Multiple levels of cyclin specificity in cell-cycle control

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Cyclins regulate the cell cycle by binding to and activating cyclin-dependent kinases (Cdks). Phosphorylation of specific targets by cyclin–Cdk complexes sets in motion different processes that drive the cell cycle in a timely manner. In budding yeast, a single Cdk is activated by multiple cyclins. The ability of these cyclins to target specific proteins and to initiate different cell-cycle events might, in some cases, reflect the timing of the expression of the cyclins; in others, it might reflect intrinsic properties of the cyclins that render them better suited to target particular proteins.

Spindle pole body (SPB). The yeast equivalent of the centrosome, which nucleates microtubules, including those that will form the spindle.

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Cyclin-dependent kinases (Cdks) control progression through the eukaryotic cell cycle. Cdks are serine and threonine kinases, and their actions are dependent on associations with their activating subunits, cyclins. Cyclin abundance is regulated by protein synthesis and degradation; the activity of Cdks is therefore regulated to a large degree by the presence of different cyclins. In the budding yeast *Saccharomyces cerevisiae*, a single Cdk, Cdc28 (which is equivalent to Cdk1 in other organisms), associates with multiple cyclins to regulate the cell cycle. By contrast, animal cells possess multiple cell-cycle-regulatory Cdks, which are each regulated by multiple cyclins. The rationale for possessing multiple cyclins is not fully understood, although the evolution of multiple cyclins has been partially characterized (BOX 1).

Multiple cyclins are probably advantageous because they allow for flexible control of the cell cycle. Different cyclins are independently regulated transcriptionally and post-transcriptionally, providing regulatory flexibility at the level of input. Also, cyclins possess overlapping, but distinct, functional activities, allowing further refinement of control and probably the timely and irreversible occurrence of cell-cycle transitions. In this review, we focus on the budding yeast cyclins that activate Cdc28 as a model for cyclin specificity. In some cases, this information will probably be directly transferable to other eukaryotic systems thanks to the close conservation of the molecules involved; in other cases, the yeast system might provide general principles even when the details differ.

Cyclin function during the cell cycle

Cyclin specificity can be deduced from a genetic requirement for a specific subset of cyclins for a cell-cycle event to occur (FIG. 1). For example, advancement through G1 phase of the cell cycle, which involves bud emergence, spindle pole body (SPB) duplication and the activation of subsequently expressed cyclins, requires at least one of the G1-phase cyclins — Cln1, Cln2 or Cln3. In the absence of *CLN1–3*, G1 arrest occurs. Following Cln function, efficient initiation of DNA replication and progression through S phase requires the early-expressed B-type cyclins Clb5 and Clb6. In their absence, the B-type cyclins Clb1–4 will drive a late initiation of DNA replication. The B-type cyclins Clb1–4 are required for mitotic events, such as spindle morphogenesis; these cyclins also prevent mitotic exit and cytokinesis, and therefore, mitotic cyclin activity must be downregulated for cell division to be completed. As noted above, a simplification in budding yeast is that all of these cyclins bind to and activate the same Cdk subunit, Cdc28. In instances when this was tested, all of the cyclin-dependent cell-cycle steps required Cdc28 activity as well.

All of the G1 and B-type cyclins can bind to and activate Cdc28. The levels of all the cyclins have been quantified on the scale of molecules per cell[1], and they were found to range from a few hundred to several thousand molecules per cell. These numbers do not take into account possible variations in the specific activity of different cyclin–Cdc28 complexes; indeed, Clb5 has been shown to have a lower ability to activate Cdc28 than Clb2, and this difference has been proposed to contribute to the ordering of cell-cycle events[2].

Mechanisms for cyclin specificity

Cyclin specificity can be achieved in various ways: cyclins are expressed or are present at stable levels at different times; they are differentially sensitive to cell-cycle-regulated inhibitors; they are differentially

Box 1 | **Evolutionary perspective on cyclin diversity**

Analysis of yeast B-type cyclin phylogeny has revealed that they have undergone several rounds of duplication[101]. The deduced ancestor common to *Saccharomyces cerevisiae* and *Candida albicans* probably had only two B-type cyclin genes, *CLB1256* and *CLB34*. *CLB1256* split into *CLB12* and *CLB56* in the ancestors of *S. cerevisiae* and *Ashbya gossypii*; a subsequent genome duplication in a recent ancestor of *S. cerevisiae* yielded the six B-type cyclin genes *CLB1–6*.

Examination of the hydrophobic patch in B-type cyclins shows that the Clb5 hydrophobic patch is ancestral to *CLB1256*; a variant Clb12-specific hydrophobic patch is detected with the split of *CLB1256* into *CLB12* and *CLB56* (REF. 101). Therefore, Clb1, Clb2 and Clb12 have a unique hydrophobic patch, which might correlate with the hydrophobic-patch-dependent cytoplasmic and bud-neck localization of a portion of Clb2 (REF. 50). Whereas the hydrophobic patch of Clb5 is associated with substrate binding, the variant hydrophobic patch of Clb2, although functionally important[12,50], is not associated with efficient substrate recognition *in vitro*[2].

This phylogeny could be consistent with an ancestral eukaryote with a single B-type cyclin driving S phase and M phase[126]. The independent derivation of multiple cyclins by gene duplication in most eukaryotic lineages indicates that cyclin specificity is likely to have important advantages for regulatory flexibility over the hypothetical single-cyclin ancestor. Remarkably, however, a single-cyclin solution is experimentally accessible even in modern organisms[127].

The phylogeny of G1 cyclins has not been well studied; yeast and animal G1 cyclins might not be phylogenetically related, but they still carry out similar functions, such as the activation of G1–S transcription by the phosphorylation of the transcriptional inhibitors Whi5 and Rb and the reinforcement of B-type cyclin function and accumulation by the phosphorylation and subsequent degradation of Sic1 and p27. Similarly, Whi5 and Rb are not thought to be homologous, and Sic1 and p27 also lack significant sequence homology. If verified through more complete bioinformatics, G1 control in yeast and animals might therefore represent a remarkable case of convergent evolution.

restricted to specific subcellular locations; or they bind specifically to only some phosphorylation targets. In some cases, intrinsic cyclin specificity has been traced to specific modular sequences in the cyclin protein (for example, nuclear localization sequences (NLS), destruction boxes that regulate proteolysis or hydrophobic patches that can mediate interactions with substrates). However, the transfer of cyclin specificity by the interchange of modules has been, at best, incompletely demonstrated.

Cyclin-specific targeting of Cdk activity is unlikely to be essential. In budding yeast, ectopically expressed Clb1 can rescue a strain in which all other B-type cyclins have been deleted[3]. Although cells that carry only the B-type cyclins Clb1 and Clb2 (*clb3–6*Δ cells) are not viable, early expression of Clb2 from the *CLB5* promoter coupled with the deletion of the gene that encodes Swe1, an inhibitor of early-expressed Clb2–Cdc28 (see below), can rescue the viability of *clb3–6*Δ cells[4]. Also, the overexpression of *CLB5* or the deletion of the gene that encodes Sic1, an inhibitor of B-type cyclin activity, rescues cells that lack all three G1 cyclins[5,6]. Similarly, in fission yeast, deletion of the cyclins *cig1*, *cig2* and *puc1* allows DNA replication and mitosis to be driven by a single cyclin, Cdc13 (REF. 7). These data indicate that there is significant overlap in the ability of cyclins to target different substrates. A mutant Cdc28 was identified with partial cyclin-independent kinase activity, and it can bypass the genetic requirement for G1 cyclins, but not the requirement for B-type cyclins. This indicates that there is no strict requirement for cyclin targeting of any kind for a number of G1 cyclin functions[8].

By contrast, other experiments in which cyclins are ectopically expressed so that they can replace others in cell-cycle phases in which they are not normally present indicate that cyclins do possess intrinsic substrate specificity. When the mitotic cyclin Clb2 is substituted for the S-phase cyclin Clb5 by placing *CLB2* under the control of the *CLB5* promoter, the initiation of DNA replication is less effective[9]. Moreover, Clb5 is unable to block exit from mitosis (a normal function of Clb2), even when it is highly overexpressed[10]. Additionally, proteins that associate specifically with Cln2, Clb2, Clb3 or Clb5 have been identified by affinity purification and mass-spectrometric analysis[11]. This approach yielded proteins that function upstream, as well as downstream, of different cyclin–Cdk complexes, which indicates that both the regulation of and the targeting by cyclin–Cdk complexes is specialized. More recently, the identification of a subset of Cdc28 substrates that are better phosphorylated by Clb5–Cdc28 than by Clb2–Cdc28 *in vitro*[2] and the dependence of a substrate-targeting domain of Clb5, the hydrophobic patch, for substrate phosphorylation[2,12] indicate that cyclins contain elements that are necessary to target Cdc28 to particular substrates.

Below, we discuss mechanisms that lead to cyclin specificity in several aspects of cell-cycle control. It is conceptually convenient to divide cell-cycle regulation into three domains in which the issue of cyclin specificity has been examined: the engine, signalling pathways and downstream events. The cell-cycle engine is the set of reactions that provide a rise and fall of Cdk activity and a nearly reciprocal fall and rise of anaphase-promoting complex (APC) activity. Signalling pathways start from either extra- or intracellular events and end with the regulation of the engine. Downstream events are cell-cycle processes that are driven by the engine. We illustrate how different mechanisms of cyclin specificity contribute to each of these domains.

Specificity in the cell-cycle engine

Mechanisms that contribute to cyclin specificity in the yeast cell-cycle engine include the differential transcriptional activation of cyclins, the degradation of cyclins, the association of cyclin–Cdk complexes with different Cdk inhibitors, the localization of cyclins and the inhibitory phosphorylation of Cdk.

Anaphase promoting complex
(APC). A multicomponent ubiquitin ligase that targets proteins for degradation by the proteasome.

Forkhead transcription factor
A member of a protein family that consists of more than 40 members. This family belongs to the winged-helix class of DNA-binding proteins and its members are involved in diverse cellular functions, including glucose metabolism, apoptosis and cell-cycle regulation.

26S proteasome
A large multisubunit protease complex that selectively degrades intracellular proteins. Targeting to proteasomes occurs through the attachment of polyubiquitin tags.

SCF complex
A multisubunit ubiquitin ligase that contains Skp1, a member of the cullin family (Cdc53) and an F-box protein, as well as a RING-finger-containing protein (Roc1; also known as Rbx1).

F-box protein
A component of the machinery for the ubiquitin-dependent degradation of proteins. F-box proteins recognize specific substrates and, with the help of other subunits of the E3 ubiquitin ligase, deliver them to the E2 ubiquitin-conjugating enzyme.

Transcriptional regulation of cyclins. A crucial mechanism for cyclin specificity is the differential regulation of G1 and B-type cyclins at the level of transcription during the cell cycle. Transcription of the *CLN3* gene is detectable throughout the cell cycle, but peaks in late M–early G1, whereas the transcription of the *CLN1* and *CLN2* genes peaks during G1–S. Transcription of the *CLB5* and *CLB6* genes also peak at G1–S, followed by the transcription of the *CLB3* and *CLB4* genes and then the transcription of the *CLB1* and *CLB2* genes. Transcription of early-expressed cyclins is largely controlled by the heterodimeric transcription factor SBF, which is composed of Swi4 and Swi6, and the related MBF transcription factor, which is composed of Mbp1 and Swi6. *CLN1* and *CLN2* expression has been primarily attributed to SBF[13,14], and the transcriptional activation of the *CLB5* and *CLB6* genes is primarily attributed to MBF[15]. However, recent evidence indicates that there is a high degree of overlap for these transcription factors in gene activation[16].

Cln3–Cdc28 activity is important for SBF-mediated gene activation[17–19], and this might reflect some intrinsic specialization of Cln3 relative to another G1 cyclin, Cln2 (REF. 20). Cln3–Cdc28 phosphorylates Whi5, a transcriptional repressor of SBF, to induce its nuclear export and allows for *CLN1* and *CLN2* transcription to be induced[21,22]. The phosphorylation of Whi5 by Cln3–Cdc28 early in the cell cycle probably reflects, at least in part, the fact that Cln3 is the only cyclin that is expressed at this time. Cln1-, Cln2- and Clb5-directed Cdc28 activity can also phosphorylate Whi5, which has the potential to provide a positive-feedback loop for their expression[23,24] (FIG. 2a).

An additional level of cyclin-specific regulation of SBF and MBF is provided by Clb6–Cdc28, which phosphorylates Swi6 to promote its nuclear export. Biochemical evidence indicates that this is due to the intrinsic specificity of Clb6, although the responsible sequences in Clb6 have not been mapped[25]. Therefore, the ability of different cyclins to bind to specific targets is an additional cyclin-specific mechanism for transcriptional control.

Later in the cell cycle, Clb2 can specifically inactivate SBF-mediated gene expression, which correlates with the ability of Clb2 to bind to Swi4 (REFS 26,27). Clb3 and Clb4, which are expressed at this time[28] and in levels that are comparable to Clb2 (REF. 1), are much less capable of turning off SBF, which indicates some intrinsic difference in the protein structure of Clb2. Clb2 also positively regulates its own expression. The transcription factor Mcm1 recruits the forkhead transcription factor Fkh2 and the co-activator Ndd1 to regulate the expression of *CLB2*. Clb2–Cdc28 phosphorylates Ndd1, which is important for its recruitment to the *CLB2* gene promoter[29,30], and phosphorylates Fkh2, which enhances the interaction of Fkh2 with Ndd1 (REF. 31). Transcriptional control of *CLB2* is highly specific because Clb2 is probably specialized in Ndd1 phosphorylation[30] and because *CLB2* is also a specific cyclin target of this circuit (FIG. 2b).

Degradation of cyclins. Cyclins are regulated at the level of protein degradation by ubiquitin-mediated proteolysis. Proteins that are tagged with a chain of ubiquitin molecules are degraded by the 26S proteasome. The sensitivity of different cyclins to different ubiquitin ligases constitutes an important mechanism for cyclin specificity in controlling the cell-cycle engine. The ubiquitylation and degradation of Cln1 and Cln2 is mediated by an SCF complex that contains the F-box protein Grr1 (SCF^{Grr1})[32,33]. Clb6 is the only B-type cyclin that has been shown to be degraded in an SCF-dependent manner in yeast; it is degraded earlier than Clb5 by an SCF complex that contains the F-box protein Cdc4 (SCF^{Cdc4})[34] (FIG. 3a). These contrasting modes of Clb5 and Clb6 proteolysis might explain, in part, the observation that despite substantial overlap of Clb5 and Clb6 function in promoting DNA replication, strains that lack the *CLB5* gene activate predominantly early origins of replication[35]; Clb6 might be depleted by the time later origins are normally activated.

The other B-type cyclins are ubiquitylated by the APC (reviewed in REF. 36; FIG. 3a). During metaphase, APC is bound to Cdc20 and targets Clb5 and mitotic B-type cyclins for degradation[37,38]. Later in mitosis, APC that is bound to the adaptor protein Cdh1 completes the degradation of mitotic B-type cyclins, including the main mitotic cyclin, Clb2. APC^{Cdc20} and APC^{Cdh1} are differentially regulated by Cdc28-mediated phosphorylation. Several subunits of the APC are phosphorylated by Clb–Cdk complexes *in vivo* and can be phosphorylated by Clb2–Cdc28 specifically *in vitro*, which activates APC^{Cdc20} (REF. 39). By contrast, Cln–Cdc28 and Clb–Cdc28 complexes phosphorylate Cdh1 to inactivate APC^{Cdh1} (REFS 40,41). Differential degradation of cyclins by APC^{Cdc20} and APC^{Cdh1} has clear functional consequences. For example, the immunity of Clb5 to Cdh1-mediated proteolysis might allow Clb5–Cdc28 to inactivate Cdh1 at G1–S, thereby allowing the subsequent accumulation of Clb2 (REFS 37,42,43).

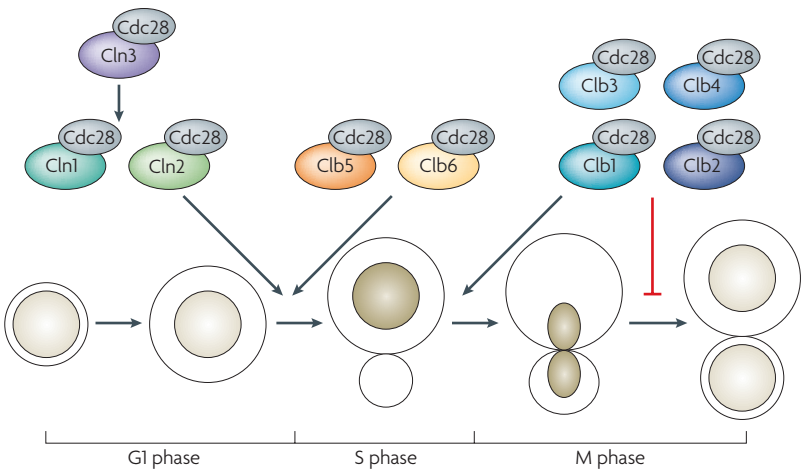

Figure 1 | **Cyclins in the budding yeast cell cycle.** Budding yeast cyclins activate a single cyclin-dependent kinase (Cdc28). The G1-phase cyclins (Cln1, Cln2 and Cln3) promote bud emergence, spindle pole body duplication (not shown) and activation of the B-type cyclins. The S-phase cyclins (Clb5, Clb6) advance DNA replication (shaded nucleus), and the M-phase cyclins (Clb1, Clb2, Clb3 and Clb4) promote spindle formation and the initiation of mitosis. Mitotic cyclins inhibit mitotic exit and cell division. Following cytokinesis, a mother and daughter cell are generated.

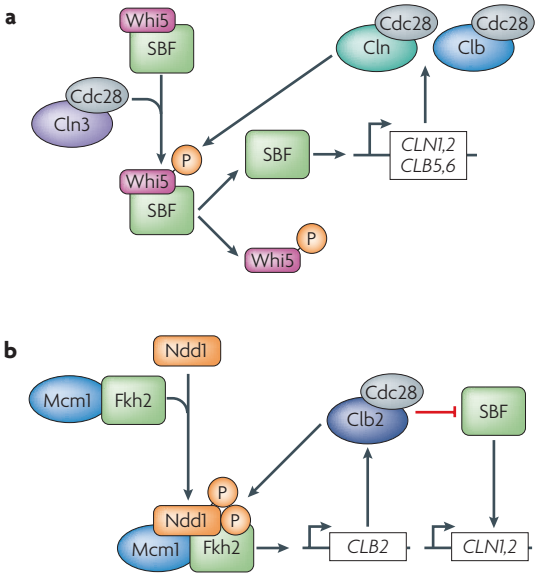

Figure 2 | **Transcriptional regulation of cyclins.** **a** | The transcription repressor Whi5 inhibits the activity of the SBF transcription factor. Phosphorylation of Whi5 by Cln3–Cdc28 induces the nuclear export of Whi5 and activates SBF, which induces the transcription of the genes that encode Cln1, Cln2, Clb5 and Clb6. Cln–Cdc28 and Clb–Cdc28 phosphorylate Whi5, which might provide a positive-feedback loop. **b** | The co-activator Ndd1 recruits minichromosome maintenance-1 (Mcm1) and the transcription factor Fkh2 for the activation of the gene that encodes Clb2. Phosphorylation of Ndd1 and Fkh2 by Clb2–Cdc28 promotes Ndd1-dependent recruitment of Mcm1–Fkh2 to the promoter of *CLB2*. Clb2–Cdc28 also phosphorylates and inhibits SBF to repress the transcription of G1-phase cyclins. P, phosphate.

Inhibition of cyclin–Cdk complexes. Specific cyclin–Cdk complexes are negatively regulated by binding to stoichiometric inhibitors. Clb–Cdc28 complexes, but not Cln–Cdc28 complexes, are blocked when bound to Sic1 (REF. 44). Similarly to cyclins, Sic1 is controlled by ubiquitin-dependent protein degradation. Cln2–Cdc28 and Clb–Cdc28 can phosphorylate Sic1, which allows Sic1 to be recognized by the SCF^{Cdc4} ubiquitin ligase[33,45,46] (FIG. 3B). This degradation of Sic1 relieves the inhibition of Clb–Cdc28 and allows cells to enter into S phase[44]. There is no known intrinsic cyclin specificity in the ability to phosphorylate Sic1 and target it for degradation, but the efficient inhibition of B-type Cdk activity by Sic1 probably accounts for the need for G1 cyclins to enable efficient Sic1 proteolysis *in vivo*[6].

Cyclin localization. The localization of individual cyclins to different subcellular compartments provides a means for cyclin-specific targeting. The G1 cyclin Cln3 is primarily nuclear, whereas Cln2 is primarily cytoplasmic and can localize to sites of polarized growth[47–49]. This localization pattern contributes to the abilities of Cln2 and Cln3 to regulate different substrates. The role of Cln3 in promoting transcription by phosphorylation and nuclear exclusion of the transcriptional repressor Whi5 (see above) is probably promoted by the nuclear localization of Cln3. In fact, the introduction of a nuclear export signal into Cln3 renders the Cln3–Cdc28 complex largely non-functional for this role[47,49]. Similarly, the function of Cln2 is compromised when a nuclear export signal is added, which indicates that Cln2 has an important role in the nucleus despite being largely cytoplasmic[47–49]. However, cytoplasmic Cln2, but not nuclear Cln2, can rescue the phenotypes of mutations in proteins that are involved in budding, which indicates that cytoplasmic Cln2 might be involved in this pathway[47–49].

The localization of Cln2 is regulated by Cdc28-mediated phosphorylation. When the Cdc28 consensus phosphorylation sites in Cln2 are mutated, Cln2 is exclusively nuclear, which indicates that phosphorylation can conceal an NLS or expose a nuclear export signal[47–49]. Subcellular localization can restrict function as well as enhance it because cytoplasmically targeted Cln3 has a limited ability to carry out some cytoplasmic roles of Cln2 (REFS 48,49). This partial reversal of cyclin specificity by altering its subcellular targeting is one of the few examples of modular transferable domains that might account for genetic cyclin specificity.

Clb1–4 are localized primarily to the nucleus, with portions associated with the mitotic spindle and SPBs[50]. Clb2 is the only mitotic cyclin that is also distributed to the bud neck[50,51]. Localization of Clb2 to the bud neck is independent of its binding to Cdc28, but is dependent on its hydrophobic patch. Deletion of the gene that encodes the bud-neck protein Bud3 eliminates bud-neck-localized Clb2 and delays cytokinesis, which indicates that Bud3 is important for targeting Clb2 to the bud neck, and this might positively regulate cell division[50].

Inhibition of Clb2–Cdc28 complexes by Swe1. Swe1 has been shown to phosphorylate and inactivate Clb2–Cdc28, but not Cln2–Cdc28, *in vitro*[52]. Genetic evidence indicates that Swe1 inhibits different Clb–Cdc28 complexes to varying degrees, with Clb2–Cdc28 being strongly inhibited, Clb3 and Clb4–Cdc28 being moderately inhibited, and Clb5 and Clb6–Cdc28 being unaffected when these cyclins are expressed during S phase[4]. This implies that when the temporal control of cyclins is compromised, inappropriately expressed cyclin–Cdc28 complexes are differentially sensitive to Swe1 inhibition.

Cdc28 reciprocally regulates Swe1. Mitotic Clb–Cdc28 complexes phosphorylate Swe1, priming it for degradation by the APC[53,54]. In addition, Cdc28 activity has recently been shown to both positively and negatively regulate the association of Swe1 with Cdc28. Clb2–Cdc28 phosphorylates Swe1 *in vivo*, and removal of the Cdc28 phosphorylation sites in Swe1 (Swe1–18A) causes premature entry into mitosis[55]. This indicates that the phosphorylation of Swe1 is important for its inhibitory activity towards Clb–Cdc28. This can be explained by the observation that Swe1–18A cannot form a complex with Clb2–Cdc28. Later, when the tyrosine phosphatase Mih1 removes the inhibitory phosphorylation on Cdc28, Swe1 becomes hyperphosphorylated and dissociates from the Clb2–Cdc28 complex to allow for the full activation of Clb2–Cdc28.

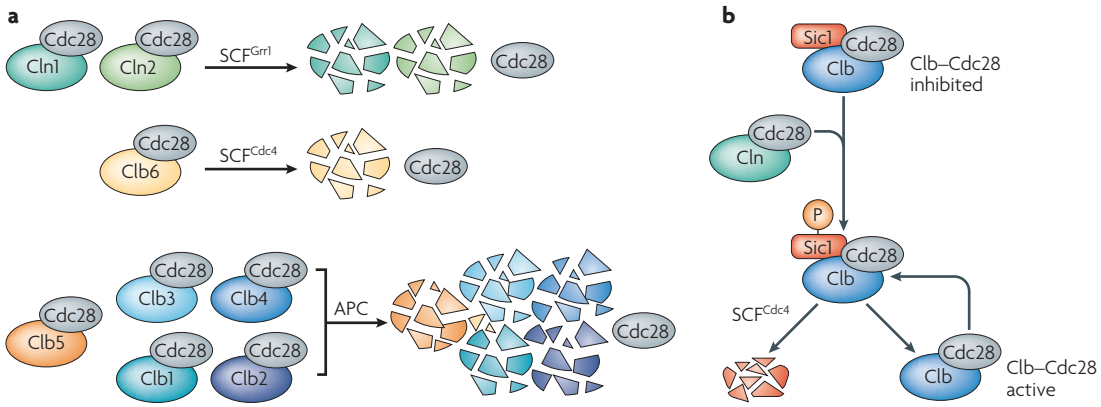

Figure 3 | **Degradation and inhibition of cyclins. a** | Cyclins are ubiquitylated by different ubiquitin ligases and degraded by 26S proteasomes. The G1-phase cyclins (Cln1 and Cln2) are ubiquitylated by SCF^{Grr1}, Clb6 is ubiquitylated by SCF^{Cdc4}, and the other B-type cyclins (Clb1, Clb2, Clb3, Clb4 and Clb5) are ubiquitylated by the anaphase promoting complex (APC). **b** | Sic1 inhibits the activity of Clb–Cdc28 complexes. Cln–Cdc28 phosphorylates Sic1, which promotes SCF^{Cdc4}-mediated ubiquitylation and subsequent degradation of Sic1, allowing for Clb–Cdc28 activation and S-phase entry. Clb–Cdc28 complexes also phosphorylate Sic1 to induce its proteolysis. P, phosphate.

Specificity in signalling pathways

Signal-transduction pathways impinge on specific cyclins. Examples of these include the mating-factor response and associated G1 arrest, and various checkpoint systems that block mitosis until structural requirements are met. These checkpoints include the morphogenesis checkpoint, the DNA-damage response, the Mad2-dependent spindle-integrity-sensing pathway and the Bub2-dependent nuclear-position-sensing pathway.

Pheromone-induced cell-cycle arrest. Binding of the pheromone α-factor to its cognate receptor, Ste2, causes a mitogen-activated protein kinase (MAPK) cascade that ultimately leads to the phosphorylation of the MAPK Fus3 and the subsequent regulation of the Cdk inhibitor Far1 (reviewed in REF. 56). Far1 that is phosphorylated by Fus3 inhibits the kinase activity of Cln2–Cdc28 and Cln3–Cdc28 complexes[57,58]. More recent work showed that the phosphorylation of Far1 by Fus3 promotes the binding of Far1 to Cln2–Cdc28, but does not affect the activity of Cln2–Cdc28 (REF. 59). Therefore, the mechanism by which Cln–Cdc28 complexes are inhibited through α-factor remains unclear. However, it is likely that Far1-mediated inhibition only of G1 cyclin–Cdk complexes contributes to the G1-phase specificity of mating-factor arrest (FIG. 4a).

Far1 is itself regulated by Cdc28-dependent phosphorylation and subsequent proteolysis, and it is modified by Cln2–Cdc28 (REFS 60,61). Even in the absence of α-factor, Far1 protein levels are regulated by SCF-dependent ubiquitylation during the cell cycle[61], and Far1 levels peak in G1 (REF. 62). As Cln–Cdk complexes, and not Clb–Cdk complexes, are active during this phase of the cell cycle, it not clear whether Cln2-mediated phosphorylation and the subsequent degradation of Far1 represents an intrinsically cyclin-specific pathway. Overexpression of Cln3 or Clb5 is sufficient to trigger efficient Far1 proteolysis in the absence of other G1 cyclins, although the pattern of Far1 phosphorylation before degradation is different in the case of overexpressed Clb5 (REF. 63).

A clear case of intrinsic functional cyclin specificity is found in the ability of Cln1 and Cln2, but not of Cln3 or any of the B-type cyclins, to inactivate the mating-factor signal-transduction pathway by interfering with MAPK-cascade activation[64]. The mechanism of this regulation is unknown, but is mapped to a point in the pathway between the Ste20 p21-activated kinase and the Ste11 MAPK kinase kinase[65]. It correlates with Cln2-specific phosphorylation of Ste20 (REFS 65,66), but this phosphorylation might not be causal[67].

Morphogenesis checkpoint. The morphogenesis checkpoint delays nuclear division in cells that have not formed a bud[68]. This pathway induces the inhibitory phosphorylation of Cdc28 by the kinase Swe1 (REF. 69). It has been proposed that Swe1 is instead involved in a size checkpoint and causes a G2–M delay in cells that have a small or medium-sized bud[55], although this might reflect a response to cytoskeletal stresses that also affect bud growth[70]. Swe1 can also inhibit Cdc28 in a phosphorylation-independent manner. A Swe1 mutant that lacks catalytic activity can still block the activity of Cdc28, and is thought to do so by directly binding to Cdc28 (REF. 71) (FIG. 4b).

Spindle-assembly and spindle-position checkpoints. The spindle-assembly checkpoint delays the metaphase–anaphase transition when duplicated chromosomes have not been properly attached to the mitotic spindle. The Mad and Bub proteins associate with kinetochores and monitor their attachment to spindle microtubules[72–74]. Mad1, Mad2 and Mad3 can bind to the Cdc20 adaptor protein for the APC[75]. Overexpression of Cdc20 allows cells to overcome the spindle checkpoint, indicating that this signalling pathway ultimately results in the Mad-protein-mediated inhibition of Cdc20. As APC^{Cdc20} is responsible for the ubiquitylation of B-type cyclins, the spindle checkpoint prevents the degradation of specific cyclins and delays the inactivation of Clb–Cdc28 (FIG. 4c).

α-factor
A peptide that is secreted by yeast cells of the α-mating type that causes cells of the a-mating type to prepare for mating by inducing arrest in G1, morphological changes and the transcription of genes involved in mating.

Spindle-assembly checkpoint
A checkpoint that monitors the correct attachment of chromosomes to spindles in the metaphase–anaphase transition. Activation of this checkpoint causes cell-cycle arrest as a result of the inhibition of the anaphase-promoting complex (APC).

Kinetochore
A multiprotein complex that assembles on centromeric DNA and mediates the attachment and movement of chromosomes along the microtubules of the mitotic spindle.

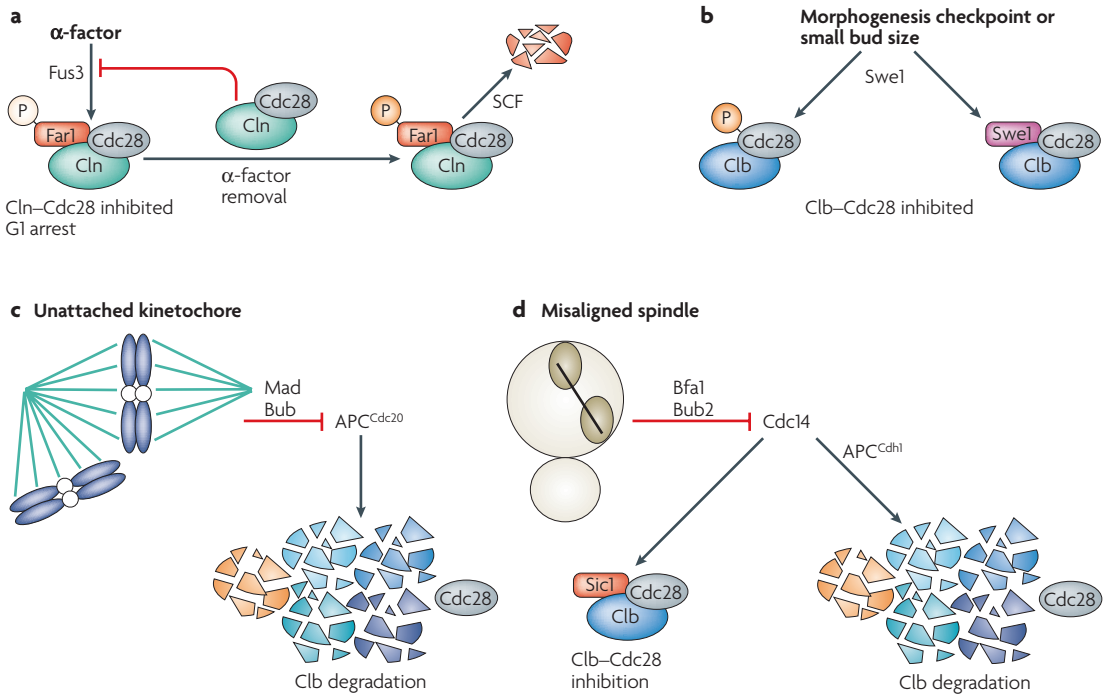

Figure 4 | **Signalling pathways that regulate cyclins. a** | The pheromone α-factor induces a mitogen-activated protein kinase cascade that leads to the activation of the kinase Fus3 and the inhibition of Cln–Cdc28 complexes by the Fus3-dependent phosphorylation of Far1, which causes G1 arrest. Upon the removal of α-factor, Far1 is phosphorylated by Cln–Cdc28, ubiquitylated by an SCF complex and degraded. Exceptionally among all cyclins, Cln1–Cdc28 and Cln2–Cdc28 can also inhibit the mating-factor pathway upstream of Far1 activation; these mechanisms can combine to provide a tight transition from mating-factor sensitivity to mating-factor resistance as cells proceed through the G1 phase. **b** | The kinase Swe1 blocks Clb–Cdc28 activity by the inhibitory phosphorylation of Cdc28 and/or by binding directly to Clb–Cdc28 complexes in cells that have not formed a proper bud. **c** | APC^{Cdc20} is inhibited by Mad and Bub proteins in cells in which duplicated chromosomes have not properly attached to the mitotic spindle in order to delay the degradation of B-type cyclins and entry into anaphase. **d** | Improper alignment of the mitotic spindle causes Bub2- and Bfa1-dependent inhibition of the phosphatase Cdc14 to prevent the antagonism of mitotic B-type cyclins by multiple mechanisms, including the degradation of mitotic B-type cyclins and the association of Sic1 with Clb–Cdc28 complexes. P, phosphate (dark orange and light orange represent Cdc28-dependent and Fus3-dependent phosphorylation, respectively).

The spindle-position checkpoint delays the activation of the mitotic exit network (MEN) until the spindle is properly aligned along the mother–bud axis. The MEN (see below) ultimately causes the activation of the phosphatase Cdc14. Cdc14 promotes mitotic exit by activating the APC^{Cdh1} for degradation of mitotic B-type cyclins, and by stabilizing Sic1, for the inhibition of Clb–Cdc28 complexes[41,76]. Components of the MEN are restricted to the daughter SPB or the bud cortex, which prevents the initiation of this signalling cascade until the nucleus has entered the bud[77]. Bub2, which is part of the Bfa1–Bub2 GTPase-activating complexes, is localized to SPBs throughout the cell cycle[78] and inhibits the MEN in response to defects in spindle alignment[79]. This pathway, therefore, stabilizes specific cyclin–Cdc28 complexes and prevents their inhibition by Sic1 (FIG. 4d).

These pathways are not known to have any direct effects on G1 cyclins, although the accumulation of Clb2 when the checkpoints are activated can repress transcription of *CLN1* and *CLN2*. The high cyclin specificity of these pathways promotes specific arrest in the appropriate stage of the cell cycle.

Mitotic exit network
(MEN). A signal-transduction pathway that is required for sustained Cdc14 release during anaphase. This allows for the degradation of mitotic cyclins and the accumulation of the Clb–Cdc28 inhibitor Sic1.

Bfa1–Bub2 GTPase-activating complex
A complex that localizes to spindle pole bodies and inhibits the activation of the mitotic exit network until the mitotic spindle is properly aligned along the mother–bud axis.

Specificity in downstream events

Downstream events are driven by the cell-cycle engine, but they do not contribute directly to engine function. These include budding and morphogenesis, SPB duplication, DNA replication, mitotic spindle formation, orientation and elongation, and activation of the MEN. Cyclin specificity in many of these cases is achieved by the ability of different cyclins to bind to particular targets, although in other cases it could reflect only the timing of expression of these cyclins.

Budding and morphogenesis. Pre-bud site assembly correlates with the activation of the G1 cyclins, which promote actin polymerization at the pre-bud site[80]. Deletion of the *CLN1* and *CLN2* genes causes delayed bud emergence. Furthermore, a screen for genetic mutations that limit bud formation in the absence of *CLN1* and *CLN2* yielded *BUD2* (REFS 81,82), a GTPase-activating protein that had previously been shown to have a role in bud positioning[83]. Deletion of the *CLN1* and *CLN2* genes, together with deletion of the *PCL1* and *PCL2* genes — which encode cyclins that activate the Pho85 Cdk, which is involved in the response to environmental

stress — causes cells to fail to form buds and to have wide necks and amorphous shapes[84]. These cells can still degrade Sic1, replicate their DNA and duplicate their SPBs, which indicates that the Cln1, Cln2, Pcl1 and Pcl2 cyclins are crucial for budding and morphogenesis but not other cell-cycle events. The inability of Cln3 and B-type cyclins to promote bud emergence in the absence of Cln1, Cln2, Pcl1 and Pcl2 indicates a high functional specificity. Related observations from mutant *cln1*, *cln2* synthetic lethal screens indicate a role for Cln1 and Cln2 in septin ring function[81,82,85].

The mitotic cyclin Clb2 is important for controlling bud growth[80,86]. During mitosis, the bud starts to grow over its entire surface, indicating a switch from polarized growth to isotropic growth; Clb2 contributes to this change by promoting the depolarization of actin in the bud[80]. Nap1, a protein that interacts specifically with B-type cyclins[87], is important for the ability of Clb2 to promote isotropic bud growth[88]. Cells that are dependent on Clb2–Cdc28 (due to the deletion of the *CLB1*, *CLB3* and *CLB4* genes) and that lack the *NAP1* gene continue polar growth to form highly elongated cells, indicating that Nap1 functions in this Clb2-dependent pathway[88]. A screen to identify additional mutations that affect Clb2-controlled bud growth yielded the protein kinase Gin4 (REF. 86). Deletion of *GIN4* in cells that are dependent on Clb2–Cdc28 also resulted in highly elongated bud morphology. Gin4 is phosphorylated during mitosis in a Clb2- and Nap1-dependent manner, and phosphorylation activates the kinase activity of Gin4 (REF. 86). Gin4 and Nap1 associate with septins during mitosis, an event that is dependent on Cdc28-mediated phosphorylation[89]. Together, Clb2-dependent phosphorylation might indirectly regulate septin function through Gin4; Gin4 regulation might be a specialized function of Clb2, as the deletion of the *CLB1*, *CLB3* and *CLB4* genes, alone or in combination, does not affect isotropic growth[80].

The opposing effects of G1 cyclins in promoting bud emergence and polarized growth, compared with mitotic cyclins (especially Clb2), which promote isotropic growth, is a clear example of the functional specificity of cyclins that cannot be accounted for by the timing of expression[80], although the mechanistic basis for this specificity is unknown.

DNA replication. Cdc28-mediated phosphorylation is important both for the initiation of DNA synthesis and for limiting DNA replication to a single round per cell cycle. Initiation of replication is a two-step process. First, the origin recognition complex (ORC), which is present on replication origins throughout the cell cycle, recruits the minichromosome maintenance (Mcm) proteins and Cdc6 to form the pre-replicative complex (pre-RC). Second, the pre-RC is activated by S-phase-dependent Cdc28 and the Dbf4–Cdc7 kinase complex. This allows for the unwinding of DNA and the recruitment of DNA polymerases. Given that Clb5 and Clb6 are important for the timely progression through S phase, it is possible that Clb5–Cdc28 and Clb6–Cdc28 target proteins that are involved in DNA synthesis. In fact, substrates identified in a screen for proteins that are preferentially phosphorylated by Clb5–Cdc28 rather than by Clb2–Cdc28 include the DNA-replication proteins Orc6, Orc2, Mcm3, Cdc6 and Sld2, and the phosphorylation of these substrates is dependent on the presence of the hydrophobic patch in Clb5 (REF. 2).

Sld2 is an essential Cdc28 substrate. Phosphorylation of Sld2 promotes its binding to the replication protein Dpb11, and the Sld2–Dpb11 complex recruits DNA polymerases to origins of replication[90,91]. Sld2 is phosphorylated in a cell-cycle-regulated manner and phosphorylation is maximal during S phase. In cells that lack both the *CLB5* and *CLB6* genes, the phosphorylation of Sld2 is significantly delayed[91]. Sld2 binds specifically to Clb5 (REF. 11), which might account for the requirement for Clb5 or Clb6 for efficient Sld2 phosphorylation. Similarly, Dpb2, a subunit of DNA polymerase ε, is phosphorylated during the cell cycle, and phospho-Dpb2 is first detectable as cells enter S phase[92]. Unlike Sld2, Dpb2 can be phosphorylated by G1 cyclin–Cdc28 as well as by S-phase cyclin–Cdc28. Dpb2 phosphorylation is not essential and its function is unclear, although it might promote its binding to the catalytic subunit of DNA polymerase ε[92] (FIG. 5a).

Multiple Cdc28-mediated phosphorylation events prevent cells from re-replicating their DNA (FIG. 5b). Pre-RCs are disassembled upon origin firing and do not form again until after mitosis has occurred. This ensures that replication origins fire only once per cell cycle. Cdc6, a component of the pre-RC, binds to and is phosphorylated by Clb–Cdc28 complexes *in vitro*[93]. This phosphorylation promotes the ubiquitylation and subsequent degradation of Cdc6 (REFS 94,95), thereby removing it from origins. In addition, Clb2–Cdc28 can bind to Cdc6 that has been phosphorylated on Cdk consensus sites; this binding inhibits pre-RC assembly and, therefore, provides another mode of re-replication control[96]. The Mcm proteins are also removed from origins by Cdc28-dependent phosphorylation[97,98]. Clb–Cdc28 complexes promote the nuclear exclusion of Mcm proteins during G2–M, and the ectopic expression of Clb2 or Clb5 causes the export of Mcm proteins[98]. Cln–Cdk complexes drive Mcm export during G1 phase to block pre-RC formation until B-type cyclins are active and able to initiate origin firing[97].

In addition, the ORC subunits are inhibited by phosphorylation after origin firing. Clb–Cdc28 phosphorylates Orc2 and Orc6 during late G1 (REF. 99). Orc6 has been shown to bind specifically to Clb5, and this interaction is dependent on the hydrophobic patch in Clb5 and the RXL motif in Orc6 (REF. 100). Mutations in these sites strongly decrease the phosphorylation of the ORC complex and Cdc6 *in vitro*[101]. Clb5 associates with origins when cells initiate replication and persists at origins until mitosis, indicating that it has a specific role in preventing origins from re-firing. Interestingly, the disruption of any one of these mechanisms — that is, the degradation of Cdc6, the nuclear export of Mcm proteins or the phosphorylation of Orc proteins — does not cause cells to re-replicate their DNA, but when all three pathways are deregulated, cells begin to re-initiate DNA synthesis[99,100].

Septin ring
A complex of seven septin proteins that forms a ring at the incipient bud site before bud emergence. The septin ring determines bud-site selection and serves as a scaffold for proteins, including those involved in cell polarity, cell-wall synthesis and cytokinesis.

Origin recognition complex
(ORC). A six-subunit complex that associates with replication origins throughout the cell cycle and recruits additional replication factors to initiate DNA replication.

Minichromosome maintenance (Mcm) proteins
Six minichromosome maintenance (Mcm) proteins form a complex that binds DNA at origins of replication and helps unwind DNA to initiate replication.

RXL motif
A protein sequence in Cdc28 substrates that mediates the interaction with the hydrophobic patch of cyclins.

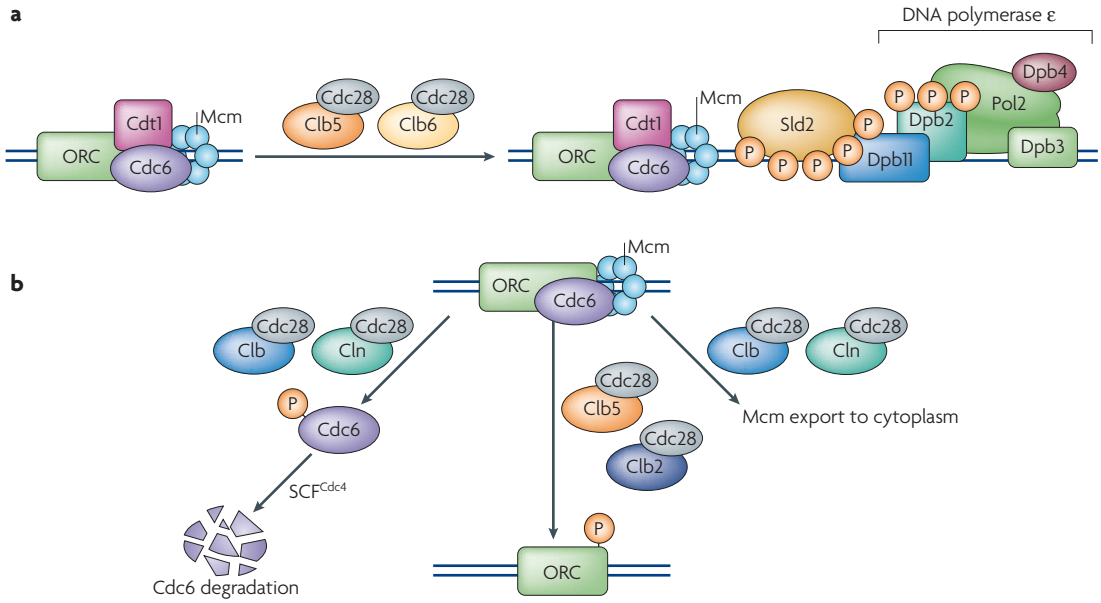

Figure 5 | **Cyclin control of DNA replication. a** | The origin recognition complex (ORC) recruits Cdc6, the minichromosome maintenance (Mcm) complex and the DNA-licensing factor Cdt1 to DNA to form the pre-replicative complex (pre-RC). Cdc28, in association with the S-phase cyclins Clb5 and Clb6, promotes loading of Cdc45 (not shown), which is essential for replication initiation, and of replication protein A (RPA; not shown). Clb5–Cdc28 and Clb6–Cdc28 phosphorylate the replication protein Sld2, which allows for the Dpb11-dependent recruitment of DNA polymerases to origins. Dpb2, a subunit of DNA polymerase ε, is also phosphorylated by cyclin–Cdk complexes. Pol2 is the catalytic subunit of DNA polymerase ε, which also comprises Dpb2–4. **b** | Inhibition of DNA replication to avoid re-replication is achieved by cyclin–Cdc28 complexes. Clb–Cdc28 and Cln–Cdc28 complexes phosphorylate Cdc6 to induce SCF^{Cdc4}-mediated ubiquitylation and subsequent degradation of Cdc6. Clb5–Cdc28 and Clb2–Cdc28 associate with and phosphorylate Orc6, which is part of the ORC. Clb–Cdc28 and Cln–Cdc28 complexes phosphorylate Mcm proteins to promote nuclear export. P, phosphate.

SPB duplication. Similarly to their control of DNA replication, specific cyclin–Cdk complexes regulate SPB duplication and restrict SPB duplication to one round per cell cycle. Cells that lack all six B-type cyclins duplicate their SPBs once, whereas cells that lack mitotic cyclins, but contain either *CLB6* alone or both *CLB5* and *CLB6*, accumulate three or four SPBs[102]. These results indicate that Clns can initiate SPB duplication and that Clb5 and Clb6 can promote SPB re-duplication in the absence of mitotic cyclins. Therefore, mitotic cyclins are thought to restrict SPB re-duplication. Instead, Clb5 and Clb6 are proposed to promote a 'maturation' step that leads to SPB separation, although mitotic cyclins can also promote SPB separation[102]. It is not fully clear whether the regulation of different aspects of the SPB cycle by individual cyclin–Cdk complexes is due to an intrinsic specificity of these cyclins, or whether it is due to the presence or absence of different cyclins at relevant times in the SPB cycle.

More recent work identified SPB components that are regulated by specific cyclin–Cdk complexes to promote SPB duplication during G1 phase. Spc42, a subunit of the central plaque of the SPB that serves as a scaffold for other components to promote SPB duplication[103], was identified as a substrate of Cdc28 and is phosphorylated *in vitro* by Cln1–Cdc28 and Cln2–Cdk complexes, but not by Clb–Cdc28 complexes[104]. Interestingly, Spc42 is also phosphorylated by the kinase Mps1 (REF. 105), and Cdc28, in turn, phosphorylates and stabilizes Mps1, allowing the further phosphorylation of Spc42 (REF. 104). Mutation of the Cdc28 phosphorylation sites of both Spc42 and Mps1 causes a severe reduction in the incorporation of Spc42 into the SPB, whereas the mutation of Spc42 or Mps1 alone causes only minor effects[104]. These data indicate that the direct and indirect regulation of Spc42 by Cln–Cdc28 complexes is important for SPB duplication, and represents an intrinsic specificity of G1 cyclins. However, Clb–Cdc28 complexes can phosphorylate Spc42 at high stoichiometry[104], which could account in part for the rescue of cells that lack G1 cyclins by the overexpression of *CLB5* (REF. 5). Interestingly, multiple SPB subunits, as well as Spc42, were identified as putative Cdc28 substrates[106], which indicates that the action of Cdc28 might specifically regulate other aspects of the SPB duplication cycle.

Mitotic spindle assembly. The use of a temperature-sensitive mutation in *CDC28* (*cdc28-4*), which makes cells more sensitive to the deletion of individual cyclins, has revealed roles for specific cyclins in regulating the mitotic spindle[107,108]. *cdc28-4 clb5*Δ cells exhibited defects in nuclear positioning and spindle orientation, with short spindles and a single nucleus migrating into the bud. Interestingly, neither *CLB2* nor *CLB4*, when expressed at the G1–S boundary, could rescue these defects, which indicates that the intrinsic specificity of Clb5, rather than its temporal expression, is important for this step in the mitotic spindle pathway[107]. The phenotype might

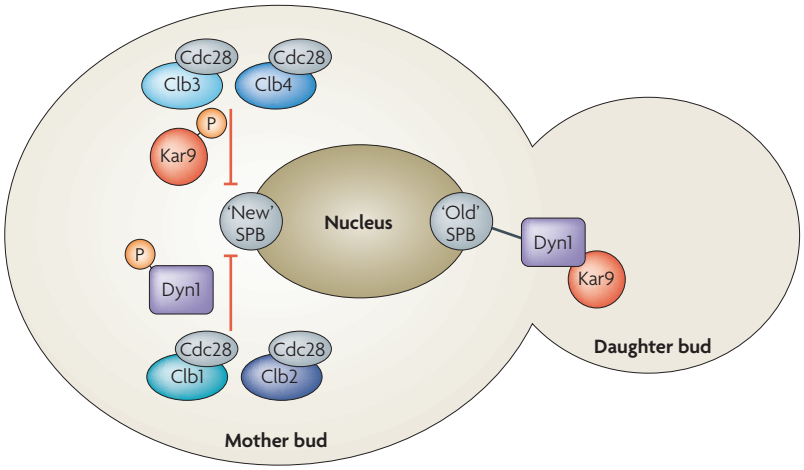

Figure 6 | **Cyclin control of mitotic spindle formation.** Clb3–Cdc28 and Clb4–Cdc28 phosphorylate the microtubule-orientation protein Kar9, which prevents its association with the 'new' or mother-bound spindle pole body (SPB). Clb1–Cdc28 and Clb2–Cdc28 phosphorylate the cytoplasmic dynein Dyn1 to prevent its association with the mother-bound SPB until anaphase. These phosphorylation events and the regulation of Kar9 and Dyn1 are probably important for establishing the orientation of the mitotic spindle. P, phosphate.

be explained by specific cyclin–Cdk complexes having roles in the asymmetrical behaviour of SPBs. The newly duplicated SPB remains in the mother cell, whereas the 'old' SPB enters the daughter cell[109]. This ensures proper orientation and elongation of the mitotic spindle. Further work with the *cdc28-4 clb5*Δ cells revealed that, in these cells, SPB polarity was not established and microtubules from both SPBs interacted with the bud cortex following SPB separation, which resulted in the spindle entering the bud[108].

Interestingly, Kar9, a cortical protein that is linked to actin cables to orient the mitotic spindle[110,111], associates only with the old SPB[112]. This asymmetrical localization of Kar9 is due to its phosphorylation by specific cyclin–Cdk complexes. When Cdc28 consensus phosphorylation sites in Kar9 are mutated, Kar9 redistributes to both SPBs, which indicates that phosphorylation restricts Kar9 to the daughter-bound SPB[113] (FIG. 6). Also, Kar9 is hypophosphorylated in cells that lack the *CLB4* gene, and ectopically expressed Clb4 associates asymmetrically with the mother SPB, thereby preventing the binding of Kar9 (REF. 113). By contrast, other work indicates that Clb4, when expressed from its endogenous promoter, is not restricted to the mother-bound SPB, and that ablation of Clb4 function, instead, redistributes Kar9 along the length of microtubules[114]. Other studies have indicated that Kar9 interacts with Clb5 in a yeast two-hybrid system[112,115], and that Clb5-dependent phosphorylation might restrict Kar9 to the daughter-bound SPB[115].

Kar9 and the cytoplasmic dynein Dyn1 are thought to function in at least partially redundant pathways[110]. Recent work has shown that dynein is asymmetrically associated with the daughter-bound SPB in pre-anaphase cells, but redistributes to both SPBs during anaphase, and this asymmetry is important for dynein-dependent spindle alignment[116]. The asymmetrical localization of Dyn1 requires the activity of Clb1- and Clb2-dependent Cdc28 (FIG. 6). The SPB component Cnm67 is essential for asymmetrical dynein distribution and is important for localizing Clb2 to SPBs[116]. This indicates the presence of a cyclin-specific regulatory signal (that is, Cnm67-mediated recruitment of Clb2 to SPBs), which, in turn, might regulate an apparently cyclin-specific output (Clb2-mediated dynein asymmetry).

Activation of the MEN. For cells to exit from mitosis, the activity of Cdc28 must be significantly decreased through the degradation of mitotic B-type cyclins and the accumulation of the G1-phase-specific Cdk inhibitor Sic1 (REF. 117), and this is coordinated by Cdc14. Cdc14 dephosphorylates Cdh1 to activate APC^{Cdh1} for the degradation of mitotic B-type cyclins[76,118]. Cdc14 dephosphorylates and activates Swi5, a transcription factor for *SIC1,* and dephosphorylates Sic1 itself, which prevents the recognition of Sic1 by ubiquitin ligases that target it for proteolysis[76]. Cdc14 is also likely to oppose Clb–Cdc28 action directly by dephosphorylating other Clb–Cdc28 phosphorylation substrates. The full spectrum of Cdc14 targets remains to be identified, and it is unknown what controls Cdc14 specificity.

Cdc14 is sequestered in the nucleolus during most of the cell cycle by the nucleolar protein Net1, but it is released during anaphase by two signalling pathways: the Cdc14 early anaphase release (FEAR) network and the MEN. Although the FEAR network can trigger the release of Cdc14 from the nucleolus in early anaphase and its accumulation at SPBs, it is not sufficient for exit from mitosis. This indicates that the MEN is required to sustain the presence of Cdc14 in the nucleus and cytoplasm[119]. The MEN is not activated until the daughter-bound SPB has entered the bud[77]. This prevents the initiation of mitotic exit before the anaphase spindle is properly oriented and nuclei are segregated, constituting the spindle-position checkpoint (reviewed in REF. 120). Given that Clb-dependent Cdc28 activity is important for the formation and orientation of the mitotic spindle (see above), Clb–Cdc28 contributes to the activation of the MEN and to Cdc14 release (FIG. 7).

The FEAR network also promotes MEN activation. This occurs, in part, because the transiently released Cdc14 dephosphorylates and activates the MEN component Cdc15 (REFS 121,122). The FEAR network also promotes the phosphorylation of Net1 by Clb1–Cdc28 and Clb2–Cdc28, which disrupts the interaction between Cdc14 and Net1 and causes the liberation of Cdc14 (REF. 123) (FIG. 7). This phosphorylation might be antagonized by $PP2A^{Cdc55}$ and activated by Esp1 separase[124]. When the Cdc28 consensus phosphorylation sites in Net1 are mutated, or when the *CLB2* gene is deleted, cells are defective for early anaphase release of Cdc14. Released Cdc14 might specifically counteract the activity of Clb5–Cdc28; cells that carry a mutation in Cdc14 that reduces its affinity for Net1 are not viable when the *CLB5* gene is deleted but are viable when the *CLB2* gene is deleted[125]. The specific pathway(s) that is antagonistically regulated by Clb5 and Cdc14 has not yet been determined.

Cdc14 early anaphase release (FEAR) network
A signal-transduction pathway that promotes the transient release of Cdc14 from the nucleolus during early anaphase. This allows for stabilization of the mitotic spindle, segregation of ribosomal DNA and activation of the mitotic exit network.

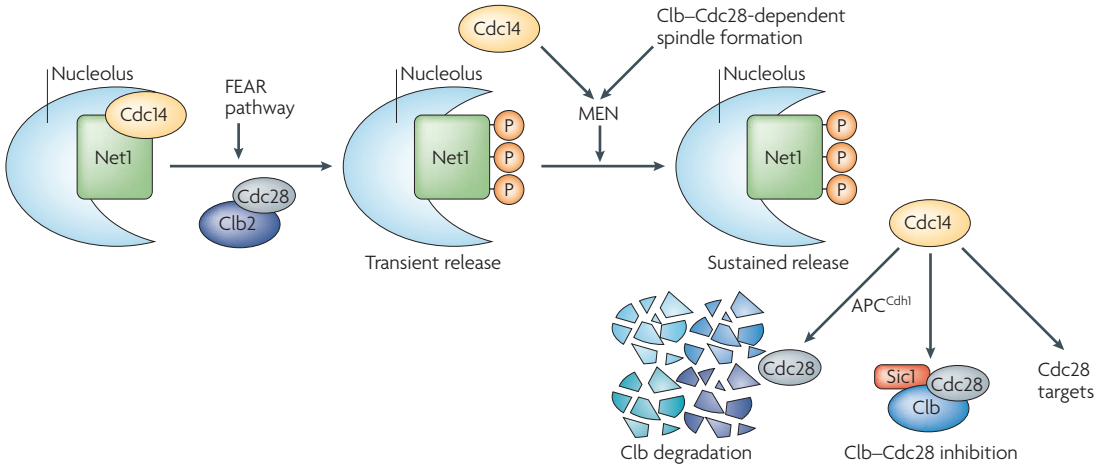

Figure 7 | **Cyclin control of exit from mitosis.** The Cdc14 phosphatase is sequestered in the nucleolus by the nucleolar protein Net1. In early anaphase, the Cdc14 early anaphase release (FEAR) pathway promotes Clb2–Cdc28-dependent phosphorylation of Net1, which correlates with the transient release of Cdc14. Released Cdc14 and Clb–Cdc28-dependent spindle formation activate the mitotic exit network (MEN), which is required for sustained release of Cdc14 from the nucleolus. Cdc14 activity promotes the activation of APC^{Cdh1} for the degradation of mitotic B-type cyclins, and the stabilization of the Sic1 inhibitor, so that it inhibits Clb–Cdc28 complexes. In addition, Cdc14 dephosphorylates Cdc28 targets. P, phosphate.

Concluding remarks

A common theme that emerges when cyclin specificity is considered is the inactivation of an inhibitor of a late-functioning cyclin by an early functioning cyclin that is immune to the inhibitor. Cln3 is immune to Whi5 transcriptional inhibition and phosphorylates Whi5, allowing the activation of Cln1 and Cln2; Cln1 and Cln2 are immune to Sic1 inhibition and phosphorylate Sic1, leading to its degradation and thereby allowing the activation of B-type cyclins; Clb5 is immune to degradation by APC^{Cdh1} and phosphorylates and inhibits APC^{Cdh1}, permitting the accumulation of Clb2. This type of specificity results in the sequential activation of different cyclins and contributes directionality to the cell-cycle engine. In turn, these early and late cyclins might be linked to appropriate early and late phosphorylation targets by intrinsic protein specialization, allowing downstream events to occur in the proper order. Cln2 promotes bud emergence by phosphorylating proteins that are involved in bud formation, such as Cdc42. By contrast, Clb5 promotes DNA replication by phosphorylating Sld2, and Clb2 promotes mitotic events, including the repression of Cln2 and isotropic bud growth, by phosphorylating Ndd1 and Nap1, respectively. Cyclin specificity could therefore trigger the sequential occurrence of events to reinforce the unidirectionality of the cell cycle, both at the level of the cell-cycle engine and at the level of downstream events. In turn, these cyclin-specific roles allow signal-transduction pathways to produce cell-cycle-specific outcomes by targeting individual cyclin classes — it is surely not an 'accident' that no known signal-transduction pathway directly inactivates all cyclin–Cdc28 complexes. Therefore, although cyclin specificity is not an absolute requirement for a viable cell cycle, it clearly can contribute to the construction of a robust, unidirectional system with flexible regulatory inputs.

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