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CLOSING MITOSIS: The Functions of the Cdc14 Phosphatase and Its Regulation

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■ **Abstract** Completion of the cell cycle requires the temporal and spatial coordination of chromosome segregation with mitotic spindle disassembly and cytokinesis. In budding yeast, the protein phosphatase Cdc14 is a key regulator of these late mitotic events. Here, we review the functions of Cdc14 and how this phosphatase is regulated to accomplish the coupling of mitotic processes. We also discuss the function and regulation of Cdc14 in other eukaryotes, emphasizing conserved features.

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INTRODUCTION

The transmission of genetic information from one generation to the next requires the accurate replication of the DNA during S phase and the faithful partitioning of chromosomes during mitosis. Accomplishing this task requires the coordination of several cellular events. Successful completion of mitosis, for example, depends on the temporal and spatial coordination of many mitotic processes such as chromosome segregation, spindle disassembly, and cytokinesis. Errors during mitosis, most notably chromosome mis-segregation, lead to genetic instability, a molecular hallmark of cancer. The fidelity of mitotic processes is in part controlled by checkpoints that monitor key cell-cycle events and, in case of a defect, halt mitotic progression to provide time for error correction. In addition, the coordination of mitotic events is often achieved by employing the same proteins to control multiple events.

In this review, we discuss how the protein phosphatase Cdc14, an antagonist of mitotic cyclin-dependent kinases (CDKs), regulates and couples multiple mitotic events. We focus our discussion on the budding yeast *Saccharomyces cerevisiae*, where the function of this phosphatase and its regulation are best understood. We also examine Cdc14's role in other eukaryotes with the goal of highlighting conserved features.

AN OVERVIEW OF MITOSIS

Over the past two decades, it has become clear that the basic cell-cycle machinery regulating chromosome segregation is conserved in all eukaryotes. Protein kinases known as mitotic cyclin-dependent kinases (CDKs), which are composed of a catalytic kinase subunit and a regulatory cyclin subunit, promote entry into the chromosome segregation phase (reviewed in 91, 95). Preparations for chromosome segregation, however, already begin during DNA replication, when protein complexes known as cohesins are laid down between the duplicated DNA strands (sister chromatids) (reviewed in 47). During prophase, these cohesins facilitate the bipolar attachment of pairs of sister chromatids on the mitotic spindle (reviewed in 96). The dissolution of sister chromatids is triggered by cleavage of one cohesin subunit (Scc1/Mcd1 in budding yeast; Rad21 in *Schizosaccharomyces pombe*, *Drosophila*,

and mammals; SCC-1/COH-2 in *Caenorhabditis elegans*) by a CD clan family protease called separase (Esp1 in budding yeast) (reviewed in 96). Separase is kept inactive by securin (Pds1 in yeast) until all pairs of sister chromatids have attached to the mitotic spindle in a bipolar manner. Once this is achieved, an ubiquitin ligase known as the Anaphase Promoting Complex or Cyclosome (APC/C) together with the specificity factor Cdc20 mediate the ubiquitin-dependent proteolysis of securin (Figure 1) (reviewed in 105). Destruction of securin and hence activation of separase marks the onset of anaphase.

After the completion of chromosome segregation, cells exit from mitosis (Figure 1). This cell-cycle transition is characterized by mitotic spindle disassembly, chromosome decondensation, and the removal of mitotic determinants. The destruction of mitotic determinants creates conditions that are permissive for cytokinesis and assembly of protein complexes required for the initiation of DNA replication (prereplicative complexes; preRCs) onto origins of replication.

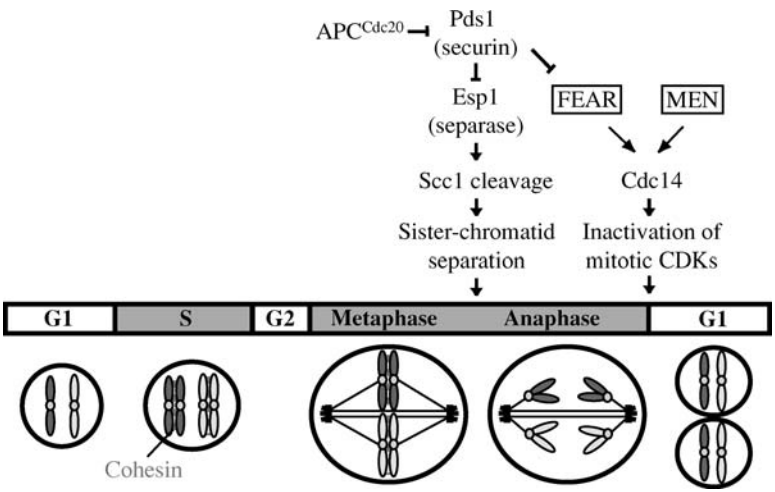

Figure 1 Regulation of mitosis in *S. cerevisiae*. After DNA replication, sister chromatids are held together by the cohesin complex. Sister-chromatid separation is initiated when a protease called separase (Esp1 in budding yeast) cleaves the cohesin subunit Scc1/Mcd1. Until the onset of anaphase, separase is kept inactive by its inhibitor securin (Pds1 in budding yeast). Securin is inactivated at the onset of anaphase by ubiquitin-dependent proteolysis mediated by the anaphase-promoting complex/cyclosome (APC/C) together with its specificity factor Cdc20. This allows for separase to become active, thereby triggering the onset of sister-chromatid separation. Exit from mitosis requires the inactivation of mitotic CDKs. Cdc14 promotes mitotic exit by antagonizing mitotic CDK activity. The activity of Cdc14 is regulated by two signaling pathways, the Cdc fourteen early anphase release (FEAR) network and the mitotic exit network (MEN).

In all organisms studied to date, exit from mitosis requires the inactivation of mitotic CDKs. When inactivation of mitotic CDKs is prevented, cells arrest in late anaphase/telophase with segregated DNA masses and an extended mitotic spindle (55, 148). Mitotic CDK inactivation is brought about primarily by the ubiquitin-mediated degradation of mitotic cyclins (Clb cyclins in yeast), which is initiated at the metaphase-anaphase transition by APC/C^{Cdc20} (reviewed in 105).

In contrast to most other eukaryotes in which the bulk of mitotic cyclin degradation occurs at the metaphase-anaphase transition, a significant amount of mitotic CDK activity persists until telophase in budding yeast (59, 115, 132). This pool of mitotic CDK activity is inactivated by APC/C complexed with the specificity factor Cdc20 or Cdh1/Hct1, which targets mitotic cyclins for ubiquitin-mediated proteolysis (105, 108, 142, 148, 153). Accumulation of a CDK inhibitor, Sic1, which directly binds to the cyclin-CDK complex, further ensures the precipitous inactivation of mitotic CDKs at the end of mitosis (28, 87, 109).

THE MOLECULAR MECHANISMS REGULATING MITOTIC CDK INACTIVATION AT THE END OF MITOSIS IN *S. CEREVISIAE*

In budding yeast, the protein phosphatase Cdc14 is essential for the inactivation of the pool of mitotic CDKs that persists until the completion of spindle elongation. Cells lacking Cdc14 function arrest in late anaphase with high mitotic CDK activity (140, 144). Conversely, overexpression of *CDC14* results in inappropriate mitotic CDK inactivation (140). Cdc14 promotes mitotic CDK inactivation by reversing CDK phosphorylation events. Cdc14 dephosphorylates Cdh1/Hct1, which promotes association with the APC/C, thereby activating it (58, 140, 158). Cdc14 also promotes Sic1 accumulation by dephosphorylating Sic1 and its transcription factor Swi5, which leads to the stabilization of Sic1 and upregulation of *SIC1* transcription, respectively (29, 58, 69, 90, 123, 135, 138, 140). Sic1, Swi5, and Cdh1/Hct1, however, are not the only substrates of Cdc14. In fact, Cdc14 has many substrates in the cell (Figure 2), and Cdc14 likely dephosphorylates many, if not all, Clb-CDK substrates. This general reversal of CDK phosphorylation is likely to further contribute to the rapid resetting of the cell cycle to the G1 state.

REGULATION OF Cdc14 DURING MITOSIS IN *S. CEREVISIAE*

The activity of Cdc14 is controlled by cell cycle–dependent changes in its association with the competitive inhibitor Cfi1/Net1 (120, 128, 137, 141). For most of the cell cycle up to metaphase, Cfi/Net1 binds to and inhibits Cdc14 activity. As Cfi1/Net1 resides in the nucleolus, Cdc14, when bound to its inhibitor, also

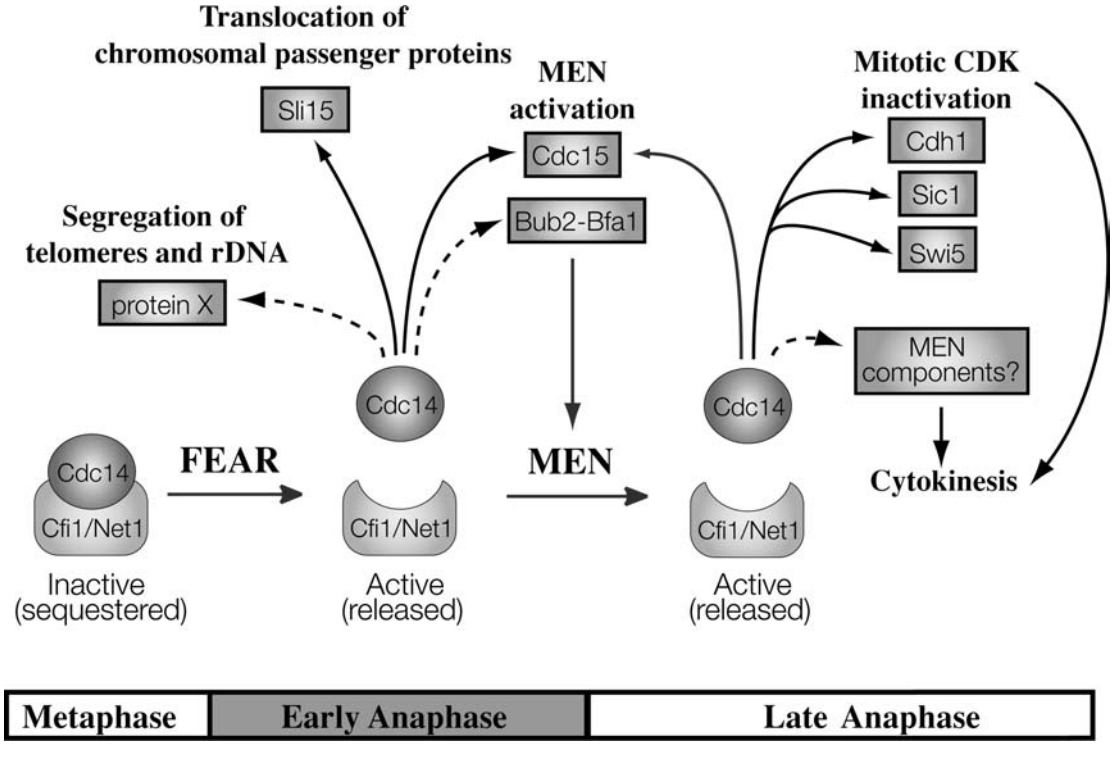

Figure 2 Substrates and functions of Cdc14 during mitosis. Cdc14 released by the FEAR network and the MEN regulates many mitotic processes. The FEAR network-activated Cdc14 promotes the segregation of telomeres and rDNA, the translocation of chromosomal passenger proteins, and activation of the MEN. Cdc14 activated by the MEN promotes the inactivation of mitotic CDKs and cytokinesis. The known targets of Cdc14 in the regulation of these processes are shown in the gray boxes. Solid arrows indicate reasonably well-established Cdc14 targets; dashed arrows indicate more speculative ones. For details refer to the main text.

localizes to this subnuclear structure. During anaphase, Cdc14 is released from its inhibitor and spreads into the nucleus and cytoplasm, allowing it to dephosphorylate its substrates (120, 141).

The dissociation of Cdc14 from its inhibitor during anaphase is controlled by at least two signaling networks. At the onset of anaphase, the FEAR (Cdc fourteen early anaphase release) network initiates the release of Cdc14 from its inhibitor and the phosphatase spreads throughout the nucleus and associates with spindle pole bodies (SPBs, the yeast centrosomes) and the mitotic spindle (102, 103, 127, 154). During later stages of anaphase, the MEN (mitotic exit network) promotes further release of Cdc14 and maintains the phosphatase in its released state. During telophase, when Cdc14 release is mediated by the MEN, the phosphatase is also found in the cytoplasm (120, 141). Whether the qualitative difference in release caused by the FEAR network (primarily nuclear) and by the MEN (nuclear and cytoplasmic) is relevant for Cdc14 regulation is at present unclear. It is, however, interesting to note that two screens aimed at discovering reduction-of-function mutants that bypass the mitotic exit defect of MEN mutants identified proteins involved in nuclear-cytoplasmic transport, such as the karyopherins Kap104 and Mtr10 (4, 119).

In addition to causing a qualitative difference in Cdc14 release, there are quantitative differences in the extent by which the FEAR network and the MEN promote exit from mitosis. The MEN is essential for mitotic exit, as MEN mutants arrest in late anaphase with high mitotic CDK activity (59, 115, 132). In contrast, the FEAR network is not essential. Cells lacking FEAR network activity delay but do not fail to exit from mitosis (102, 127, 154), which implies that Cdc14 activated by the FEAR network does not lower mitotic CDKs enough to trigger exit from mitosis. This result may be explained by the following findings: (*a*) The FEAR network appears to be active only for a brief time during early anaphase, as Cdc14 is not maintained in its released state in the absence of MEN function (102, 120, 127, 141, 154). (*b*) The FEAR network is not able to direct large amounts of Cdc14 to the cytoplasm where Cdh1, Swi5, and perhaps other critical substrates are localized (57, 90, 97).

Despite qualitative and quantitative differences in FEAR network and MEN-mediated Cdc14 activation, the question arises as to why budding yeast utilizes two pathways to regulate Cdc14 activation rather than one. Employing multiple pathways may allow for more elaborate regulation of exit from mitosis. Another, not mutually exclusive, possibility is that Cdc14 released by the FEAR network performs functions during mitosis that are different from that of Cdc14 released by the MEN. Recent studies underscore this idea. Cdc14 released by the FEAR network plays important roles in regulating chromosome segregation and the localization of chromosomal passenger proteins (see below) (25, 103, 129). Perhaps the levels of Cdc14 activity needed to accomplish these tasks are lower than that needed to promote exit from mitosis. Hence, employing a pathway to activate Cdc14 briefly during early anaphase allows the phosphatase to regulate anaphase events, whereas full activation later in the cell cycle allows it to accomplish its mitotic exit-promoting function.

TABLE 1 MEN and SIN components and homologues in other eukaryotes

	S. cerevisiae (MEN)	*S. pombe* (SIN)	*C. elegans*: putative homologue	Mammals: putative homologue
GTPase	Tem1	Spg1	?	?
Putative GEF	Lte1	?	?	?
GAP	Bub2	Cdc16	C33F10.2	GAPCenA
	Bfa1	Byr4	?	?
Protein kinase	Cdc15	Cdc7	?	?
	?	Sid1	T19A5.2	?
	Dbf2	Sid2	T20F10.1/R11G1.4	WARTS/LATS1
Associated factors	?	Cdc14 (w/Sid1)	?	?
	Mob1 (w/Dbf2)	Mob1 (w/Sid2)	T12B3.4/F38H4.10/ F09A5.4	mMob1
SPB scaffold	Nud1	Cdc11	?	Centriolin
	?	Sid4	?	?
Phosphatase	Cdc14	Clp1/Flp1	CeCDC-14	hCdc14A, hCdc14B

The Mitotic Exit Network

The Mitotic Exit Network was the first signaling network shown to regulate the subcellular localization of Cdc14 (120, 141). As this signaling cascade has been reviewed in detail recently (6, 86, 110), we restrict our discussion to the essential aspects and recent discoveries. The MEN resembles a Ras-like GTPase signaling cascade (Table 1, Figure 3) and is comprised of the GTPase Tem1; the putative GEF (guanine-nucleotide exchange factor) Lte1; the two-component GAP (GTPase activating protein) Bub2-Bfa1/Byr4; the protein kinases Cdc5, Cdc15, Dbf2; the Dbf2-associated factor Mob1; and a scaffold protein Nud1. A combination of genetic and biochemical analyses support the model for MEN signaling outlined in Figure 3. Tem1's GTPase activity is negatively regulated by the GAP complex Bub2-Bfa1 (3, 8, 13, 26, 30, 31, 34, 71, 73, 79, 101, 107, 146). Lte1 positively regulates Tem1 (8, 114, 116) but in the absence of direct biochemical evidence that Lte1 functions as a GEF for Tem1, its molecular function remains controversial (61, 155). The activated form of Tem1, which is likely but not proven to be the GTP-bound form, is thought to propagate a signal to the protein kinase Cdc15 (5, 7, 76, 88, 139). Cdc15, in turn, activates the protein kinase Dbf2 (83), which requires the Dbf2-associated factor Mob1 (Figure 3) (70, 82, 83).

Cdc5 has long been thought to be a component of the MEN signaling cascade, as cells lacking the polo kinase Cdc5 exhibit a phenotype similar to that of MEN mutants, arresting in late anaphase with Cdc14 sequestered in the nucleolus (59, 67, 76, 120, 141). Recent studies, however, suggest that Cdc5 is not a core component of the MEN signaling cascade. Instead, Cdc5 is essential for mitotic exit because it

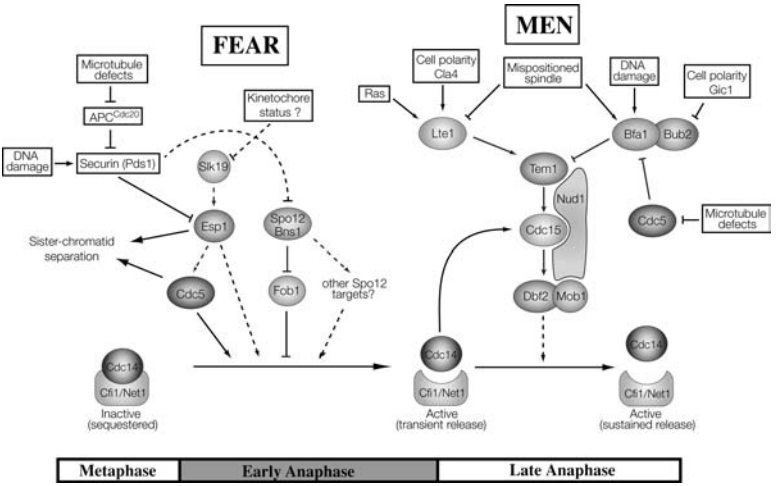

Figure 3 Components of the FEAR network and the MEN and signals controlling these pathways. The release of Cdc14 from its inhibitor Cfi1/Net1 is initiated during early anaphase by the FEAR network. During later stages of anaphase, the MEN promotes and sustains the release of the phosphatase. The signals and proteins regulating the activation of these two pathways are shown. Solid arrows indicate reasonably well-established interactions; dashed arrows indicate more speculative interactions. For details refer to the main text.

activates the MEN in multiple ways. The Bub2-Bfa1 complex is phosphorylated by Cdc5 (51, 52, 77), which inactivates its GAP activity (35, 52). Cdc5 may also regulate Lte1 (77). Lastly, Cdc5 activates the Dbf2 kinase in a *BUB2*-independent manner (76), at least in part by promoting FEAR network-induced activation of Cdc14 (see below) (60, 127, 143). Together, these MEN-activating functions of Cdc5 are likely to account for the complete loss of MEN signaling in *cdc5* mutants (143).

Activation of the MEN is regulated not only by Cdc5 but also in many additional ways. Cdc15 has a potential autoinhibitory C-terminal domain (7) but its regulation remains elusive. The spatial segregation of the MEN components Lte1 and Tem1 also contributes to the temporal coordination of MEN activation. The MEN activator Lte1 localizes to the bud cortex concomitant with bud formation while Tem1 resides on the daughter-bound SPB (8, 101). Therefore, activation of Tem1 by Lte1 occurs only after the Tem1-bearing SPB has migrated into the bud (6, 8, 101). The subcellular localization of many other MEN components is also cell cycle regulated. Tem1, Bub2, Bfa1, Cdc5, Cdc15, Dbf2, and Mob1 localize to the cytoplasmic face of the SPB (7, 8, 19, 39, 101, 139, 151, 156). The SPB component Nud1 functions as a scaffold for the Bub2-Bfa1-Tem1 complex and helps to recruit other MEN components onto the SPBs (8, 39, 101, 139). Cdc15 and Dbf2 localize to both SPBs, whereas Tem1 and the Bub2-Bfa1 complex associate preferentially

with the daughter-bound SPB (7, 8, 19, 88, 89, 101, 139, 151). Interestingly, the future destination (daughter versus mother cell) rather than age of the SPB (old versus newly synthesized) determines which SPB "recruits" the Tem1-Bub2-Bfa1 complex (104). However, the biological significance of this asymmetric localization, which curiously is conserved in fission yeast, and the molecular mechanisms that establish and/or maintain this asymmetry remain unknown. It is tempting to speculate that the Tem1-Bub2-Bfa1 complex associates with the SPB that experiences maximal pulling force during anaphase spindle elongation. As the pulling force on the daughter-bound SPB is likely to be higher during anaphase than on the SPB in the mother cell, the Tem1-Bub2-Bfa1 complex would localize to the SPB migrating into the bud. This idea is consistent with the finding that Tem1 localization on the daughter SPB is significantly decreased in cells lacking microtubule motor proteins such as *KIP2* or the dynein heavy chain *DHC1* (89) and that Tem1 localizes to both SPBs during late stages of anaphase (101). This mechanism would also predict the existence of one or multiple force-sensitive factors on the SPB.

The FEAR Network

Recent studies found that the release of Cdc14 from its inhibitor during early anaphase occurs in the absence of MEN activation (102, 127, 154). Several genes, collectively referred to as the FEAR network, are required for this activation of Cdc14 during early anaphase (127). The FEAR network is comprised of the separase Esp1, the polo-like kinase Cdc5, the kinetochore protein Slk19, the small nuclear protein Spo12 and its homologue Bns1, and the replication fork block protein Fob1 (Figure 3) (102, 126, 127, 143, 154). Genetic epistasis analyses suggest that the FEAR network consists of at least two branches (Figure 3). *ESP1* and *SLK19* appear to function in parallel to *SPO12* and *BNS1* (131, 143). The placement of Cdc5 within the FEAR network is complicated by the fact that Cdc5 is also an activator of the MEN. It is, however, likely, though not proven, that Cdc5 is the ultimate effector of the FEAR network, as its overexpression suppresses the Cdc14 release defect of cells impaired in both the *ESP1* and *SPO12* branch (143).

Little is known about the relationship among FEAR network components. Slk19 is cleaved by Esp1 at the metaphase-anaphase transition (130) but Slk19 cleavage is not required for its mitotic exit function (127, 131). Remarkably, Esp1 promotes the release of Cdc14 independently of its protease function (15, 131), but the nature of this protease-independent signaling mechanism remains elusive. Rather than Slk19 being regulated by Esp1, it appears that Slk19 functions together with Esp1 to promote Cdc14 release from the nucleolus. Slk19 forms a complex with Esp1 and is required for targeting Esp1 to kinetochores and the spindle midzone (131). Thus it is possible that Slk19 promotes the protease-independent function of Esp1.

There is also some evidence to suggest that Esp1 and Slk19 regulate Cdc5 during anaphase. Overexpression of *ESP1* lacking its proteolytic activity induces the phosphorylation of Cfi1/Net1 in metaphase-arrested cells (131). As Cfi1/Net1 phosphorylation is at least in part *CDC5* dependent, it is possible that Esp1

activates Cdc5 toward its anaphase-specific substrates. Importantly, both the protease-dependent and protease-independent functions of Esp1 are inhibited by the securin Pds1 (131), thus enabling securin to restrain both the onset of sister-chromatid separation and FEAR network-mediated activation of Cdc14.

Several studies provided insights into the mechanism by which Spo12 promotes the release of Cdc14 from its inhibitor. Spo12 was found to physically interact with the replication fork block protein Fob1 (126), which localizes to the same rDNA region as Cfi1/Net1 and Cdc14 (53, 126). *FOB1* is a negative regulator within the FEAR network, functioning downstream of or in parallel to *SPO12* (126). But how is the Spo12-Fob1 branch activated? The phosphorylation of two serine residues within the DSP-Box (double SP sites), a highly conserved C-terminal domain within Spo12, is required for its function, and phosphorylation of these sites appears to be cell cycle regulated (112, 126). Thus, it is possible that phosphorylation of Spo12 during early anaphase triggers the activation of the Spo12 branch.

Regulation of the Cdc14–Cfi1/Net1 Interaction by the FEAR Network and the MEN

Although it is clear that the FEAR network and the MEN promote the release of Cdc14 from its inhibitor during anaphase, the molecular mechanisms by which they disrupt the complex are only partially understood. Both Cdc14 and Cfi1/Net1 are phosphorylated during anaphase, raising the possibility that complex dissociation is regulated by this posttranslational modification (118, 120, 143, 157). Consistent with this notion, the phosphorylation of Cfi1/Net1 destabilizes the complex in vitro (118). Which kinases phosphorylate Cfi1/Net1 and Cdc14? Cdc5 appears to be at least in part responsible. Cdc5 can phosphorylate both Cdc14 and Cfi1/Net1 in vitro (118, 157; R.Visintin, personal communication). In vivo, the phosphorylation of Cdc14 by Cdc5 does not require the MEN, whereas Cfi1/Net1 phosphorylation is at least in part mediated by the MEN (118, 143, 157). A likely candidate for the MEN-dependent Cfi1/Net1 phosphorylation is Dbf2, the most downstream kinase within the MEN.

How does the Spo12-Fob1 branch of the FEAR network contribute to the release of Cdc14 from its inhibitor? Fob1 binds to Cfi1/Net1 and was proposed to inhibit the dissociation of the Cdc14-Cfi1/Net1 complex prior to anaphase initiation (53, 126). As Spo12 phosphorylation decreases its binding to Fob1, it is possible that the phosphorylation of Spo12 during early anaphase triggers a conformational change in the Spo12-Fob1 complex, which in turn may help to destabilize the Cdc14-Cfi1/Net1 complex (126).

We speculate that the following series of events triggers the release of Cdc14 from its inhibitor. It appears that Cdc5, perhaps activated by the Esp1 branch of the FEAR network, promotes the phosphorylation of Cdc14. Together with a Spo12-dependent destabilization of the Cdc14-Cfi1/Net1 complex, this might trigger the transient release of Cdc14 from its inhibitor during early anaphase. Activation of the MEN during later stages of anaphase then may cause Dbf2 to

phosphorylate Cfi1/Net1, thereby promoting further conformational changes that sustain the dissociation of the Cdc14-Cfi1/Net1 complex. Rigorous testing of such a model requires the identification of the in vivo phosphorylation sites within Cdc14 and Cfi1/Net1.

Inactivation of the MEN and FEAR Network

The inactivation of Cdc14 after mitotic exit has been completed is as important for successful cell division as its activation is during anaphase. This is illustrated by the severe growth defects exhibited by cells with unconstrained Cdc14 activity (120, 140, 141). Therefore, it is critical that the FEAR network and the MEN are inactivated once mitotic exit has been completed and cells have entered G1.

FEAR network activity appears to be restricted to a very brief time during early anaphase, as Cdc14 becomes resequestered into the nucleolus during late anaphase in cells lacking a functional MEN (102, 127, 154). How the activation of the FEAR network is restricted to the duration of early anaphase is unknown. Two FEAR network components, Cdc5 and Spo12, are targeted for degradation by APC/C^{Cdh1} (20, 21, 112, 117). This mechanism, although it is likely to contribute to lowering FEAR network activity after cells have exited from mitosis, cannot account for restricting FEAR network activity to early anaphase, as Cdc5 and Spo12 protein levels remain high in telophase-arrested MEN mutant cells (F. Stegmeier, unpublished data). The phosphorylation of two serine residues of Spo12 is required for its FEAR network function. Given that these phosphorylation sites conform to the Cdc14 consensus, Cdc14 itself may quench FEAR network activation by dephosphorylating Spo12 (112, 126). This hypothesis further predicts that the kinase responsible for phosphorylating Spo12 is inactivated shortly after the onset of anaphase.

The inactivation of the MEN is accomplished in multiple ways. Interestingly, in each case Cdc14 plants the seeds for its own demise. Cdc14 dephosphorylates both Bfa1 and Lte1, which presumably restores the GAP activity of Bub2-Bfa1 toward Tem1 (35, 52, 102) and triggers the release of Lte1 from the bud cortex (61, 111). The cortical release of Lte1 decreases its concentration in the bud, which is believed to be required for efficient Tem1 activation (8, 61, 111). It is also possible that Lte1 dephosphorylation decreases its activity (61, 111). Furthermore, the sustained release of Cdc14 activates APC/C^{Cdh1}, which induces the degradation of the MEN activator Cdc5 (20, 21, 117). Lastly, Amn1, which is expressed specifically in daughter cells only after Cdc14 has been activated, is thought to antagonize MEN function by competing with Cdc15 for binding to Tem1 (147).

Signals Controlling MEN and FEAR Network Activity

Many mitotic processes, such as the onset of sister-chromatid separation, mitotic spindle disassembly, and cytokinesis, are irreversible. Therefore, to ensure the successful completion of mitosis, it is essential that mitotic exit is tightly coordinated with other cell-cycle events. Recent work has identified some of the cellular signals that control the activation of the FEAR network and the MEN, shedding light

onto the mechanisms whereby Cdc14 activation and hence the coordination of late mitotic events are accomplished.

Coordination of Chromosome Segregation and Exit from Mitosis Through the FEAR Network

Faithful chromosome segregation requires that exit from mitosis is temporally coordinated with the partitioning of the genetic material between the progeny cells. The fact that two important regulators of sister-chromatid separation, the separase Esp1 and the polo-kinase Cdc5, also promote the release of Cdc14 as part of the FEAR network provides a molecular explanation as to how cells ensure that exit from mitosis does not occur prior to the onset of sister-chromatid separation (Figure 3) (127, 131). Conversely, the fact that FEAR network-induced Cdc14 activation promotes the partitioning of late-segregating DNA regions, i.e., the telomeres and the ribosomal DNA (rDNA) region (described in detail below), ensures that sister-chromatid separation is completed before cells exit from mitosis (25, 129).

Preventing Mitotic Exit in Response to Microtubule and DNA Damage Through Inhibition of the MEN and the FEAR Network

In the event of mitotic spindle or DNA damage, cells need to delay sister-chromatid separation and mitotic exit until the damage has been repaired. Two surveillance mechanisms, known as the spindle-assembly checkpoint and the DNA-damage checkpoint, monitor defects in the attachment of microtubules to kinetochores and DNA damage, respectively (reviewed in 78, 99). Both checkpoints antagonize MEN function and prevent the degradation of Pds1. The stabilization of Pds1 inhibits both sister-chromatid separation and FEAR network activation (2, 3, 22, 127, 145, 152, 154). The spindle-assembly checkpoint components Mad1, Mad2, Mad3, Bub1, and Bub3 are essential for preventing the degradation of Pds1 by inhibiting the APC/C^{Cdc20} (reviewed in 78), whereas the Bub2-Bfa1 complex is necessary to prevent MEN activation in response to spindle damage. The current view is that the Bub2-Bfa1 complex is essential to inhibit MEN activity during all mitotic arrests (3, 8, 13, 26, 30, 31, 50, 71, 77, 79, 101, 146). It has been proposed that activation of the spindle checkpoint prolongs the inhibitory function of the Bub2-Bfa1 complex (51), but direct biochemical evidence (i.e., increased Bub2-Bfa1 GAP activity) is still lacking.

Coupling Mitotic Exit with Spindle Position Through Regulating MEN Activity

Because of its unusual division pattern (budding), *S. cerevisiae* faces the unique challenge of threading the nucleus through the mother-bud neck during chromosome segregation. A surveillance mechanism, known as the spindle position

checkpoint, ensures that exit from mitosis does not occur until the nucleus has moved into the bud, the future daughter cell. But how do cells "know" whether part of the nucleus has moved into the bud during anaphase? One mechanism relies on the spatial segregation of the MEN components Lte1 and Tem1. The MEN activator Lte1 localizes to the bud cortex during bud formation while Tem1 resides on the daughter-bound SPB (8, 101). Thus, delivery of the Tem1-bearing SPB into the bud is likely to promote Tem1 activation during anaphase (6, 8, 101). Indeed, when maintenance of Lte1 in the bud is disrupted by the inactivation of septins, which form a diffusion barrier for membrane-associated proteins (10, 48, 111), cells with a mispositioned nucleus exit from mitosis (18). The Bub2-Bfa1 complex is also required to restrain mitotic exit in cells in which the nucleus and hence the mitotic spindle are not correctly positioned along the mother-bud axis (1, 8, 13, 26, 101, 146). Interestingly, the phosphorylation of Bfa1 and its asymmetric localization to the daughter-bound SPB appear to be regulated in response to spindle orientation (52, 104). The interaction of cytoplasmic microtubules with the bud neck possibly controls the activity of the Bub2-Bfa1 complex (1, 18, 104), but the molecular nature of such a signaling mechanism remains elusive.

Regulation of Mitotic Exit by Cell Polarity Determinants

Several recent studies found that Lte1 and the Bub2-Bfa1 complex are also regulated by cell polarity proteins, including the Rho-like GTPase Cdc42 and its effectors Cla4, Ste20 (both PAK-like kinases), Gic1, and Gic2 (48, 49, 61, 111). Cdc42 appears to promote MEN activation through three independent effector branches: (*a*) Cdc42-dependent activation of the PAK-like kinase Cla4 is both required and sufficient for the phosphorylation and thus targeting of Lte1 to the bud cortex (48, 111). Importantly, cells lacking *CDC42* or *CLA4* delay in exit from mitosis, supporting the notion that bud enrichment of Lte1 is important for the timely completion of mitotic exit (8, 48, 111). However, it is also possible that phosphorylation of Lte1 is required for its activation (48, 111). (*b*) The activation of Gic1 and Gic2 by Cdc42 is thought to promote mitotic exit by interfering with Bub2-Bfa1 GAP protein function (49). (*c*) The PAK-like kinase Ste20 is also activated by Cdc42 and functions in a pathway parallel to *LTE1*, but its molecular target remains elusive (48). Given that these cell polarity proteins help to establish Lte1's asymmetric bud localization (in the case of Cla4), and are thought to antagonize Bub2-Bfa1 function only after the mitotic spindle has been correctly positioned (in the case of Gic1), these pathways may well contribute to the proper function of the spindle position checkpoint.

Regulation of Mitotic Exit by Nutrient Signaling

The effects of nutrient signaling, which is likely to be mediated at least in part by the Ras-cAMP pathway, on entry into the cell cycle are well established (9, 133). The Ras pathway has, however, also been implicated in controlling exit from mitosis. When the essential function of the Ras pathway in cAMP production is bypassed,

cells lacking both *RAS1* and *RAS2* are impaired in mitotic exit at elevated or lower temperatures (92). A recent study found that active Ras is required for the proper localization of Lte1 to the bud cortex (155), which is likely to account for the mitotic exit defect of *ras1*Δ *ras2*Δ mutant cells at lower temperatures. However, additional Ras effectors must regulate exit from mitosis, as *LTE1* is dispensable for mitotic exit at elevated temperatures (1). It will be interesting to determine whether Ras signaling affects the localization or the activity of the Bub2-Bfa1 complex.

THE MULTIPLE ROLES OF Cdc14 DURING LATE STAGES OF MITOSIS

Having discussed the regulation of Cdc14 in detail, we now describe the cellular processes controlled by Cdc14. In summary, Cdc14 is the workhorse during late stages of mitosis; it reverses the CDK phosphorylation events that earlier during the cell cycle promoted progression through mitosis. In recent years, it has become clear that Cdc14 induces not only mitotic CDK inactivation and exit from mitosis but regulates a variety of other cellular events such as rDNA and telomere segregation, mitotic spindle dynamics, and cytokinesis. Remarkably, the execution of these diverse events relies on Cdc14 activation mediated by different regulatory networks.

Functions of Cdc14 Released by the FEAR Network

Although Cdc14 activated by the FEAR network is not essential for cell proliferation, it helps to coordinate several mitotic events (Figure 2), thereby contributing to the maintenance of genomic stability. The importance of the FEAR network is illustrated by a loss in viability that cells experience when progressing through anaphase in the absence of FEAR network function (25), which may at least in part be due to chromosome loss (46).

Several recent studies found that FEAR network-induced Cdc14 activation promotes the completion of chromosome segregation (Figure 2). Cells lacking *CDC14* or FEAR network activity are impaired in the segregation of telomeres and the rDNA array (15, 25, 36, 129, 134). Given that cells lacking MEN activity do not exhibit such defects, it appears that the transient activation of Cdc14 by the FEAR network is sufficient for the separation of these late-segregating chromosomal regions. Several observations point to the possibility that Cdc14 mediates rDNA segregation by promoting the enrichment of condensins, which are protein complexes required for chromosome condensation (reviewed in 44, 47), at the rDNA locus. Cells carrying mutations in subunits of the condensin complex exhibit nucleolar segregation defects similar to *cdc14* mutants (12, 32, 72, 100). Moreover, *CDC14* is required for rDNA condensation (41) and the targeting of condensins to the rDNA region during anaphase (25, 129). The observation that Cdc14 also induces the sumoylation of the condensin subunit Ycs4 during anaphase raises the interesting

possibility that Cdc14 targets condensins to the rDNA by promoting Ycs4 sumoylation. However, the molecular mechanism(s) by which Cdc14 and condensin facilitate the individualization of these genomic regions remains unknown.

Another function of Cdc14 released by the FEAR network is to regulate the subcellular localization of chromosomal passenger proteins, which presumably contributes to the stabilization of the mitotic spindle during early anaphase (Figure 2) (103). Chromosomal passenger proteins are characterized by their change in subcellular localization during anaphase. During metaphase they reside at kinetochores but translocate to the spindle midzone during anaphase (reviewed in 17). A recent study found that the translocation of the chromosomal passenger proteins Ipl1 (Aurora B kinase) and Sli15 (INCENP) to the mitotic spindle and spindle midzone is promoted by FEAR network-induced activation of Cdc14 (103). Cdc14 dephosphorylates Sli15, thereby triggering the change in Ipl1-Sli15 localization. This change in localization appears to be important for anaphase spindle stabilization. A *SLI15* mutant that mimics its dephosphorylated form partially rescues the premature spindle breakage observed in *esp1* mutants, possibly by recruiting other spindle-stabilizing proteins, such as Slk19, to the spindle midzone (103). However, another study reached the opposite conclusion, namely that Ipl1 promotes mitotic spindle disassembly (16). One possible explanation for these contradictory observations is that the Ipl1 complex first stabilizes the mitotic spindle during early anaphase but promotes its disassembly during telophase.

Lastly, FEAR network-released Cdc14 stimulates MEN activity. It does so in at least two ways (Figure 2). (*a*) Cdc14 released by the FEAR network promotes the dephosphorylation of Cdc15, thus enhancing Cdc15's mitotic exit function (60, 127). How the dephosphorylation of Cdc15 promotes its mitotic exit function remains unknown. Dephosphorylation of Cdc15 may enhance its interaction with Tem1, its localization to SPBs, or antagonize the inhibitory function of Cdc15's C-terminal domain. (*b*) Cdc14 has been proposed to inactivate the Bub2-Bfa1 complex in a phosphatase-independent manner during early anaphase (102). Cdc14 binds to Bub2 at the daughter SPB shortly after its release from the nucleolus during early anaphase (102, 154). Although the association between the Bub2-Bfa1 complex and Cdc14 has been proposed to inactivate its GAP activity during early anaphase, Bfa1's dephosphorylation by Cdc14 during later stages of mitosis is thought to reactivate the GAP complex (35, 102). Validation of this model will require the elucidation of Cdc14's phosphatase-independent inhibitory function on Bub2-Bfa1 and the identification of the mechanism(s) that prevent Cdc14 from dephosphorylating Bfa1 during early anaphase.

Cdc14 released by the FEAR network most likely regulates many other aspects of anaphase chromosome movement. What these processes may have in common is that their regulatory proteins require dephosphorylation by Cdc14 despite the presence of high mitotic CDK activity throughout the cell. Therefore, subcellular regions that contain a high local concentration of Cdc14 during early anaphase, which includes kinetochores, the mitotic spindle, and SPBs, are most likely to harbor yet unidentified targets of Cdc14 released by the FEAR network.

Functions of Cdc14 Released by the MEN

Cdc14 activated by the MEN is mainly responsible for promoting exit from mitosis, as this transition does not occur in the absence of MEN function (45, 59, 63, 115, 132). Several observations, however, indicate that Cdc14 and the MEN also regulate cytokinesis independently of their mitotic exit function. When the need for MEN function in mitotic exit is bypassed, either by weakening the Cdc14–Cfi1/Net1 interaction or by overexpression of the CDK inhibitor *SIC1*, severe cytokinesis defects become apparent (62, 80, 81, 120, 125). The notion that the MEN regulates cytokinesis is also consistent with the translocation of the MEN components Cdc15, Dbf2, Mob1, and Cdc5 to the bud neck (the site of actomyosin ring constriction in budding yeast) during late anaphase and telophase (33, 81, 124, 125, 151, 156). The cytokinesis function of at least some MEN components requires Cdc14 function, as the accumulation of Dbf2 and Mob1 at the bud neck depends on *CDC14* (33, 156). We speculate that after the MEN promoted Cdc14 activation, Cdc14 itself may dephosphorylate CDK consensus sites in MEN components to promote their localization to the bud neck, where they then regulate cytokinesis (Figure 2). However, the critical Cdc14 targets within the MEN in the regulation of cytokinesis remain to be identified.

FUNCTIONS OF Cdc14, THE FEAR NETWORK AND THE MEN DURING MEIOSIS

Meiosis is a specialized cell cycle during which a single round of DNA replication is followed by two chromosome segregation phases (reviewed in 96). Owing to this special segregation program, the transition from meiosis I to meiosis II has specific requirements. CDK activity needs to be lowered sufficiently to allow for the disassembly of the meiosis I spindle. On the other hand, complete CDK inactivation, which would allow for the assembly of prereplicative complexes (preRCs) onto the DNA, needs to be avoided to prevent an intervening S phase (56). Two studies raise the interesting possibility that meiotic cells solve this problem by employing the FEAR network rather than the MEN to promote exit from meiosis I. Cells impaired in FEAR network-mediated Cdc14 activation are severely delayed in meiosis I spindle disassembly, whereas MEN inactivation does not affect exit from the first meiotic division (15, 85). Given that Cdc14 activity released by the FEAR network is short-lived, employing the FEAR network for Cdc14 activation may lower CDK activity sufficiently for meiosis I spindle disassembly to occur but not low enough to allow assembly of preRCs onto origins of replication.

The requirement for Cdc14 and the FEAR network in exit from meiosis I is similar to its mitotic function, but another phenotype exhibited by *cdc14* and FEAR network mutants is unique to meiosis. Instead of arresting in anaphase I with homologous chromosomes segregated, *cdc14* and FEAR network mutants exhibit a "mixed" chromosome segregation pattern, which is characterized by some chromosomes segregating in a meiosis I-like pattern and others segregating

in a meiosis II-like pattern (15, 65, 68, 85, 113, 159). This unusual chromosome segregation pattern appears to result from meiotic events being uncoupled (15, 85). Despite cells arresting in anaphase I, meiosis II chromosome segregation events continue to occur, leading to some chromosomes undergoing both meiotic divisions on the same anaphase I spindle. These findings suggest that Cdc14 and the FEAR network ensure that the two meiotic divisions occur on two sequentially built spindles. Presumably, by antagonizing meiotic CDK activity, Cdc14 not only promotes meiosis I spindle disassembly, but also creates conditions that preclude the onset of the second chromosome segregation phase.

Cdc14 HOMOLOGUES IN OTHER EUKARYOTES

The Functions and Regulation of Cdc14's *S. pombe* Homologue Clp1/Flp1

Like its cousin in *S. cerevisiae*, Clp1/Flp1 antagonizes mitotic CDK activity. Furthermore, the activity of Clp1/Flp1 is at least in part regulated by the septation-initiation network (SIN, Table 1), a signaling pathway homologous to the MEN that is essential for cytokinesis in fission yeast (reviewed in detail in 6, 86, 121). Despite these similarities, the mechanisms whereby Clp1/Flp1 and Cdc14 accomplish mitotic CDK inactivation and the cellular processes regulated by these two phosphatases appear to be quite different. In contrast to Cdc14, Clp1/Flp1 is not essential for mitotic exit but predominantly antagonizes mitotic CDK activity during G2. In the absence of $clp1^{+}/flp1^{+}$ cells enter mitosis prematurely (23, 136). The importance of Clp1/Flp1 in maintaining low mitotic CDK activity during G2 is further revealed by its role in the cytokinesis checkpoint. Activation of the cytokinesis checkpoint in fission yeast leads to cell-cycle arrest in G2. This cell-cycle arrest requires the continuous inhibition of mitotic CDKs, which is at least in part mediated by Clp1/Flp1 (23, 136).

Although Clp1/Flp1's main function appears to be the regulation of the G2/M transition, it was recently shown that Clp1/Flp1 also contributes to the inhibition of mitotic CDKs during exit from mitosis (43, 150). Furthermore, cells lacking $clp1^{+}/flp1^{+}$ exhibit defects in cytokinesis (23, 136). As mitotic CDKs inhibit SIN activation by antagonizing the localization of the SIN component Sid1 to SPBs (42), Clp1/Flp1 activation during mitosis may help to maintain Cdk1 activity below a certain threshold that allows the SIN to stay active and properly execute cytokinesis (150).

Unlike Cdc14 in budding yeast, which dephosphorylates and activates the APC/C activator Cdh1/Hct1 and the Cdk1 inhibitor Sic1 to inhibit mitotic CDKs, Clp1/Flp1 antagonizes mitotic CDK activity by preventing the dephosphorylation of a conserved tyrosine residue (Y15 in *S. pombe*) in Cdk1 (136). Clp1/Flp1 promotes Y15 phosphorylation by activating the protein kinase Wee1, which phosphorylates Y15, and by down-regulating the Cdc25 phosphatase, which dephosphorylates Y15 (136, 150). The dual regulation of Cdc25 and Wee1 by Clp1/Flp1

not only causes down-regulation of CDKs during G2 (23, 136) but it may also help to inactivate CDKs during exit from mitosis (150).

Similar to its budding yeast ortholog, Clp1/Flp1 localizes to the nucleolus during some cell-cycle stages and is released during others, raising the possibility that some aspects of Cdc14 and Clp1/Flp1 regulation are shared between the two yeasts. During G1 and S phase, Clp1/Flp1 localizes predominantly to the nucleolus, but a small portion resides at SPBs (23, 136). While Cdc14 is released from the nucleolus during anaphase, Clp1/Flp1 is already released from the nucleolus during prophase and localizes to the mitotic spindle, the SPBs, and the medial ring (23, 136). It is still unclear whether the release of Clp1/Flp1 from the nucleolus alone is sufficient for its activation. It will be important to determine whether Clp1/Flp1 is bound to an inhibitor when it resides in the nucleolus and if so, whether the release of Clp1/Flp1 from the nucleolus disrupts the interaction with this inhibitor. The activity of Clp1/Flp1 might also be regulated by changes in phosphorylation, as Clp1/Flp1 phosphorylation is maximal during mitosis (23, 136).

Although it does not appear to be the sole mechanism of regulation, the changes in subcellular localization of Clp1/Flp1 are likely to contribute to its activation. How is the release of Clp1/Flp1 from the nucleolus regulated? SIN, a regulatory network homologous to the MEN (Table 1), appears to control Clp1/Flp1 localization in a manner similar to the MEN (23, 136). Although the SIN does not regulate Clp1/Flp1 localization during an unperturbed cell cycle, this signaling network is required to maintain Clp1/Flp1 in its released state when the cytokinesis checkpoint has been activated (23, 136). Thus, maintenance of Cdc14 and Clp1/Flp1 in its released state relies on conserved pathways. Whether a pathway similar to the FEAR network regulates the release of Clp1/Flp1 during early stages of mitosis in *S. pombe* is not known but initial observations indicate that this might not be the case. *cut1* mutants (*cut1*$^+$ encodes the *ESP1* homologue in *S. pombe*) are not impaired in the release of Clp1/Flp1 from the nucleolus (D. McCollum, personal communication). Furthermore, overexpression of the fission yeast polo-like kinase *plo1*$^+$ does not promote the release of Clp1/Flp1 from the nucleolus (D. McCollum, personal communication). Identifying the regulatory mechanisms that promote Clp1/Flp1 release from the nucleolus during early mitosis will be an important task for future studies, as this might also provide insights into the mechanisms controlling the localization of metazoan homologues, which are also released from the nucleolus during prophase (see below).

The Functions of Cdc14 Homologues and their Regulation in Metazoans

Homologues of Cdc14 exist in most if not all eukaryotes and have been characterized at least to some extent in *C. elegans* and mammals. In *C. elegans*, depletion of CeCDC-14 by RNAi causes defects in cytokinesis, most likely due to a failure to form an intact central spindle (40). How CeCDC-14 promotes central spindle formation is not known, but CeCDC-14 is required for the localization of ZEN-4,

a kinesin-related motor protein that is also required for central spindle formation, to the mitotic spindle (40). Consistent with a role in cytokinesis and central spindle function, CeCDC-14 localizes to the central spindle and the midbody, similar to Clp1/Flp1 in *S. pombe*, but the phosphatase has been detected neither in the nucleolus nor on centrosomes (40). Whether CeCDC-14 is regulated by signaling pathways homologous to the MEN and SIN is also unclear. A recent study has explored the function of several potential MEN and SIN homologues in *C. elegans*, using RNA-mediated interference (RNAi) (40). Surprisingly, depletion of Dbf2, Mob1, and Sid1 homologues (Table 1) by RNAi did not lead to embryonic lethality (40). It is possible that the RNAi-mediated depletion was incomplete or that redundant factors exist in *C. elegans*. Alternatively, the MEN/SIN components may not be necessary during embryonic divisions or these pathways may have evolved to fulfill functions specific to yeast.

The human genome encodes two Cdc14 homologues, hCdc14A and hCdc14B. The roles of these two phosphatases are poorly understood, but an involvement in mitotic exit and cytokinesis is possible. In vitro studies have shown that hCdc14A, just like its yeast counterparts, has a clear preference for substrates of proline-directed kinases (64, 136, 140), which is further supported by the crystal structure of the core domain of hCdc14B (37). Furthermore, hCdc14A can dephosphorylate Cdh1 and reconstitute active APC^{Cdh1} in vitro (11). Although this finding indicates that hCdc14A can in principle bring about mitotic CDK inactivation, this Cdc14 isoform appears to primarily regulate centrosome function in vivo. Overexpression of hCdc14A leads to premature centriole splitting in S phase and formation of an excessive number of aberrant mitotic spindles (64, 84). Conversely, depletion of hCdc14A by RNAi leads to centrosome duplication, mitotic and cytokinesis defects (84). The latter defects may, however, be an indirect consequence of the centrosome malfunction.Consistent with its proposed role in regulating centrosome function, hCdc14A localizes to this organelle (11, 64, 84). Much less is known about the functions of hCdc14B. Intriguingly, however, hCdc14B localizes to the nucleolus during interphase but not during mitosis (11, 64). The dramatic differences in the localization of hCdc14A and B suggest that these isoforms perform different functions in the cell.

Potential homologues of MEN/SIN pathway components have been identified in mammals (Table 1) but their cellular functions remain largely unexplored. Interestingly, some of these putative homologues, such as GAPCenA, WARTS/LATS1 and centriolin (24, 38, 98), localize to centrosomes. Furthermore, the centrosome has been implicated in the regulation of cytokinesis. Ablation of centrosomes leads to defects in cytokinesis (66, 106). Thus, the centrosome, like the SPBs in budding and fission yeast, may anchor signaling networks needed for the completion of the cell cycle. In support of this hypothesis, the Nud1 homolog centriolin is important for the final stages of cytokinesis (38).

Metazoan genomes also harbor homologues of the FEAR network components Cdc5, Esp1, and Spo12 (Table 2). Several studies suggest that polo kinases are required to control mitotic exit and cytokinesis in most if not all

TABLE 2 FEAR network components and potential homologues in other eukaryotes

	S. cerevisiae	*S. pombe*	*C. elegans*	**Mammals**
Separase	Esp1	Cut1	SEP-1	Separase
Polo kinase	Cdc5	Plo1	PLK-1/PLK-2	Polo-kinase family
Spo12 family	Spo12	Wis3	4D376	?
Kinetochore protein	Slk19	?	?	?
Fork block protein	Fob1	?	?	?

organisms (27, 54, 74, 75, 93), but whether polo kinases regulate these processes through activation of Cdc14 in mammals remains unknown. Separase may also be needed for cell-cycle steps after cohesin removal in at least some metazoans. Reduction of separase function by RNAi in *C. elegans* not only prevents sister-chromatid separation but also slows down subsequent cell-cycle events (122). Clearly, further studies will be needed to elucidate the role of homologues of Cdc14, the MEN, and the FEAR network during cell-cycle progression in mammalian cells.

CONCLUSIONS AND PERSPECTIVES

There has been significant progress in the past few years in elucidating the mechanisms that control late mitotic events in budding yeast. The Cdc14 phosphatase takes center stage during late mitosis and promotes several mitotic processes by reversing mitotic CDK phosphorylation events. Besides its essential role in exit from mitosis, this phosphatase also regulates certain aspects of sister-chromatid separation, mitotic spindle dynamics, and cytokinesis. Given its important role in coordinating several mitotic events, it is not surprising that the activation of Cdc14 is intricately controlled by at least two signaling networks, the FEAR network and the MEN, which integrate different cellular signals into the decision whether or not to activate Cdc14.

While the ability of Cdc14 homologues to antagonize mitotic CDK activity is likely to be conserved in all eukaryotes, the cellular functions of Cdc14 homologues appears to differ significantly between species. For example, the absolute requirement for Cdc14 in mitotic exit appears unique to budding yeast. We speculate that the reason for this might be founded in the physiology of budding yeast. Because of its unusual division pattern (budding), this yeast might have evolved a delay in mitotic CDK inactivation and re-engineered Cdc14's regulatory circuits to ensure that the spindle has been correctly positioned between the mother cell and the bud axis before committing to exit from mitosis. This safeguard mechanism could be unnecessary in cells dividing by fission.

Another conserved feature of Cdc14 homologues in all species appears to be the regulation of several mitotic events. In budding and fission yeast, Cdc14

regulates chromosome segregation, spindle dynamics, cytokinesis, and mitotic exit. The complex phenotype of human cells lacking or overexpressing hCdc14A and hCdc14B homologues is consistent with Cdc14 regulating many mitotic processes. Thus, we speculate that all Cdc14 homologues coordinate multiple mitotic processes by reversing CDK phosphorylation events but that each organism re-engineered its regulatory circuits according to its particular needs.

A specific example of conserved Cdc14 function is its regulation of cytokinesis in all organisms studied to date (23, 33, 40, 84, 136, 156). Could other mitotic functions of Cdc14 be conserved across species? It is possible that Cdc14 regulates the subcellular localization of chromosomal passenger proteins also in mammals. INCENP (Sli15 in yeast) is phosphorylated prior to mitosis (14) and the translocation of the chromosomal passenger proteins aurora B (Ipl1 in yeast) and TD-60 (not conserved in yeast) from kinetochores to the spindle midzone is prevented by high levels of mitotic CDK activity (94, 149). These findings suggest that protein dephosphorylation is necessary for this translocation to occur. However, whether one of the mammalian Cdc14 phosphatases regulates this process remains to be determined.

In budding and fission yeast, great strides have been made in dissecting the role of the Cdc14 phosphatase in several mitotic processes and in deciphering its complex regulation by the FEAR network and the MEN/SIN. In contrast, our molecular understanding of Cdc14 function and the regulation of late mitotic events in metazoans is still limited. Systematic RNAi screens in a variety of systems are likely to lead to the identification and characterization of the pathways regulating Cdc14 activity in these organisms. In addition, the identification of relevant physiological substrates of Cdc14 in diverse species will help to unearth further mitotic processes controlled by this conserved phosphatase.

NOTE ADDED IN PROOF

A recent study (160) provided significant insight into the molecular mechanisms that promote the release of Cdc14 from its inhibitor Cfi1/Net1 during early anaphase. Azzam et al. mapped the in vivo phosphorylation sites of Cfi1/Net1 and found that the phosphorylation of a subset of these sites by Clb2–CDK (or Clb1-CDK) is required for the release of Cdc14 during early anaphase. Interestingly, the phosphorylation of Cfi1/Net1 occurs significantly later than the activation of Clb-CDKs, which peaks already during metaphase. The authors propose that the other FEAR network components are required for the timely phosphorylation of Cfi1/Net1 by Clb-CDKs, but the molecular mechanisms remain to be identified. It is important to note that the phosphorylation of Cfi1/Net1 alone is not sufficient for Cdc14 release from the nucleolus. Cdc14 becomes re-sequestered into the nucleolus in *cdc15-2* mutants despite Cfi1/Net1 still being highly phosphorylated (160). We speculate that the phosphorylation of Cdc14 by Cdc5 synergizes with Cfi1/Net1 phosphorylation to weaken the interaction of Cdc14 with its inhibitor.

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LITERATURE CITED

1. Adames NR, Oberle JR, Cooper JA. 2001. The surveillance mechanism of the spindle position checkpoint in yeast. *J. Cell Biol.* 153:159–68
2. Agarwal R, Tang Z, Yu H, Cohen-Fix O. 2003. Two distinct pathways for inhibiting pds1 ubiquitination in response to DNA damage. *J. Biol. Chem.* 278:45027–33
3. Alexandru G, Zachariae W, Schleiffer A, Nasmyth K. 1999. Sister chromatid separation and chromosome re-duplication are regulated by different mechanisms in response to spindle damage. *EMBO J.* 18:2707–21
4. Asakawa K, Toh-e A. 2002. A defect of Kap104 alleviates the requirement of mitotic exit network gene functions in *Saccharomyces cerevisiae*. *Genetics* 162: 1545–56
5. Asakawa K, Yoshida S, Otake F, Toh-e A. 2001. A novel functional domain of Cdc15 kinase is required for its interaction with Tem1 GTPase in *Saccharomyces cerevisiae*. *Genetics* 157:1437–50
6. Bardin AJ, Amon A. 2001. Men and sin: What's the difference? *Nat. Rev. Mol. Cell Biol.* 2:815–26
7. Bardin AJ, Boselli MG, Amon A. 2003. Mitotic exit regulation through distinct domains within the protein kinase Cdc15. *Mol. Cell Biol.* 23:5018–30
8. Bardin AJ, Visintin R, Amon A. 2000. A mechanism for coupling exit from mitosis to partitioning of the nucleus. *Cell* 102:21–31
9. Baroni MD, Monti P, Alberghina L. 1994. Repression of growth-regulated G1 cyclin expression by cyclic AMP in budding yeast. *Nature* 371:339–42
10. Barral Y, Mermall V, Mooseker MS, Snyder M. 2000. Compartmentalization of the cell cortex by septins is required for maintenance of cell polarity in yeast. *Mol. Cell* 5:841–51
11. Bembenek J, Yu H. 2001. Regulation of the anaphase-promoting complex by the dual specificity phosphatase human Cdc14a. *J. Biol. Chem.* 276:48237–42
12. Bhalla N, Biggins S, Murray AW. 2002. Mutation of YCS4, a budding yeast condensin subunit, affects mitotic and nonmitotic chromosome behavior. *Mol. Biol. Cell* 13:632–45
13. Bloecher A, Venturi GM, Tatchell K. 2000. Anaphase spindle position is monitored by the BUB2 checkpoint. *Nat. Cell Biol.* 2:556–58
14. Bolton MA, Lan W, Powers SE, McCleland ML, Kuang J, Stukenberg PT. 2002. Aurora B kinase exists in a complex with survivin and INCENP and its kinase activity is stimulated by survivin binding and phosphorylation. *Mol. Biol. Cell* 13:3064–77
15. Buonomo SB, Rabitsch KP, Fuchs J, Gruber S, Sullivan M, et al. 2003. Division of the nucleolus and its release of CDC14 during anaphase of meiosis I depends on separase, SPO12, and SLK19. *Dev. Cell* 4:727–39

16. Buvelot S, Tatsutani SY, Vermaak D, Biggins S. 2003. The budding yeast Ipl1/Aurora protein kinase regulates mitotic spindle disassembly. *J. Cell Biol.* 160: 329–39
17. Carmena M, Earnshaw WC. 2003. The cellular geography of aurora kinases. *Nat. Rev. Mol. Cell Biol.* 4:842–54
18. Castillon GA, Adames NR, Rosello CH, Seidel HS, Longtine MS, et al. 2003. Septins have a dual role in controlling mitotic exit in budding yeast. *Curr. Biol.* 13:654–58
19. Cenamor R, Jimenez J, Cid VJ, Nombela C, Sanchez M. 1999. The budding yeast Cdc15 localizes to the spindle pole body in a cell-cycle-dependent manner. *Mol. Cell Biol. Res. Commun.* 2:178–84
20. Charles JF, Jaspersen SL, Tinker-Kulberg RL, Hwang L, Szidon A, Morgan DO. 1998. The Polo-related kinase Cdc5 activates and is destroyed by the mitotic cyclin destruction machinery in *S. cerevisiae*. *Curr. Biol.* 8:497–507
21. Cheng L, Hunke L, Hardy CF. 1998. Cell cycle regulation of the *Saccharomyces cerevisiae* polo-like kinase cdc5p. *Mol. Cell Biol.* 18:7360–70
22. Cohen-Fix O, Peters JM, Kirschner MW, Koshland D. 1996. Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes Dev.* 10:3081–93
23. Cueille N, Salimova E, Esteban V, Blanco M, Moreno S, et al. 2001. *S. pombe* cdc14 orthologue. *J. Cell Sci.* 114 (Part14):2649–64
24. Cuif MH, Possmayer F, Zander H, Bordes N, Jollivet F, et al. 1999. Characterization of GAPCenA, a GTPase activating protein for Rab6, part of which associates with the centrosome. *EMBO J.* 18:1772–82
25. D'Amours D, Stegmeier F, Amon A. 2004. Cdc14 and condensin control the dissolution of cohesin-independent chromosome linkages at repeated DNA. *Cell* 117(4):455–69
26. Daum JR, Gomez-Ospina N, Winey M, Burke DJ. 2000. The spindle checkpoint of *Saccharomyces cerevisiae* responds to separable microtubule-dependent events. *Curr. Biol.* 10:1375–78
27. Descombes P, Nigg EA. 1998. The polo-like kinase Plx1 is required for M phase exit and destruction of mitotic regulators in Xenopus egg extracts. *EMBO J.* 17:1328–35
28. Donovan JD, Toyn JH, Johnson AL, Johnston LH. 1994. P40SDB25, a putative CDK inhibitor, has a role in the M/G1 transition in *Saccharomyces cerevisiae*. *Genes Dev.* 8:1640–53
29. Feldman RM, Correll CC, Kaplan KB, Deshaies RJ. 1997. A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. *Cell* 91:221–30
30. Fesquet D, Fitzpatrick PJ, Johnson AL, Kramer KM, Toyn JH, Johnston LH. 1999. A Bub2p-dependent spindle checkpoint pathway regulates the Dbf2p kinase in budding yeast. *EMBO J.* 18:2424–34
31. Fraschini R, Formenti E, Lucchini G, Piatti S. 1999. Budding yeast Bub2 is localized at spindle pole bodies and activates the mitotic checkpoint via a different pathway from Mad2. *J. Cell Biol.* 145:979–91
32. Freeman L, Aragon-Alcaide L, Strunnikov A. 2000. The condensin complex governs chromosome condensation and mitotic transmission of rDNA. *J. Cell Biol.* 149:811–24
33. Frenz LM, Lee SE, Fesquet D, Johnston LH. 2000. The budding yeast Dbf2 protein kinase localises to the centrosome and moves to the bud neck in late mitosis. *J. Cell Sci.* 113(Part 19):3399–408
34. Geymonat M, Spanos A, Smith SJ, Wheatley E, Rittinger K, et al. 2002. Control of mitotic exit in budding yeast. In vitro regulation of Tem1 GTPase by Bub2 and Bfa1. *J. Biol. Chem.* 277:28439–45
35. Geymonat M, Spanos A, Walker PA, Johnston LH, Sedgwick SG. 2003. In vitro

regulation of budding yeast Bfa1/Bub2 GAP activity by Cdc5. *J. Biol. Chem.* 278: 14591–94

36. Granot D, Snyder M. 1991. Segregation of the nucleolus during mitosis in budding and fission yeast. *Cell Motil. Cytoskelet.* 20:47–54
37. Gray CH, Good VM, Tonks NK, Barford D. 2003. The structure of the cell cycle protein Cdc14 reveals a proline-directed protein phosphatase. *EMBO J.* 22:3524–35
38. Gromley A, Jurczyk A, Sillibourne J, Halilovic E, Mogensen M, et al. 2003. A novel human protein of the maternal centriole is required for the final stages of cytokinesis and entry into S phase. *J. Cell Biol.* 161:535–45
39. Gruneberg U, Campbell K, Simpson C, Grindlay J, Schiebel E. 2000. Nud1p links astral microtubule organization and the control of exit from mitosis. *EMBO J.* 19:6475–88
40. Gruneberg U, Glotzer M, Gartner A, Nigg EA. 2002. The CeCDC-14 phosphatase is required for cytokinesis in the *Caenorhabditis elegans* embryo. *J. Cell Biol.* 158:901–14
41. Guacci V, Hogan E, Koshland D. 1994. Chromosome condensation and sister chromatid pairing in budding yeast. *J. Cell Biol.* 125:517–30
42. Guertin DA, Chang L, Irshad F, Gould KL, McCollum D. 2000. The role of the sid1p kinase and cdc14p in regulating the onset of cytokinesis in fission yeast. *EMBO J.* 19:1803–15
43. Guertin DA, Venkatram S, Gould KL, McCollum D. 2002. Dma1 prevents mitotic exit and cytokinesis by inhibiting the septation initiation network (SIN). *Dev. Cell.* 3:779–90
44. Hagstrom KA, Meyer BJ. 2003. Condensin and cohesin: more than chromosome compactor and glue. *Nat. Rev. Genet.* 4:520–34
45. Hartwell LH, Culotti J, Reid B. 1970. Genetic control of the cell-division cycle in yeast. I. Detection of mutants. *Proc. Natl. Acad. Sci. USA* 66:352–59
46. Hartwell LH, Smith D. 1985. Altered fidelity of mitotic chromosome transmission in cell cycle mutants of *S. cerevisiae*. *Genetics* 110:381–95
47. Hirano T. 2000. Chromosome cohesion, condensation, and separation. *Annu. Rev. Biochem.* 69:115–44
48. Hofken T, Schiebel E. 2002. A role for cell polarity proteins in mitotic exit. *EMBO J.* 21:4851–62
49. Hofken T, Schiebel E. 2004. Novel regulation of mitotic exit by the Cdc42 effectors Gic1 and Gic2. *J. Cell Biol.* 164:219–31
50. Hoyt MA, Totis L, Roberts BT. 1991. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* 66:507–17
51. Hu F, Elledge SJ. 2002. Bub2 is a cell cycle regulated phospho-protein controlled by multiple checkpoints. *Cell Cycle* 1:351–55
52. Hu F, Wang Y, Liu D, Li Y, Qin J, Elledge SJ. 2001. Regulation of the Bub2/Bfa1 GAP complex by Cdc5 and cell cycle checkpoints. *Cell* 107:655–65
53. Huang J, Moazed D. 2003. Association of the RENT complex with nontranscribed and coding regions of rDNA and a regional requirement for the replication fork block protein Fob1 in rDNA silencing. *Genes Dev.* 17:2162–76
54. Hudson JW, Kozarova A, Cheung P, Macmillan JC, Swallow CJ, et al. 2001. Late mitotic failure in mice lacking Sak, a polo-like kinase. *Curr. Biol.* 11:441–46
55. Irniger S. 2002. Cyclin destruction in mitosis: a crucial task of Cdc20. *FEBS Lett.* 532:7–11
56. Iwabuchi M, Ohsumi K, Yamamoto TM, Sawada W, Kishimoto T. 2000. Residual Cdc2 activity remaining at meiosis I exit is essential for meiotic M-M transition in Xenopus oocyte extracts. *EMBO J.* 19:4513–23
57. Jaquenoud M, van Drogen F, Peter M. 2002. Cell cycle-dependent nuclear

export of Cdh1p may contribute to the inactivation of APC/C(Cdh1). *EMBO J.* 21: 6515–26

58. Jaspersen SL, Charles JF, Morgan DO. 1999. Inhibitory phosphorylation of the APC regulator Hct1 is controlled by the kinase Cdc28 and the phosphatase Cdc14. *Curr. Biol.* 9:227–36
59. Jaspersen SL, Charles JF, Tinker-Kulberg RL, Morgan DO. 1998. A late mitotic regulatory network controlling cyclin destruction in *Saccharomyces cerevisiae*. *Mol. Biol. Cell.* 9:2803–17
60. Jaspersen SL, Morgan DO. 2000. Cdc14 activates cdc15 to promote mitotic exit in budding yeast. *Curr. Biol.* 10:615–18
61. Jensen S, Geymonat M, Johnson AL, Segal M, Johnston LH. 2002. Spatial regulation of the guanine nucleotide exchange factor Lte1 in *Saccharomyces cerevisiae*. *J. Cell Sci.* 115:4977–91
62. Jimenez J, Cid VJ, Cenamor R, Yuste M, Molero G, et al. 1998. Morphogenesis beyond cytokinetic arrest in *Saccharomyces cerevisiae*. *J. Cell. Biol.* 143:1617–34
63. Johnston LH, Eberly SL, Chapman JW, Araki H, Sugino A. 1990. The product of the *Saccharomyces cerevisiae* cell cycle gene DBF2 has homology with protein kinases and is periodically expressed in the cell cycle. *Mol. Cell. Biol.* 10:1358–66
64. Kaiser BK, Zimmerman ZA, Charbonneau H, Jackson PK. 2002. Disruption of centrosome structure, chromosome segregation, and cytokinesis by misexpression of human Cdc14A phosphatase. *Mol. Biol. Cell.* 13:2289–300
65. Kamieniecki RJ, Shanks RM, Dawson DS. 2000. Slk19p is necessary to prevent separation of sister chromatids in meiosis I. *Curr. Biol.* 10:1182–90
66. Khodjakov A, Rieder CL. 2001. Centrosomes enhance the fidelity of cytokinesis in vertebrates and are required for cell cycle progression. *J. Cell Biol.* 153:237–42
67. Kitada K, Johnson AL, Johnston LH, Sugino A. 1993. A multicopy suppressor gene of the *Saccharomyces cerevisiae* G1 cell cycle mutant gene dbf4 encodes a protein kinase and is identified as CDC5. *Mol. Cell. Biol.* 13:4445–57
68. Klapholz S, Esposito RE. 1980. Isolation of SPO12-1 and SPO13-1 from a natural variant of yeast that undergoes a single meiotic division. *Genetics* 96:567–88
69. Knapp D, Bhoite L, Stillman DJ, Nasmyth K. 1996. The transcription factor Swi5 regulates expression of the cyclin kinase inhibitor p40SIC1. *Mol. Cell Biol.* 16:5701–7
70. Komarnitsky SI, Chiang YC, Luca FC, Chen J, Toyn JH, et al. 1998. DBF2 protein kinase binds to and acts through the cell cycle-regulated MOB1 protein. *Mol. Cell Biol.* 18:2100–7
71. Krishnan R, Pangilinan F, Lee C, Spencer F. 2000. *Saccharomyces cerevisiae* BUB2 prevents mitotic exit in response to both spindle and kinetochore damage. *Genetics* 156:489–500
72. Lavoie BD, Tuffo KM, Oh S, Koshland D, Holm C. 2000. Mitotic chromosome condensation requires Brn1p, the yeast homologue of Barren. *Mol. Biol. Cell.* 11:1293–304
73. Lee J, Hwang HS, Kim J, Song K. 1999. Ibd1p, a possible spindle pole body associated protein, regulates nuclear division and bud separation in *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta.* 1449: 239–53
74. Lee KS, Grenfell TZ, Yarm FR, Erikson RL. 1998. Mutation of the polo-box disrupts localization and mitotic functions of the mammalian polo kinase Plk. *Proc. Natl. Acad. Sci. USA* 95:9301–6
75. Lee KS, Song S, Erikson RL. 1999. The polo-box-dependent induction of ectopic septal structures by a mammalian polo kinase, plk, in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 96:14360–65
76. Lee SE, Frenz LM, Wells NJ, Johnson AL, Johnston LH. 2001. Order of function of the budding-yeast mitotic exit-network

proteins Tem1, Cdc15, Mob1, Dbf2, and Cdc5. *Curr. Biol.* 11:784–88
77. Lee SE, Jensen S, Frenz LM, Johnson AL, Fesquet D, Johnston LH. 2001. The Bub2-dependent mitotic pathway in yeast acts every cell cycle and regulates cytokinesis. *J. Cell Sci.* 114:2345–54
78. Lew DJ, Burke DJ. 2003. The spindle assembly and spindle position checkpoints. *Annu. Rev. Genet.* 37:251–82
79. Li R. 1999. Bifurcation of the mitotic checkpoint pathway in budding yeast. *Proc. Natl. Acad. Sci. USA* 96:4989–94
80. Lippincott J, Shannon KB, Shou W, Deshaies RJ, Li R. 2001. The Tem1 small GTPase controls actomyosin and septin dynamics during cytokinesis. *J. Cell. Sci.* 114:1379–86
81. Luca FC, Mody M, Kurischko C, Roof DM, Giddings TH, Winey M. 2001. *Saccharomyces cerevisiae* Mob1p is required for cytokinesis and mitotic exit. *Mol. Cell Biol.* 21:6972–83
82. Luca FC, Winey M. 1998. MOB1, an essential yeast gene required for completion of mitosis and maintenance of ploidy. *Mol. Biol. Cell.* 9:29–46
83. Mah AS, Jang J, Deshaies RJ. 2001. Protein kinase Cdc15 activates the Dbf2-Mob1 kinase complex. *Proc. Natl. Acad. Sci. USA* 98:7325–30
84. Mailand N, Lukas C, Kaiser BK, Jackson PK, Bartek J, Lukas J. 2002. Deregulated human Cdc14A phosphatase disrupts centrosome separation and chromosome segregation. *Nat. Cell Biol.* 4:317–22
85. Marston AL, Lee BH, Amon A. 2003. The Cdc14 phosphatase and the FEAR network control meiotic spindle disassembly and chromosome segregation. *Dev. Cell* 4:711–26
86. McCollum D, Gould KL. 2001. Timing is everything: regulation of mitotic exit and cytokinesis by the MEN and SIN. *Trends Cell Biol.* 11:89–95
87. Mendenhall MD, Jones CA, Reed SI. 1987. Dual regulation of the yeast CDC28-p40 protein kinase complex: cell cycle, pheromone, and nutrient limitation effects. *Cell* 50:927–35
88. Menssen R, Neutzner A, Seufert W. 2001. Asymmetric spindle pole localization of yeast Cdc15 kinase links mitotic exit and cytokinesis. *Curr. Biol.* 11:345–50
89. Molk JN, Schuyler SC, Liu JY, Evans JG, Salmon ED, et al. 2004. The differential roles of budding yeast Tem1p, Cdc15p, and Bub2p protein dynamics in mitotic exit. *Mol. Biol. Cell* 15(4):1519–32
90. Moll T, Tebb G, Surana U, Robitsch H, Nasmyth K. 1991. The role of phosphorylation and the CDC28 protein kinase in cell cycle-regulated nuclear import of the *S. cerevisiae* transcription factor SWI5. *Cell* 66:743–58
91. Morgan DO. 1997. Cyclin-dependent kinases: engines, clocks, and microprocessors. *Annu. Rev. Cell Dev. Biol.* 13:261–91
92. Morishita T, Mitsuzawa H, Nakafuku M, Nakamura S, Hattori S, Anraku Y. 1995. Requirement of *Saccharomyces cerevisiae* Ras for completion of mitosis. *Science* 270:1213–15
93. Moutinho-Santos T, Sampaio P, Amorim I, Costa M, Sunkel CE. 1999. In vivo localisation of the mitotic POLO kinase shows a highly dynamic association with the mitotic apparatus during early embryogenesis in Drosophila. *Biol. Cell* 91:585–96
94. Murata-Hori M, Wang YL. 2002. The kinase activity of aurora B is required for kinetochore-microtubule interactions during mitosis. *Curr. Biol.* 12:894–99
95. Nasmyth K. 1996. Viewpoint: putting the cell cycle in order. *Science* 274:1643–45
96. Nasmyth K. 2001. Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu. Rev. Genet.* 35:673–745
97. Nasmyth K, Adolf G, Lydall D, Seddon A. 1990. The identification of a second cell cycle control on the HO promoter in yeast: cell cycle regulation of SW15 nuclear entry. *Cell* 62:631–47

98. Nishiyama Y, Hirota T, Morisaki T, Hara T, Marumoto T, et al. 1999. A human homolog of Drosophila warts tumor suppressor, h-warts, localized to mitotic apparatus and specifically phosphorylated during mitosis. *FEBS Lett.* 459:159–65
99. Nyberg KA, Michelson RJ, Putnam CW, Weinert TA. 2002. Toward maintaining the genome: DNA damage and replication checkpoints. *Annu. Rev. Genet.* 36:617–56
100. Ouspenski II, Cabello OA, Brinkley BR. 2000. Chromosome condensation factor Brn1p is required for chromatid separation in mitosis. *Mol. Biol. Cell* 11:1305–13
101. Pereira G, Hofken T, Grindlay J, Manson C, Schiebel E. 2000. The Bub2p spindle checkpoint links nuclear migration with mitotic exit. *Mol. Cell* 6:1–10
102. Pereira G, Manson C, Grindlay J, Schiebel E. 2002. Regulation of the Bfa1p-Bub2p complex at spindle pole bodies by the cell cycle phosphatase Cdc14p. *J. Cell Biol.* 157:367–79
103. Pereira G, Schiebel E. 2003. Separase regulates INCENP-Aurora B anaphase spindle function through Cdc14. *Science* 302(5653):2120–24
104. Pereira G, Tanaka TU, Nasmyth K, Schiebel E. 2001. Modes of spindle pole body inheritance and segregation of the Bfa1p-Bub2p checkpoint protein complex. *EMBO J.* 20:6359–70
105. Peters JM. 2002. The anaphase-promoting complex: proteolysis in mitosis and beyond. *Mol. Cell* 9:931–43
106. Piel M, Nordberg J, Euteneuer U, Bornens M. 2001. Centrosome-dependent exit of cytokinesis in animal cells. *Science* 291:1550–53
107. Ro HS, Song S, Lee KS. 2002. Bfa1 can regulate Tem1 function independently of Bub2 in the mitotic exit network of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 99:5436–41
108. Schwab M, Lutum AS, Seufert W. 1997. Yeast Hct1 is a regulator of Clb2 cyclin proteolysis. *Cell* 90:683–93
109. Schwob E, Bohm T, Mendenhall MD, Nasmyth K. 1994. The B-type cyclin kinase inhibitor p40SIC1 controls the G1 to S transition in *S. cerevisiae*. *Cell* 79:233–44
110. Seshan A, Amon A. 2004. Linked for life: temporal and spatial coordination of late mitotic events. *Curr. Opin. Cell Biol.* 16:41–48
111. Seshan A, Bardin AJ, Amon A. 2002. Control of Lte1 localization by cell polarity determinants and Cdc14. *Curr. Biol.* 12:2098–110
112. Shah R, Jensen S, Frenz LM, Johnson AL, Johnston LH. 2001. The Spo12 protein of *Saccharomyces cerevisiae*: a regulator of mitotic exit whose cell cycle-dependent degradation is mediated by the anaphase-promoting complex. *Genetics* 159:965–80
113. Sharon G, Simchen G. 1990. Mixed segregation of chromosomes during single-division meiosis of *Saccharomyces cerevisiae*. *Genetics* 125:475–85
114. Shirayama M, Matsui Y, Tanaka K, Toh-e A. 1994. Isolation of a CDC25 family gene, MSI2/LTE1, as a multicopy suppressor of ira1. *Yeast* 10:451–61
115. Shirayama M, Matsui Y, Toh EA. 1994. The yeast TEM1 gene, which encodes a GTP-binding protein, is involved in termination of M phase. *Mol. Cell Biol.* 14: 7476–82
116. Shirayama M, Matsui Y, Toh-e A. 1996. Dominant mutant alleles of yeast protein kinase gene CDC15 suppress the lte1 defect in termination of M phase and genetically interact with CDC14. *Mol. Gen. Genet.* 251:176–85
117. Shirayama M, Zachariae W, Ciosk R, Nasmyth K. 1998. 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.* 17:1336–49

118. Shou W, Azzam R, Chen SL, Huddleston MJ, Baskerville C, et al. 2002. Cdc5 influences phosphorylation of Net1 and disassembly of the RENT complex. *BMC Mol. Biol.* 3:3
119. Shou W, Deshaies RJ. 2002. Multiple telophase arrest bypassed (tab) mutants alleviate the essential requirement for Cdc15 in exit from mitosis in *S. cerevisiae*. *BMC Genet.* 3:4
120. Shou W, Seol JH, Shevchenko A, Baskerville C, Moazed D, et al. 1999. Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. *Cell* 97:233–44
121. Simanis V. 2003. Events at the end of mitosis in the budding and fission yeasts. *J. Cell Sci.* 116:4263–75
122. Siomos MF, Badrinath A, Pasierbek P, Livingstone D, White J, et al. 2001. Separase is required for chromosome segregation during meiosis I in *Caenorhabditis elegans*. *Curr. Biol.* 11:1825–35
123. Skowyra D, Craig KL, Tyers M, Elledge SJ, Harper JW. 1997. F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* 91:209–19
124. Song S, Grenfell TZ, Garfield S, Erikson RL, Lee KS. 2000. Essential function of the polo box of Cdc5 in subcellular localization and induction of cytokinetic structures. *Mol. Cell Biol.* 20:286–98
125. Song S, Lee KS. 2001. A novel function of *Saccharomyces cerevisiae* CDC5 in cytokinesis. *J. Cell Biol.* 152:451–69
126. Stegmeier F, Huang J, Rahal R, Zmolik J, Moazed D, Amon A. 2004. The replication fork block protein Fob1 functions as a negative regulator of the FEAR network. *Curr. Biol.* 14:467–80
127. Stegmeier F, Visintin R, Amon A. 2002. Separase, polo kinase, the kinetochore protein Slk19, and Spo12 function in a network that controls Cdc14 localization during early anaphase. *Cell* 108:207–20
128. Straight AF, Shou W, Dowd GJ, Turck CW, Deshaies RJ, et al. 1999. Net1, a Sir2-associated nucleolar protein required for rDNA silencing and nucleolar integrity. *Cell* 97:245–56
129. Sullivan M, Higuchi T, Katis V, Uhlmann F. 2004. Cdc14 phosphatase induces rDNA condensation to resolve cohesin-independent cohesion during budding yeast anaphase. *Cell* 117(4):471–82
130. Sullivan M, Lehane C, Uhlmann F. 2001. Orchestrating anaphase and mitotic exit: separase cleavage and localization of Slk19. *Nat. Cell Biol.* 3:771–77
131. Sullivan M, Uhlmann F. 2003. A non-proteolytic function of separase links the onset of anaphase to mitotic exit. *Nat. Cell Biol.* 5:249–54
132. Surana U, Amon A, Dowzer C, McGrew J, Byers B, Nasmyth K. 1993. Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. *EMBO J.* 12:1969–78
133. Tokiwa G, Tyers M, Volpe T, Futcher B. 1994. Inhibition of G1 cyclin activity by the Ras/cAMP pathway in yeast. *Nature* 371:342–45
134. Torres-Rosell J, Machin F, Jarmuz A, Aragon L. 2004. Nucleolar segregation lags behind the rest of the genome and requires Cdc14p activation by the FEAR network. *Cell. Cycle* 3:496–502
135. Toyn JH, Johnson AL, Donovan JD, Toone WM, Johnston LH. 1997. The Swi5 transcription factor of *Saccharomyces cerevisiae* has a role in exit from mitosis through induction of the cdk-inhibitor Sic1 in telophase. *Genetics* 145:85–96
136. Trautmann S, Wolfe BA, Jorgensen P, Tyers M, Gould KL, McCollum D. 2001. Fission yeast Clp1p phosphatase regulates G2/M transition and coordination of cytokinesis with cell cycle progression. *Curr. Biol.* 11:931–40
137. Traverso EE, Baskerville C, Liu Y, Shou W, James P, et al. 2001. Characterization

of the Net1 cell cycle-dependent regulator of the Cdc14 phosphatase from budding yeast. *J. Biol. Chem.* 276:21924–31

138. Verma R, Annan RS, Huddleston MJ, Carr SA, Reynard G, Deshaies RJ. 1997. Phosphorylation of Sic1p by G1 Cdk required for its degradation and entry into S phase. *Science* 278:455–60
139. Visintin R, Amon A. 2001. Regulation of the mitotic exit protein kinases Cdc15 and Dbf2. *Mol. Biol. Cell* 12:2961–74
140. Visintin R, Craig K, Hwang ES, Prinz S, Tyers M, Amon A. 1998. The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. *Mol. Cell* 2:709–18
141. Visintin R, Hwang ES, Amon A. 1999. Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus. *Nature* 398:818–23
142. Visintin R, Prinz S, Amon A. 1997. CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. *Science* 278:460–63
143. Visintin R, Stegmeier F, Amon A. 2003. The role of the polo kinase cdc5 in controlling cdc14 localization. *Mol. Biol. Cell* 14:4486–98
144. Wan J, Xu H, Grunstein M. 1992. CDC14 of *Saccharomyces cerevisiae*. Cloning, sequence analysis, and transcription during the cell cycle. *J. Biol. Chem.* 267:11274–80
145. Wang H, Liu D, Wang Y, Qin J, Elledge SJ. 2001. Pds1 phosphorylation in response to DNA damage is essential for its DNA damage checkpoint function. *Genes Dev.* 15:1361–72
146. Wang Y, Hu F, Elledge SJ. 2000. The Bfa1/Bub2 GAP complex comprises a universal checkpoint required to prevent mitotic exit. *Curr. Biol.* 10:1379–82
147. Wang Y, Shirogane T, Liu D, Harper JW, Elledge SJ. 2003. Exit from exit: resetting the cell cycle through Amn1 inhibition of G protein signaling. *Cell* 112:697–709
148. Wasch R, Cross FR. 2002. APC-dependent proteolysis of the mitotic cyclin Clb2 is essential for mitotic exit. *Nature* 418:556–62
149. Wheatley SP, Hinchcliffe EH, Glotzer M, Hyman AA, Sluder G, Wang Y. 1997. CDK1 inactivation regulates anaphase spindle dynamics and cytokinesis in vivo. *J. Cell Biol.* 138:385–93
150. Wolfe BA, Gould KL. 2004. Fission yeast Clp1p phosphatase affects G(2)/M transition and mitotic exit through Cdc25p inactivation. *EMBO J.* 23:919–29
151. Xu S, Huang HK, Kaiser P, Latterich M, Hunter T. 2000. Phosphorylation and spindle pole body localization of the Cdc15p mitotic regulatory protein kinase in budding yeast. *Curr. Biol.* 10:329–32
152. Yamamoto A, Guacci V, Koshland D. 1996. Pds1p is required for faithful execution of anaphase in the yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.* 133:85–97
153. Yeong FM, Lim HH, Padmashree CG, Surana U. 2000. Exit from mitosis in budding yeast: biphasic inactivation of the Cdc28-Clb2 mitotic kinase and the role of Cdc20. *Mol. Cell.* 5:501–11
154. Yoshida S, Asakawa K, Toh-e A. 2002. Mitotic exit network controls the localization of Cdc14 to the spindle pole body in *Saccharomyces cerevisiae*. *Curr. Biol.* 12:944–50
155. Yoshida S, Ichihashi R, Toh-e A. 2003. Ras recruits mitotic exit regulator Lte1 to the bud cortex in budding yeast. *J. Cell Biol.* 161:889–97
156. Yoshida S, Toh-e A. 2001. Regulation of the localization of Dbf2 and mob1 during cell division of *Saccharomyces cerevisiae*. *Genes Genet. Syst.* 76:141–47
157. Yoshida S, Toh-e A. 2002. Budding yeast Cdc5 phosphorylates Net1 and assists Cdc14 release from the nucleolus. *Biochem. Biophys. Res. Commun.* 294:687–91
158. Zachariae W, Schwab M, Nasmyth K,

Seufert W. 1998. Control of cyclin ubiquitination by CDK-regulated binding of Hct1 to the anaphase promoting complex. *Science* 282:1721–24

159. Zeng X, Saunders WS. 2000. The *Saccharomyces cerevisiae* centromere protein Slk19p is required for two successive divisions during meiosis. *Genetics* 155:577–87
160. Azzam R, Chen SL, Shou W, Mah AS, Alexandru G, et al. 2004. Phosphorylation by cyclin B-cdk underlies release of mitotic exit activator Cdc14 from the nucleolus. *Science.* In press

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