 532, 7-11 (2002)
26. Jacobs H, D. Richter, T. Venkatesh & C. Lehner: Completion of mitosis requires neither *fzr/rap* nor *fzr2*, a male germline-specific *Drosophila* Cdh1 homolog. *Curr Biol* 12, 1435-1441 (2002)
27. Schwab M, A. S. Lutum & W. Seufert: Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. *Cell* 90, 683-693 (1997)
28. Shirayama M, W. Zachariae, R. Ciosk & K. Nasmyth: The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in *Saccharomyces cerevisiae*. *Embo J* 17, 1336-1349 (1998)
29. Charles J F, S. L. Jaspersen, R. L. Tinker-Kulberg, L. Hwang, A. Szidon & D. O. Morgan: The Polo-related kinase Cdc5 activates and is destroyed by the mitotic cyclin destruction machinery in *S. cerevisiae*. *Curr Biol* 8, 497-507 (1998)
30. Jaspersen S L, J. F. Charles & D. O. Morgan: Inhibitory phosphorylation of the APC regulator Hct1 is

- controlled by the kinase Cdc28 and the phosphatase Cdc14. *Curr Biol* 9, 227-236 (1999)
31. Zachariae W, M. Schwab, K. Nasmyth & W. Seufert: Control of cyclin ubiquitination by CDK-regulated binding of Hct1 to the anaphase promoting complex. *Science* 282, 1721-1724 (1998)
32. Kramer E R, N. Scheuringer, A. V. Podtelejnikov, M. Mann & J. M. Peters: Mitotic regulation of the APC activator proteins CDC20 and CDH1. *Mol Biol Cell* 11, 1555-1569 (2000)
33. Jaspersen S L, J. F. Charles, R. L. Tinker-Kulberg & D. O. Morgan: A late mitotic regulatory network controlling cyclin destruction in *Saccharomyces cerevisiae*. *Mol Biol Cell* 9, 2803-2817 (1998)
34. Cueille N, E. Salimova, V. Esteban, M. Blanco, S. Moreno, A. Bueno & V. Simanis: Flp1, a fission yeast orthologue of the *s. cerevisiae* CDC14 gene, is not required for cyclin degradation or rum1p stabilisation at the end of mitosis. *J Cell Sci* 114, 2649-2664 (2001)
35. Trautmann S, B. A. Wolfe, P. Jorgensen, M. Tyers, K. L. Gould & D. Mccollum: Fission yeast Clp1p phosphatase regulates G2/M transition and coordination of cytokinesis with cell cycle progression. *Curr Biol* 11, 931-940 (2001)
36. Bardin A J & A. Amon: Men and sin: what's the difference? *Nat Rev Mol Cell Biol* 2, 815-826 (2001)
37. Mccollum D & K. L. Gould: Timing is everything: regulation of mitotic exit and cytokinesis by the MEN and SIN. *Trends Cell Biol* 11, 89-95 (2001)
38. Yeong F M, H. H. Lim & U. Surana: MEN, destruction and separation: mechanistic links between mitotic exit and cytokinesis in budding yeast. *Bioessays* 24, 659-666 (2002)
39. Le Goff X, S. Utzig & V. Simanis: Controlling septation in fission yeast: finding the middle, and timing it right. *Curr Genet* 35, 571-584 (1999)
40. Balasubramanian M K, D. Mccollum & U. Surana: Tying the knot: linking cytokinesis to the nuclear cycle. *J Cell Sci* 113 ( Pt 9), 1503-1513 (2000)
41. Geymonat M, A. Spanos, S. J. Smith, E. Wheatley, K. Rittinger, L. H. Johnston & S. G. Sedgwick: Control of mitotic exit in budding yeast. *In vitro* regulation of Tem1 GTPase by Bub2 and Bfa1. *J Biol Chem* 277, 28439-28445 (2002)
42. Hoyt M A, L. Totis & B. T. Roberts: *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* 66, 507-517 (1991)
43. Li R & A. W. Murray: Feedback control of mitosis in budding yeast. *Cell* 66, 519-531 (1991)
44. Alexandru G, W. Zachariae, A. Schleiffer & K. Nasmyth: Sister chromatid separation and chromosome re-duplication are regulated by different mechanisms in response to spindle damage. *Embo J* 18, 2707-2721 (1999)
45. Shirayama M, Y. Matsui & E. A. Toh: The yeast TEM1 gene, which encodes a GTP-binding protein, is involved in termination of M phase. *Mol Cell Biol* 14, 7476-7482 (1994)
46. Pereira G, T. Hofken, J. Grindlay, C. Manson & E. Schiebel: The Bub2p spindle checkpoint links nuclear migration with mitotic exit. *Mol Cell* 6, 1-10 (2000)
47. Bardin A J, R. Visintin & A. Amon: A mechanism for coupling exit from mitosis to partitioning of the nucleus. *Cell* 102, 21-31 (2000)
48. Xu S, H. K. Huang, P. Kaiser, M. Latterich & T. Hunter: Phosphorylation and spindle pole body localization of the Cdc15p mitotic regulatory protein kinase in budding yeast. *Curr Biol* 10, 329-332 (2000)
49. Jaspersen S L & D. O. Morgan: Cdc14 activates cdc15 to promote mitotic exit in budding yeast. *Curr Biol* 10, 615-618 (2000)
50. Visintin R & A. Amon: Regulation of the mitotic exit protein kinases Cdc15 and Dbf2. *Mol Biol Cell* 12, 2961-2974 (2001)
51. Mah A S, J. Jang & R. J. Deshaies: Protein kinase Cdc15 activates the Dbf2-Mob1 kinase complex. *Proc Natl Acad Sci U S A* 98, 7325-7330 (2001)
52. Frenz L M, S. E. Lee, D. Fesquet & L. H. Johnston: The budding yeast Dbf2 protein kinase localises to the centrosome and moves to the bud neck in late mitosis. *J Cell Sci* 113 Pt 19, 3399-3408 (2000)
53. Menssen R, A. Neutzner & W. Seufert: Asymmetric spindle pole localization of yeast Cdc15 kinase links mitotic exit and cytokinesis. *Curr Biol* 11, 345-350 (2001)
54. Alexandru G, F. Uhlmann, K. Mechtler, M. A. Poupart & K. Nasmyth: Phosphorylation of the cohesin subunit Scc1 by Polo/Cdc5 kinase regulates sister chromatid separation in yeast. *Cell* 105, 459-472 (2001)
55. F. Hu, Y. Wang, D. Liu, Y. Li, J. Qin & S. J. Elledge: Regulation of the Bub2/Bfa1 GAP complex by Cdc5 and cell cycle checkpoints. *Cell* 107, 655-665 (2001)
56. Shou W, R. Azzam, S. L. Chen, M. J. Huddleston, C. Baskerville, H. Charbonneau, R. S. Annan, S. A. Carr & R. J. Deshaies: Cdc5 influences phosphorylation of Net1 and disassembly of the RENT complex. *BMC Mol Biol* 3, 3 (2002)
57. Yoshida S & A. Toh-E: Budding yeast Cdc5 phosphorylates Net1 and assists Cdc14 release from the nucleolus. *Biochem Biophys Res Commun* 294, 687-691 (2002)
58. Song S, T. Z. Grenfell, S. Garfield, R. L. Erikson & K. S. Lee: Essential function of the polo box of Cdc5 in subcellular localization and induction of cytokinetic structures. *Mol Cell Biol* 20, 286-298 (2000)
59. Shou W, J. H. Seol, A. Shevchenko, C. Baskerville, D. Moazed, Z. W. Chen, J. Jang, H. Charbonneau & R. J. Deshaies: Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. *Cell* 97, 233-244 (1999)
60. Visintin R, E. S. Hwang & A. Amon: Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. *Nature* 398, 818-823 (1999)
61. Visintin R, K. Craig, E. S. Hwang, S. Prinz, M. Tyers & A. Amon: The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. *Mol Cell* 2, 709-718 (1998)
62. Jensen S, M. Geymonat, A. L. Johnson, M. Segal & L. H. Johnston: Spatial regulation of the guanine nucleotide exchange factor Lte1 in *Saccharomyces cerevisiae*. *J Cell Sci* 115, 4977-4991 (2002)
63. Seshan A, A. J. Bardin & A. Amon: Control of lte1 localization by cell polarity determinants and cdc14. *Curr Biol* 12, 2098-2110 (2002)
64. Tinker-Kulberg R L & D. O. Morgan: Pds1 and Esp1 control both anaphase and mitotic exit in normal cells and after DNA damage. *Genes Dev* 13, 1936-1949 (1999)
65. Cohen-Fix O & D. Koshland: Pds1p of budding yeast has dual roles: inhibition of anaphase initiation and regulation of mitotic exit. *Genes Dev* 13, 1950-1959 (1999)

66. Shah R, S. Jensen, L. M. Frenz, A. L. Johnson & L. H. Johnston: The Spo12 protein of *Saccharomyces cerevisiae*: a regulator of mitotic exit whose cell cycle-dependent degradation is mediated by the anaphase-promoting complex. *Genetics* 159, 965-980 (2001)
67. Stegmeier F, R. Visintin & A. Amon: Separase, polo kinase, the kinetochore protein Slk19, and Spo12 function in a network that controls Cdc14 localization during early anaphase. *Cell* 108, 207-220 (2002)
68. Pereira G, C. Manson, J. Grindlay & E. Schiebel: Regulation of the Bfa1p-Bub2p complex at spindle pole bodies by the cell cycle phosphatase Cdc14p. *J Cell Biol* 157, 367-379 (2002)
69. Yoshida S, K. Asakawa & A. Toh-E: Mitotic exit network controls the localization of Cdc14 to the spindle pole body in *Saccharomyces cerevisiae*. *Curr Biol* 12, 944-950 (2002)
70. Sullivan M & F. Uhlmann: A non-proteolytic function of separase links the onset of anaphase to mitotic exit. *Nat Cell Biol* (2003)
71. Hofken T & E. Schiebel: A role for cell polarity proteins in mitotic exit. *Embo J* 21, 4851-4862 (2002)
72. Luca F C & M. Winey: MOB1, an essential yeast gene required for completion of mitosis and maintenance of ploidy. *Mol Biol Cell* 9, 29-46 (1998)
73. Jimenez J, V. J. Cid, R. Cenamor, M. Yuste, G. Molero, C. Nombela & M. Sanchez: Morphogenesis beyond cytokinetic arrest in *Saccharomyces cerevisiae*. *J Cell Biol* 143, 1617-1634 (1998)
74. Park C J, S. Song, P. R. Lee, W. Shou, R. J. Deshaies & K. S. Lee: Loss of CDC5 Function in *Saccharomyces cerevisiae* Leads to Defects in Swl1p Regulation and Bfa1p/Bub2p-Independent Cytokinesis. *Genetics* 163, 21-33 (2003)
75. Jwa M & K. Song: Byr4, a dosage-dependent regulator of cytokinesis in *S. pombe*, interacts with a possible small GTPase pathway including Spg1 and Cdc16. *Mol Cells* 8, 240-245 (1998)
76. Furge K A, K. Wong, J. Armstrong, M. Balasubramanian & C. F. Albright: Byr4 and Cdc16 form a two-component GTPase-activating protein for the Spg1 GTPase that controls septation in fission yeast. *Curr Biol* 8, 947-954 (1998)
77. Schmidt S, M. Sohrmann, K. Hofmann, A. Woollard & V. Simanis: The Spg1p GTPase is an essential, dosage-dependent inducer of septum formation in *Schizosaccharomyces pombe*. *Genes Dev* 11, 1519-1534 (1997)
78. Fankhauser C, J. Marks, A. Raymond & V. Simanis: The *S. pombe* cdc16 gene is required both for maintenance of p34cdc2 kinase activity and regulation of septum formation: a link between mitosis and cytokinesis? *Embo J* 12, 2697-2704 (1993)
79. Song K, K. E. Mach, C. Y. Chen, T. Reynolds & C. F. Albright: A novel suppressor of ras1 in fission yeast, byr4, is a dosage-dependent inhibitor of cytokinesis. *J Cell Biol* 133, 1307-1319 (1996)
80. Guertin D A, L. Chang, F. Irshad, K. L. Gould & D. Mccollum: The role of the sid1p kinase and cdc14p in regulating the onset of cytokinesis in fission yeast. *Embo J* 19, 1803-1815 (2000)
81. Mulvihill D P & J. S. Hyams: Cytokinetic actomyosin ring formation and septation in fission yeast are dependent on the full recruitment of the polo-like kinase Plo1 to the spindle pole body and a functional spindle assembly checkpoint. *J Cell Sci* 115, 3575-3586 (2002)
82. Heitz M J, J. Petersen, S. Valovin & I. M. Hagan: MTOC formation during mitotic exit in fission yeast. *J Cell Sci* 114, 4521-4532 (2001)
83. Mulvihill D P, J. Petersen, H. Ohkura, D. M. Glover & I. M. Hagan: Plo1 kinase recruitment to the spindle pole body and its role in cell division in *Schizosaccharomyces pombe*. *Mol Biol Cell* 10, 2771-2785 (1999)
84. Tanaka K, J. Petersen, F. Maciver, D. P. Mulvihill, D. M. Glover & I. M. Hagan: The role of Plo1 kinase in mitotic commitment and septation in *Schizosaccharomyces pombe*. *Embo J* 20, 1259-1270 (2001)
85. Murone M & V. Simanis: The fission yeast dma1 gene is a component of the spindle assembly checkpoint, required to prevent septum formation and premature exit from mitosis if spindle function is compromised. *Embo J* 15, 6605-6616 (1996)
86. Fournier N, L. Cerutti, N. Beltraminelli, E. Salimova & V. Simanis: Bypass of the requirement for cdc16p GAP function in *Schizosaccharomyces pombe* by mutation of the septation initiation network genes. *Arch Microbiol* 175, 62-69 (2001)
87. Kang D, J. Chen, J. Wong & G. Fang: The checkpoint protein Chfr is a ligase that ubiquitinates Plk1 and inhibits Cdc2 at the G2 to M transition. *J Cell Biol* 156, 249-259 (2002)
88. Guertin D A, S. Venkatram, K. L. Gould & D. Mccollum: Dma1 prevents mitotic exit and cytokinesis by inhibiting the septation initiation network (SIN) *Dev Cell* 3, 779-790 (2002)
89. Yamaguchi S, H. Okayama & P. Nurse: Fission yeast Fizzy-related protein srw1p is a G(1)-specific promoter of mitotic cyclin B degradation. *Embo J* 19, 3968-3977 (2000)
90. Kominami K, H. Seth-Smith & T. Toda: Apc10 and Ste9/Srw1, two regulators of the APC-cyclosome, as well as the CDK inhibitor Rum1 are required for G1 cell-cycle arrest in fission yeast. *Embo J* 17, 5388-5399 (1998)
91. Stern B & P. Nurse: Cyclin B proteolysis and the cyclin-dependent kinase inhibitor rum1p are required for pheromone-induced G1 arrest in fission yeast. *Mol Biol Cell* 9, 1309-1321 (1998)
92. Kitamura K, H. Maekawa & C. Shimoda: Fission yeast Ste9, a homolog of Hct1/Cdh1 and Fizzy-related, is a novel negative regulator of cell cycle progression during G1-phase. *Mol Biol Cell* 9, 1065-1080 (1998)
93. Chang L, J. L. Morrell, A. Feoktistova & K. L. Gould: Study of cyclin proteolysis in anaphase-promoting complex (APC) mutant cells reveals the requirement for APC function in the final steps of the fission yeast septation initiation network. *Mol Cell Biol* 21, 6681-6694 (2001)
94. Cuif M H, F. Possmayer, H. Zander, N. Bordes, F. Jollivet, A. Couedel-Courteille, I. Janoueix-Lerosey, G. Langsley, M. Bornens & B. Goud: Characterization of GAPCenA, a GTPase activating protein for Rab6, part of which associates with the centrosome. *Embo J* 18, 1772-1782 (1999)
95. Dutartre H, J. Davoust, J. P. Gorvel & P. Chavrier: Cytokinesis arrest and redistribution of actin-cytoskeleton regulatory components in cells expressing the Rho GTPase CDC42Hs. *J Cell Sci* 109 ( Pt 2), 367-377 (1996)
96. Justice R W, O. Zilian, D. F. Woods, M. Noll & P. J. Bryant: The *Drosophila* tumor suppressor gene warts

encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. *Genes Dev* 9, 534-546 (1995)

97. Hori T, A. Takaori-Kondo, Y. Kamikubo & T. Uchiyama: Molecular cloning of a novel human protein kinase, kpm, that is homologous to warts/lats, a *Drosophila* tumor suppressor. *Oncogene* 19, 3101-3109 (2000)

98. Tao W, S. Zhang, G. S. Turenchalk, R. A. Stewart, M. A. St John, W. Chen & T. Xu: Human homologue of the *Drosophila melanogaster* lats tumour suppressor modulates CDC2 activity. *Nat Genet* 21, 177-181 (1999)

99. Moreno C S, W. S. Lane & D. C. Pallas: A mammalian homolog of yeast MOB1 is both a member and a putative substrate of striatin family-protein phosphatase 2A complexes. *J Biol Chem* 276, 24253-24260 (2001)

100. Golsteyn R M, H. A. Lane, K. E. Mundt, L. Arnaud & E. A. Nigg: The family of polo-like kinases. *Prog Cell Cycle Res* 2, 107-114 (1996)

101. Li L, B. R. Ernsting, M. J. Wishart, D. L. Lohse & J. structurally and functionally conserved in humans and yeast. *J Biol Chem* 272, 29403-29406 (1997)

102. Kaiser B K, Z. A. Zimmerman, H. Charbonneau & P. K. Jackson: Disruption of centrosome structure, chromosome segregation, and cytokinesis by misexpression of human Cdc14A phosphatase. *Mol Biol Cell* 13, 2289-2300 (2002)

103. Listovsky T, A. Zor, A. Laronne & M. Brandeis: Cdk1 is essential for mammalian cyclosome/APC regulation. *Exp Cell Res* 255, 184-191 (2000)

104. Mailand N, C. Lukas, B. K. Kaiser, P. K. Jackson, J. Bartek & J. Lukas: Deregulated human Cdc14A phosphatase disrupts centrosome separation and chromosome segregation. *Nat Cell Biol* 4, 317-322 (2002)

105. O'connell C B & Y. L. Wang: Mammalian spindle orientation and position respond to changes in cell shape in a dynein-dependent fashion. *Mol Biol Cell* 11, 1765-1774 (2000)

106. Piel M, J. Nordberg, U. Euteneuer & M. Bornens: Centrosome-dependent exit of cytokinesis in animal cells. *Science* 291, 1550-1553 (2001)

107. Gruneberg U, M. Glotzer, A. Gartner & E. A. Nigg: The CeCDC-14 phosphatase is required for cytokinesis in the *Caenorhabditis elegans* embryo. *J Cell Biol* 158, 901-914 (2002)

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