Mitotic phosphatases: no longer silent partners

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Recent work has highlighted the important role played by protein phosphatase complexes in the regulation of mitosis from yeast to mammals. There have been important advances in defining the roles of the protein serine/threonine phosphatases PP1 and PP2A and the dual specificity protein tyrosine phosphatases CDC25 and Cdc14. Three independent studies defined a regulatory role for PP2A in the control of sister chromatid cohesion, involving a direct interaction with shugoshin. A chromatin targeting subunit has been identified for PP1 and the complex shown to play an essential role in chromosome segregation. Key regulatory residues within CDC25 have been mapped and its activity tied both to the initial activation of cyclin-dependent kinases at the centrosome and to DNA damage checkpoints. Novel roles have been defined for Cdc14, including regulation of rDNA and telomere segregation and participation in spindle assembly. These exciting advances show that protein phosphatases are not merely silent partners to kinases in regulating the control of cell division.

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Introduction

The process of cell division is complex and involves multiple independent regulatory steps, most of which are controlled by reversible protein phosphorylation. In higher eukaryotes, mitosis involves condensation of chromosomes, disassembly of the nuclear lamina, breakdown of the nuclear envelope and disassembly of many forms of nuclear bodies, including nucleoli. Completion of mitosis requires alignment and proper segregation of chromosomes into daughter cells followed by reassembly of nuclei and cytokinesis. These and many other events, such as centrosome separation and spindle assembly, are tightly regulated, and several critical checkpoints occur during mitosis to ensure fidelity. Failure to complete any of the key steps can trigger apoptosis and cell death.

While the important role of protein phosphorylation in regulating mitotic events has long been recognized, much of the work in this area has focused on the kinases, primarily the Cdk/Cyclin, Aurora, Polo-like and NIMA families (see [1] for review). An often unspoken assumption is that the corresponding phosphatase activity needed to reverse these processes may largely be constitutive. However, this view of phosphatases as silent partners of kinases in regulation is changing, particularly in the light of recent studies showing critical roles for regulated phosphatases. For example, a recent large-scale RNA interference (RNAi) approach [2••] found that while only 11% of the 650 cellular kinases tested appear to be survival kinases (i.e. decreasing their levels led to increased cell death by apoptosis), a surprising 32% of the 222 phosphatases and phosphatase regulatory proteins screened were required for cell survival. Conversely, an additional 5% were termed 'death phosphatases' for their apparent roles as tumour suppressors. This provides an elegant demonstration that regulation of phosphatase activity is a key point of control throughout the cell and highlights their potential value as therapeutic targets.

This review will focus on recent advances in understanding the contributions of four major classes of protein phosphatases to the regulation of processes involved in controlling cell division, specifically the protein serine/threonine phosphatases PP1 and PP2A and the dual-specificity protein tyrosine phosphatases (DUSPs) CDC25 and Cdc14. We will draw on examples from species as diverse as yeast, insects and mammals, reflecting the high evolutionary conservation of these regulated events.

Serine/threonine phosphatases

Both PP1 (termed Glc7 in budding yeast and Dis2 in fission yeast) and PP2A are serine/threonine-specific protein phosphatase catalytic subunits that form holoenzyme complexes with one or more regulatory subunits. These regulatory subunits can affect cellular location and/or substrate specificity. In contrast with most kinases, the PP1 and PP2A catalytic subunits can potentially act on a wide range of substrates and thus substrate specificity is conferred by their interaction partners. Thus, a critical step in understanding the role of PP1 and PP2A holoenzymes is to define their regulatory subunits and the mechanism by which they are targeted to their physiological substrates. Much of the literature ascribing specific roles to PP1 or PP2A has relied on differential effects of inhibitors such as okadaic acid, which *in vitro* blocks PP2A activity at lower concentrations than are required to inhibit PP1 [3]. While these inhibitor studies are

informative, it is difficult to draw clear conclusions about the *in vivo* roles of PP1 and PP2A from them, mainly because the global suppression of phosphatase activity affects many cellular processes and potentially results in indirect effects. Dissecting the *in vivo* roles of the respective PP1 and PP2A complexes therefore requires detailed molecular analyses of their composition and targeted disruption of specific phosphatase holoenzymes.

PP2A

PP2A plays a prominent role in the regulation of mitosis and signalling pathways. In addition to its interaction with both scaffolding and variable subunits (termed 'A' and 'B' subunits, respectively) in a trimeric complex (see Figure 1), the activity of PP2A can also be controlled by phosphorylation and methylation (see [4] for review). Recent studies have provided compelling evidence that PP2A plays a critical role in the regulation of sister chromatid cohesion. The association of sister chromatids during mitosis depends on a multi-subunit complex called cohesin [5]. During mitosis in metazoa, cohesin is released from sister chromatids in two stages. First, in prophase and prometaphase, it is released from chromosome arms by a process mediated by phosphorylation of the cohesin subunit SA2. At this stage, centromeric cohesin is retained, and therefore SA2 must be protected from phosphorylation. This protection is provided by the Shugoshin (Sgo) protein family, and hSgo1 has been shown to act by preventing phosphorylation of SA2 [6••].

Using immunoprecipitation and yeast two-hybrid studies, several groups independently identified a specific PP2A trimeric complex that interacts with Sgo1 [7•,8••,9•]. On the basis of RNAi studies and analysis of a non-PP2A-binding hSgo1 mutant, Tang and colleagues [7•] proposed that interaction with PP2A is required for centromeric localization of hSgo1 and proper chromosome segregation. Independently, the same PP2A complex was immunopurified from HEK 293T cells using Flag-tagged hSgo1 [8••]. Immunofluorescence studies by Kitajima and colleagues showed colocalization of hSgo1 and the B56 PP2A regulatory subunit at mammalian centromeres. Using RNAi in mammals, they also reported that knockdown of hSgo2, but not of hSgo1, resulted in loss of centromeric PP2A. Conversely, knockdown of PP2A led to a loss of centromeric hSgo1 [8••]. Studies on both budding and fission yeast undergoing meiosis also showed that Sgo1 interacts with PP2A at centromeres and serves to protect the cohesin Rec8 subunit from phosphorylation and cleavage [9•].

Interestingly, tethering of yeast PP2A at specific sites on chromosome arms preserved cohesion at these sites even after meiosis I, when arm cohesin should dissociate, showing an intrinsic ability of PP2A to protect cohesin, independent of Sgo1 [8••,9•]. The PP2A complex may thus work both directly at centromeres to maintain cohesion and by facilitating accumulation of Sgo1, which also acts to prevent cleavage of cohesin. Taken together, these studies point to an important new role for PP2A in the control of chromosome cohesion, mediated, at least in part, through interactions with shugoshins (Figure 1).

PP2A has also been implicated in regulating mitotic exit. Wang and Ng [10] provided evidence suggesting that a PP2A–Cdc55 complex dephosphorylates the mitotic exit network (MEN) activator Tem1 in budding yeast. This prevents mitotic exit by blocking release of Cdc14 from

Figure 1
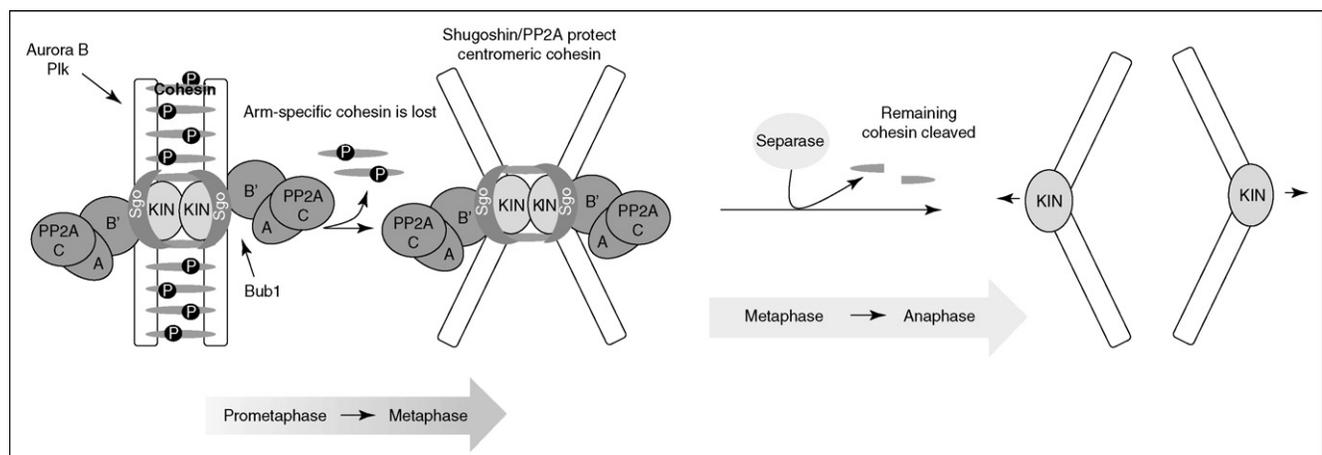
Role of PP2A in maintenance of chromosome cohesion. This diagram summarizes three recent studies that identified a specific PP2A trimeric complex acting with shugoshin to protect cohesin at centromeres from phosphorylation and cleavage until the metaphase–anaphase transition. In metazoan mitosis, cohesin is removed from chromosome arms at prometaphase but remains at the centromere regions, protected by shugoshin and PP2A. At the metaphase–anaphase transition, separase is activated and cleaves this residual cohesin, resulting in a loss of cohesion and separation of sister chromatids.

the nucleolus. An alternative mechanism to explain the block in mitotic exit was proposed by Yellman and Burke [11], who argue that the role of PP2A–Cdc55 occurs either downstream or independently of Tem1. The protein Net1 functions to sequester Cdc14 in the nucleolus until anaphase onset, at which point it is phosphorylated, releasing Cdc14 into the nucleus where it triggers mitotic exit. Yellman and Burke propose that PP2A–Cdc55 promotes retention of Cdc14 in the nucleolus by reversing Net1 phosphorylation. This view is supported by work from Queralt and colleagues, who report that the down-regulation of PP2A–Cdc55 at anaphase onset is mediated by its direct interaction with separase, resulting in Net1 phosphorylation and subsequent release of Cdc14 from the nucleolus [12•].

Although there is no known mammalian homologue for Net1, interestingly, PP2A has also been implicated in the control of mitotic exit in mammalian cells. A recent study used a yeast two-hybrid screen to show that the B55δ regulatory subunit of mammalian PP2A interacts with hSecurin. The PP2A–B55δ complex, directly or indirectly, appears to stabilize the levels of hypophosphorylated hSecurin and hence prevents premature mitotic exit [13].

PP1

PP1 has been shown to contribute to the regulation of multiple cellular processes including glycogen metabolism and muscle contraction, mediated by interaction of the PP1 catalytic domain with regulatory proteins termed 'targeting subunits'. Over 50 have been described to date, and they have the potential to regulate both the localization and the catalytic activity of PP1 (see [14] for review). Most targeting subunits share a common 'RVXF' motif that mediates direct binding to PP1 (Figure 2b). While substrate binding is normally dependent on targeting subunits, in some cases PP1 has been shown to bind directly to substrates that share this RVXF motif, e.g. the pRb protein [15] and *Xenopus* CDC25 [16].

Roles for PP1 in mitosis have been uncovered by genetic evidence in yeast, other fungi and *Drosophila*, and by various methods in higher species (see [17] for review).

PP1 is expressed in mammalian cells as three closely related isoforms, α, β/δ and γ, which despite their extensive homology (Figure 2a) show largely distinct localization patterns throughout the cell cycle [18••,19–21], suggesting isoform-specific roles in cellular regulation.

Figure 2
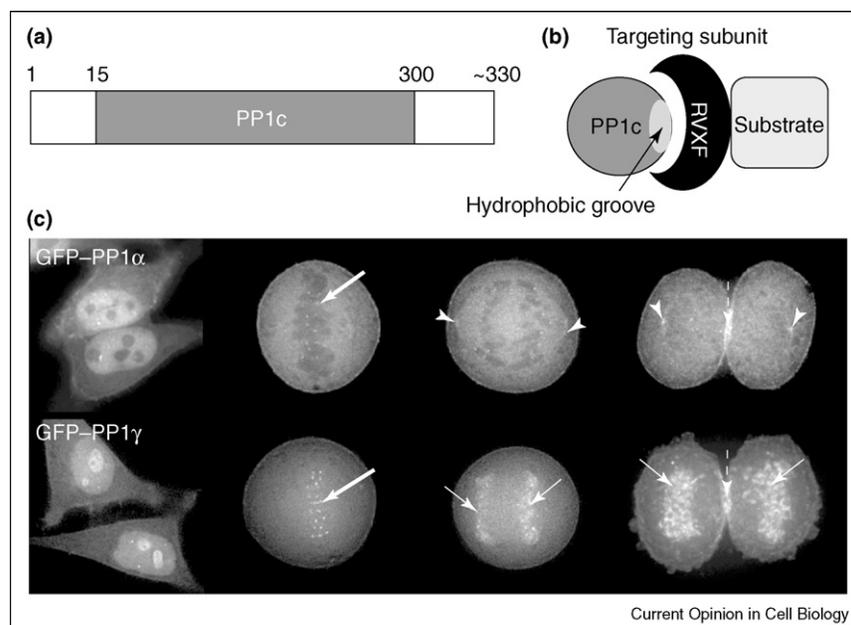
Targeting of mammalian PP1. **(a)** The three mammalian isoforms of PP1 are highly homologous, differing only at their extreme N- and C-termini. **(b)** The localization and activity of PP1 is regulated primarily through its association with a large group of diverse targeting subunits. Most of these contain a conserved RVXF binding motif that interacts with a hydrophobic groove on the catalytic subunit of PP1. **(c)** Differences in affinity for these targeting subunits may account for the distinct and dynamic localization patterns observed for different PP1 isoforms throughout the cell cycle. During interphase, both GFP–PP1α and GFP–PP1γ are nuclear and cytoplasmic, with an additional nucleolar accumulation of GFP–PP1γ. Upon entry into mitosis, both are found at kinetochores in metaphase (arrows). PP1α is also found at centrosomes at all cell cycle stages, as seen most clearly here during anaphase and telophase (arrowheads). During the metaphase-to-anaphase transition, there is a dramatic recruitment of PP1γ to chromosomes (small arrows), where the protein remains throughout telophase and into the following interphase. At telophase both PP1α and PP1γ also show accumulations at the cortex and midbody (dashed arrows).

Using stable isotope labelling and quantitative proteomics, it was shown that the differing localization patterns for GFP–PP1α and GFP–PP1γ (Figure 2c) reflect their association with different pools of targeting subunits [18••]. This study also identified Repo-Man (recruits PP1 onto mitotic chromatin at anaphase) as an essential protein that acts as a novel targeting subunit responsible for the isoform-specific recruitment of PP1 to chromatin at the metaphase–anaphase transition. The Repo-Man–PP1 complex was recently shown to play a critical role in the maintenance of chromosome architecture during mitosis [22]. PP1 is also proposed to regulate chromatin decondensation at the end of mitosis, mediated by the targeting subunit p99/PNUTS [23].

An elegant series of experiments has described a role for PP1 in controlling nuclear envelope assembly at the end of mitosis [24–26]. When cells enter mitosis, nuclear lamina disassembly is promoted by phosphorylation of B-type lamins. AKAP149, an ER and nuclear membrane protein, was shown to target PP1 (via an RVXF motif) to dephosphorylate B-type lamins at telophase, enabling their polymerization and thus lamina reassembly. A short peptide from AKAP149 containing the RVXF motif can displace PP1 and induce mislocalization of B-type lamins to the cytoplasm. Although the cells were able to complete mitosis, they died by apoptosis within six hours, suggesting that disruption of lamin assembly may directly trigger apoptosis.

The association of PP1 isoforms with centrosomes, kinetochores and the cellular cortex and midbody region (see Figure 2c), along with results from inhibitor and RNAi studies, suggest roles for as-yet-undiscovered targeting subunits and regulatory pathways. Bud14, for example, was recently identified as a Glc7 (PP1) targeting subunit in *Saccharomyces cerevisiae* that directs the phosphatase to the bud cortex to stabilize microtubule interactions [27]. A similar role in mammalian cells is suggested by the presence of both PP1α and PP1γ at the cortex at telophase [18••]. GFP–Glc7 has also been shown to accumulate in the nucleolus during interphase (like mammalian PP1γ) and at the spindle pole bodies (centrosomes) during anaphase (like mammalian PP1α) [28]. It remains to be established which substrates are being dephosphorylated at these sites.

Pinsky *et al.* [29•] took advantage of the regulation of Glc7 by targeting subunits to explore its interaction with Ipl1 (Aurora B) in budding yeast. Glc7 is known to antagonize Ipl1 activity, but it was unclear whether it dephosphorylates its substrates or regulates the kinase directly. Although the targeting subunit has not been identified, titratation of Glc7 away from Ipl1 by overexpression of Glc7 binding proteins that do not play roles in chromosome segregation led to increased phosphorylation of Ipl1 substrates, leading the authors to propose that Glc7 acts to ensure accurate chromosome segregation by dephosphorylating Ipl1 targets.
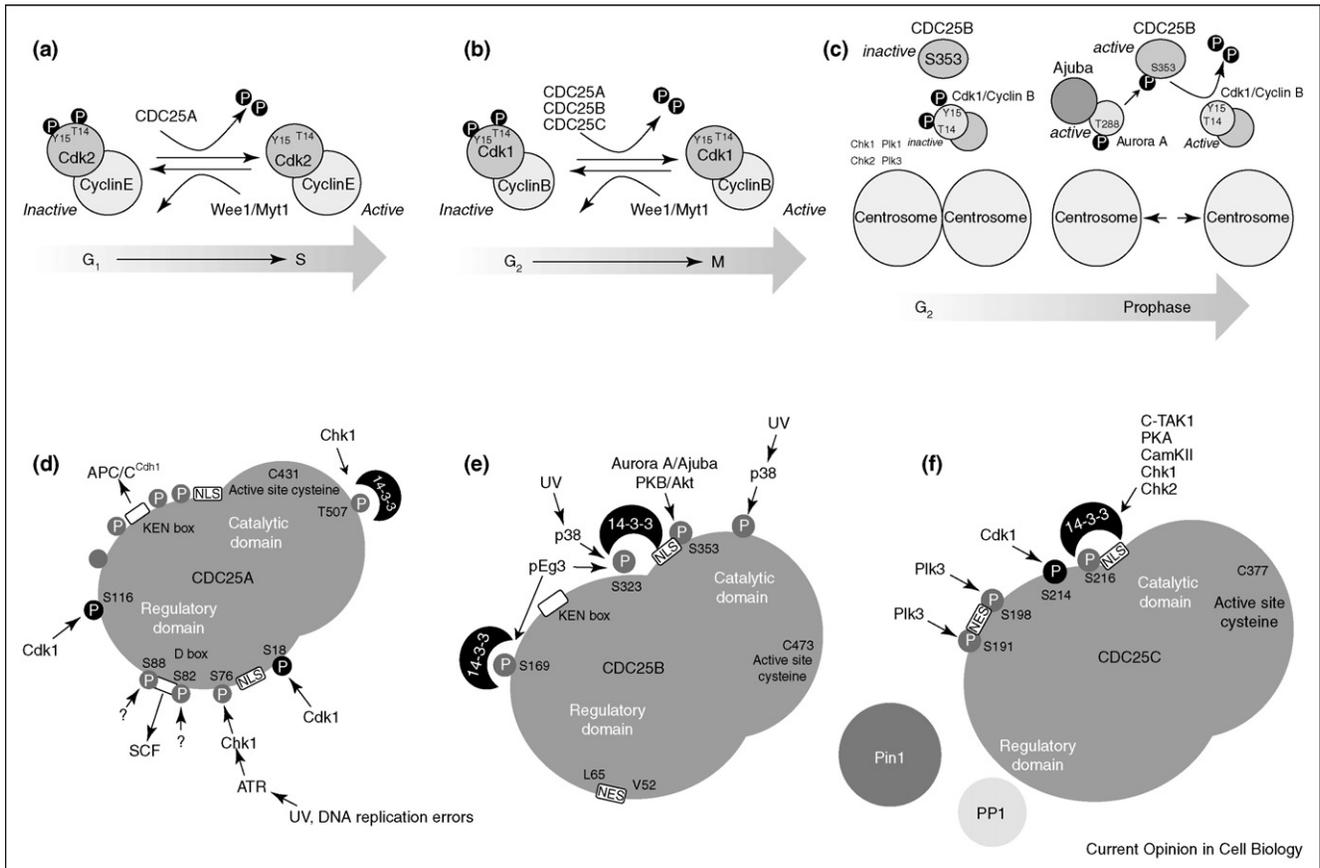
CDC25

CDC25 was first identified in fission yeast 20 years ago as a factor required for entry into mitosis [30]. It is now known to activate cyclin-dependent kinases (Cdks) by removing inhibitory phosphates, which leads to Cdk phosphorylation of multiple substrates that drive the cell division process forward (see Figure 3a,b). Regulation of CDC25 includes control of its protein levels (by a balance of synthesis and degradation), control of its localization within the cell relative to its substrates and modulation of both its catalytic activity and its substrate binding affinity. All of these mechanisms work together to tightly control the activity of this 'mitotic trigger' and to ensure that cell division occurs with proper timing.

Three mammalian genes were identified that complement the yeast cdc25 knockout strain. The proteins encoded by these genes, termed CDC25A, CDC25B and CDC25C, are ∼60% identical in their C-terminal regions, which include the catalytic core containing the CX_5R motif common to all protein tyrosine phosphatases. In contrast to the reasonably high homology of their catalytic domains, they are only 20–25% identical in their N-terminal regulatory domains, which contain sites for various post-translational modifications and site-specific protein interactions, including phosphorylation of key serine and threonine residues, ubiquitination, phosphorylation-dependent binding of 14-3-3 proteins and Pin1-dependent prolyl isomerization (see Figure 3d–f). These modifications are involved both in normal cell cycle control and in the response to checkpoint signals.

There is a dramatic hyperphosphorylation of CDC25 during the transition from interphase to mitosis, and several mitotic phosphorylation sites have been mapped (see Figure 3d–f). A key site for regulation is the dual Serine-Proline-Serine phosphorylation motif (shown in Figure 3f for CDC25C), containing overlapping substrate recognition sites for Cdk1 (S214; activating) and stress family kinases (S216; inhibitory). Cdk1/cyclinB phosphorylates and activates CDC25, and also initiates a feedback activation loop to ensure the rapid initiation of mitosis. This feedback loop has recently been shown to involve recruitment of PP1 activity to CDC25 [31•], triggered by phosphorylation of the activating serine residue by Cdk1. Phosphorylation of CDC25 also controls its localization relative to its substrates. All three isoforms contain nuclear import and export signals, which can be disrupted by phosphorylation of residues within them by kinases such as Plk1 and Plk3 [32,33]. Plk1 was also shown recently to play a role, along with CDC25B, in restarting the cell cycle upon release of the DNA damage checkpoint [34•].

Figure 3

Regulation of mammalian CDC25. **(a)** The G_1/S transition is regulated by the opposing catalytic activities of the Wee1/Myt1 kinases (phosphorylating Cdk proteins at threonine 14 and tyrosine 15) and CDC25 acting on Cdk2/CyclinE. CDC25A has been shown to control this checkpoint, although roles for CDC25B and CDC25C have not yet been ruled out. **(b)** The G_2/M transition is regulated in a similar way, with CDC25 activating Cdk1/Cyclin B by dephosphorylating critical residues. All three mammalian CDC25 isoforms have been implicated in regulation of this pathway. **(c)** The initial activation of Cdk1/Cyclin B has been shown to occur at centrosomes as they begin to separate during prophase, and involves the phosphorylation and activation of CDC25B by the Ajuba–Aurora A complex. The divergent N-terminal regulatory domains of the three mammalian CDC25 isoforms contain a variety of regulatory sites, including phosphorylation sites, 14-3-3 binding sites, domains that regulate degradation and nuclear import and export signals. Several of these known and recently described regulatory sites have been summarized here for **(d)** CDC25A, **(e)** CDC25B and **(f)** CDC25C.

Regulation of CDC25 by targeted degradation occurs both during mitosis (mediated by the anaphase-promoting complex/cyclosome, or APC/C) and in response to DNA damage/stalled replication (mediated by the Skp1/Cul1/F-box or SCF complex). The latter is under the control of several kinases, including the ataxia-telangiectasia mutated (ATM) and ATM-related (ATR) pathway transducer kinases Chk1 and Chk2 (see Figure 3d). In addition, phosphorylation of CDC25A on T507 by Chk1 creates a 14-3-3 binding site that sterically hinders the phosphatase's interaction with its Cdk/cyclin targets [35,36], while phosphorylation of CDC25B and CDC25C on other residues by Chk1 or Chk2 and binding of 14-3-3 disrupts nuclear localization signals. This leads to sequestration of the phosphatase in the cytosol, away from its nuclear substrates. More recently, p38 SAPK and its

downstream substrate MAPKAP kinase-2 have also been implicated as DNA damage checkpoint kinases [37] (Figure 3e).

While all three mammalian CDC25 phosphatases activate their Cdk substrates in the same manner, they appear to have distinct roles in regulating cell cycle transitions (see [38] for review). Results from RNAi studies implicate CDC25B and CDC25C in the regulation of the G_2/M transition, whereas CDC25A appears to play a more general role, being involved both in early (G_1/S) and late (G_2/M) cell cycle transitions (see Figure 3a,b). There is still some debate over possible redundancy of the three isoforms, and the absolute requirement of all three for regulation of mitotic entry, particularly in light of results in CDC25 knockout mice, in which a lack of both

CDC25B and CDC25C did not hinder normal development, cell cycle progression or response to DNA damage [39$^\bullet$].

The observation that the activity of CDC25B at the G_2/M transition peaks before that of CDC25C led to the suggestion that CDC25B initiates the Cdk1/cyclin B activation feedback loop [40]. CDC25B is the likeliest candidate for this initiation role. A pool of CDC25B, which is phosphorylated and activated by Aurora A kinase, accumulates at centrosomes [41], where initial activation of Cdk1/cyclin B in early prophase has been shown to take place [42] (see Figure 3c). Using a phosphospecific antibody as a marker for active Cdk1/cyclin B, Lindqvist and colleagues [43$^{\bullet\bullet}$] showed that CDC25B specifically activates this substrate at centrosomes. In addition to positive regulation by Aurora A, pEg3 kinase was found to negatively regulate centrosomal CDC25B on a different residue [44]. The DNA damage response kinase Chk1 has also been implicated in the regulation of both centrosomal CDC25 and Cdk1/cyclin B, both under normal conditions and as part of the checkpoint response [45$^\bullet$].

Cdc14
While Cdc25 is a key regulator of initiation of mitosis (and hence DNA damage checkpoint control), Cdc14 is a key regulator of late mitotic events, coordinating the temporal and spatial control of chromosome segregation with mitotic spindle disassembly and cytokinesis.

In the budding yeast *S. cerevisiae*, Cdc14p plays a key role in exit from mitosis by dephosphorylating Cdk targets (reviewed in [46]). Cdc14p is primarily regulated by localization, being sequestered during interphase in the nucleolus by Cfi/Net1 and then released in two stages, coordinated by the FEAR (Cdc fourteen early anaphase release) network and the MEN (mitotic exit network). Upon its initial release by FEAR, Cdc14p is found in the nucleus and at the spindle pole body and spindle (Figure 4). After activation of the pathway in telophase (which promotes further Cdc14p release from nucleoli) it is also found in the cytoplasm [46].

FEAR-controlled release of Cdc14p in budding yeast is also important for division of nucleoli and resolution of highly repetitive rDNA and telomere regions, as demonstrated in two recent studies. These regions separate at mid-anaphase, long after cohesin is cleaved. D'Amours and colleagues [47$^{\bullet\bullet}$] showed that Cdc14p promotes condensin recruitment at the rDNA locus and triggers rDNA segregation, while Sullivan *et al.* [48$^{\bullet\bullet}$] demonstrated that Cdc14p induces condensin- and Aurora B-dependent resolution of rDNA linkages. The final stage of resolution also relies on Cdc14p-mediated axial compaction of rDNA, to reduce its linear length [49].

Figure 4

	S. cerevisiae	S. pombe	C. elegans	H. sapiens
Name	Cdc14p	Clp1/Flp1	CeCdc-14	hCdc14A hCdc14B
Localization *Interphase*	Nucleolus	Nucleolus	Diffuse	A: Centrosomes B: Nucleolus
Mitotic function(s)	Mitotic exit Central spindle formation Spindle stability rDNA segregation	Chromosome segregation Kinetochore biorientation Cytokinesis checkpoint	Central spindle formation G$_1$ quiescence	**A** Centrosome cycle Chromosome segregation Cytokinesis **B** Microtubule bundling and stabilizing

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Cross-species comparison of Cdc14 localization and function. Cdc14 homologues from four different eukaryotes are listed, showing their localization during interphase and throughout mitosis. Nuclei are shown in green, spindle pole bodies (centrosomes) in red, microtubules in pink and chromosomes in blue. The localization of Cdc14 at these sites is shown in yellow. Known mitotic functions for these homologues are also listed.

Interestingly, the essential role of Cdc14 in mitotic exit control is not conserved in other species, although a common process that it does appear to control in all investigated organisms is cytokinesis (see [50] for review). A comparison of localization patterns for Cdc14 homologues in different species supports this essential role (see Figure 4).

In the fission yeast *Schizosaccharomyces pombe*, Cdc14 (termed Clp1/Flp1) functions primarily to regulate the G_2/M transition and cytokinesis. It achieves the former by antagonizing Cdk activity and inactivating Cdc25p [51] and the latter via the MEN counterpart SIN (septation initiation network). Clp1/Flp1 is released from the nucleolus earlier in mitosis than *S. cerevisiae* Cdc14p, localizing to kinetochores in prometaphase and functioning together with Aurora kinase to regulate chromosome biorientation [52]. In the nematode *Caenorhabditis elegans*, CeCdc14 is also required for cytokinesis but not mitotic exit, and plays a unique role in G_1 in preventing cell cycle re-entry during prolonged periods of developmentally regulated quiescence [53$^{\bullet\bullet}$]. During mitosis, CeCdc14 appears at the central spindle and midbody during anaphase and telophase, respectively, where it is involved in spindle formation and cytokinesis [54].

Mammalian cells express two homologs of Cdc14, termed hCdc14A and hCdc14B, which are both functional homologs of yeast Cdc14 [55]. Although these two proteins are still poorly understood, recent evidence points to isoform-specific roles in centrosome separation/maturation and spindle stability, with the possibility of additional roles in mitotic exit and cytokinesis. Until recently, most studies focused on hCdc14A, which was shown to interact with interphase centrosomes and to regulate the centrosome duplication cycle [56,57]. Recently, hCdc14B, which is nucleolar at interphase and then found at the central spindle and midbody during mitosis, has been shown to play a role in spindle stability, through direct binding and bundling of microtubules [58$^{\bullet}$].

Conclusions

Over the past few years the importance of regulated protein phosphatases in the control of cell division, proliferation and survival has become apparent. Their therapeutic potential is also now appreciated. A common theme is a mechanism of regulation whereby the localization of phosphatases determines their access to substrates. In the case of PP1 and PP2A phosphatases this is mediated by targeting subunits (e.g. Repo-Man targeting of PP1 to chromosomes), while in other cases phosphatases can be denied access to their substrates by sequestration and then subsequently activated by release (e.g. Cdc14p sequestered in nucleolus by Net1 and released during mitosis). A particularly interesting example of phosphatase regulation is provided by the shugoshin–PP2A interaction at centromeres, which is important

for the regulation of chromosome cohesion (see Figure 1). It is likely that control of phosphatase activity will also be shown in future to regulate other important biological processes.

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authors propose the existence of a second factor that regulates chromosome architecture early in mitosis, but is then inactivated at anaphase onset. This factor, which they term RCA (regulator of chromosome architecture), appears to be regulated, at least in part, by phosphorylation. When endogenous Repo-Man was prevented from targeting PP1 to chromosomes, either by maintaining a high level of CDK activity, or by expressing a dominant-negative mutant of Repo-Man that is recruited to chromatin but cannot bind PP1, dephosphorylation and inactivation of RCA was prevented, leading to preservation of compact chromosome structure and rescue of anaphase chromosome segregation in the condensin-depleted cells.

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Using Cdc14 and FEAR mutants in budding yeast, Cdc14p released by the FEAR network in early anaphase is shown to be required for efficient segregation of repetitive regions of chromosomes such as rDNA and telomeres and involves recruitment of condensin to these regions. This is the first study to suggest a cohesin-independent mechanism whereby the FEAR network can couple initiation of mitotic exit to completion of chromosome segregation.