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REVIEW ARTICLE

Orchestrating the cell cycle in yeast: sequential localization of key mitotic regulators at the spindle pole and the bud neck

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Overview

Coordination of nuclear and morphogenetic events is crucial for ensuring successful completion of each round of the cell division cycle. In the budding yeast *Saccharomyces cerevisiae*, progression through the different stages of the cell cycle is driven by the action of a single cyclin-dependent kinase (CDK), Cdc28. Both the onset of mitosis and exit from mitosis depend, respectively, on activation and inactivation of Cdc28–cyclin B complexes. Numerous proteins are involved in controlling accurately the sequence of events that couple nuclear division with bud formation. Remarkably, recent studies have shown that many of these key regulators localize transiently to the cytoplasmic face of the spindle pole body (SPB), to the septin-filament-based structure at the bud neck, or to both structures, usually in a sequential manner. Proteins that localize to these sites include components of several signalling and checkpoint pathways, as well as most members of the mitotic exit network (MEN). In general, the dynamic subcellular localization observed for each of these proteins follows a temporal pattern that is consistent with the stage of the cell cycle in which it plays a role. Comparison with the fission yeast *Schizosaccharomyces pombe* indicates that these regulatory proteins, processes, and localization patterns are largely conserved, although there are some differences attributable perhaps to the different cell shapes and division strategies of these two organisms. In this review, we focus on the pivotal role of two, still rather poorly understood, cellular substructures, the SPB and the septin filaments, as organizing centres for cell cycle signalling. Also highlighted are the role and logic of sequential localization of cell cycle regulators to these sites in the control of budding and cytokinesis and in their coupling to nuclear partition and mitotic exit, respectively.

Co-regulation of the timing of nuclear division and budding in *S. cerevisiae* by the CDK Cdc28

Cdc28, the CDK of *S. cerevisiae*, associates with different sets of cyclins to control the cell cycle. The START landmark is triggered by association of the kinase with G_1 cyclins, whereas mitosis coincides with its association with B cyclins. This mitotic CDK activity is responsible for sister chromatid separation during anaphase (reviewed by Nasmyth *et al.*, 2000), which involves destruction of the anaphase inhibitor Pds1 by a specific ubiquitin conjugation system known as the anaphase-promoting complex (APC) or cyclosome; this is driven by a factor for substrate specificity named Cdc20. Once chromatids have segregated, the mitotic CDK is inactivated by two joint mechanisms: (i) the degradation of B cyclins by the APC itself, this time requiring Cdh1/Hct1 for substrate recognition (Yeong *et al.*, 2000; Schwab *et al.*, 2001; Jaspersen *et al.*, 1998; reviewed by Zachariae & Nasmyth, 1999); and (ii) the expression and activation of the CDK inhibitor Sic1 (Kramer *et al.*, 1998; Schwob *et al.*, 1994; Donovan *et al.*, 1994; reviewed by Mendenhall, 1998). Only when mitotic CDK has been inactivated can the cell exit from mitosis and commit itself to septation.

Nuclear dynamics are orchestrated by astral microtubules, which emanate from the SPB, a functional equivalent to the centrosome of higher eukaryotes (Shaw *et al*., 1997). Recent work has highlighted the role of interactions between cytoplasmic microtubules and the cortex via specific anchoring proteins to coordinate these dynamics (Heil-Chapdelaine *et al*., 1999; Beach *et al*., 2000; Korinek *et al*., 2000; Lee *et al*., 2000; Segal *et al*., 2000). In G_1, the SPB orientates towards the future bud site, driven by cytoplasmic astral microtubules. SPB duplication occurs early in the cell cycle and depends on G_1 cyclins (see the pictures at the top in Fig. 3a to illustrate the topics presented). Next, in synchrony with S phase, SPB separation, which requires B cyclin–CDK activity (Lim *et al*., 1996), leads to the formation of a short premitotic spindle, which remains in the mother cell. One of the poles is oriented to the bud neck, probably by means of prevalent interactions of the astral microtubules with the neck and the bud cortex that depend on dynein and dynactin (Li *et al*., 1993; Adames *et al*., 2001). Thus oriented, the nucleus lies in wait for the onset of anaphase, which triggers spindle elongation through the bud neck towards the daughter cell. Finally, disassembly of the spindle at telophase relies on mitotic CDK inactivation by the APC and Sic1.

Cortical dynamics, based both on the highly dynamic actin cytoskeleton and on a septin-based ring that permanently marks the bud neck, are accurately synchronized with nuclear dynamics to achieve proper morphogenesis through budding (Lew & Reed, 1993; Cid *et al*., 2001a; see Gladfelter *et al*., 2001 for a recent review on septins). Depending on G_1 cyclin–CDK activity, actin cortical patches support the emergence of a bud within a site marked by the appearance of a septin ring. As the bud emerges, the septins mark the bud neck, acting as a kind of submembrane barrier between the mother and the daughter (Barral *et al*., 2000). Through the S phase and beyond, isotropic bud growth is monitored from this septin structure, since in its absence the bud will grow in length instead of adopting its typical ellipsoidal shape (Hartwell, 1971; Cid *et al*., 1998). The B cyclin–CDK complex itself is responsible for the switch from polar to isotropic growth, since *cdc28* point mutations cause the switch to fail (Ahn *et al*., 2001). The bud keeps on growing, supported by scattered cortical patches, until actin is depolarized in late mitosis. Again, mitotic CDK inactivation by the APC is essential to relocate actin to the neck to form an actomyosin contractile ring in the middle section of the septin hourglass-like scaffold at the bud neck. Immediately, cytokinesis is committed by contraction of the ring, leaving a chitin-rich primary septum and dividing the septin structure into two parallel rings (Lippincott & Li, 1998). Finally, repolarization of actin patches to the septation site reinforces the cell wall on both sides prior to cell separation by hydrolysis of chitin at the primary septum.

In sum, only one CDK orchestrates cytoskeletal rearrangements to coordinate the events that support cell division through budding both temporally and spatially. Many regulatory proteins are involved in the fine tuning of this CDK. In this review, we wish to refer to a recent body of evidence that suggests that the spatial distribution of CDK regulators to the SPB and the septins may play a capital role in this coordination.

The morphogenesis checkpoint (MCP) operates from the bud neck

Upon insults on bud site assembly, the cell arrests cell cycle progression by inhibitory phosphorylation of Tyr19 at the B-cyclin-complexed CDK (Barral *et al*., 1999; McMillan *et al*., 1998; Shulewitz *et al*., 1999). Such negative regulation is exerted by the protein kinase Swe1, the budding yeast homologue of the Wee1 kinase in other eukaryotes (Booher *et al*., 1993). This regulatory pathway is known as the morphogenesis checkpoint (MCP) (see Lew, 2000, for a review). Failures in the assembly of both actin – such as treatment with the inhibitor of actin polymerization latrunculin – and septin activate the checkpoint. Since the components of this pathway, Hsl1, Hsl7 and Swe1 (Table 1), are specifically associated with the daughter side of the septin structure, the pathway seems to monitor proper bud shaping shortly after bud emergence. Actually, the Ser/Thr kinase Hsl1 hierarchically drives the other components to the daughter septin ring as soon as the bud emerges, ensuring timely Swe1 degradation to eventually allow activation of the mitotic kinase (Sia *et al*., 1996, 1998; Kaiser *et al*., 1998; McMillan *et al*., 1999; Shulewitz *et al*., 1999; Longtine *et al*., 2000). When the complex fails to localize, sensing a defective morphogenetic site, Hsl1 fails to activate and Swe1 persists, leading to mitotic CDK inhibition (Fig. 1). Hsl1 persists at the neck until it is degraded in late mitosis in an APC-dependent manner (Burton & Solomon, 2000, 2001). Hsl7 contains a central domain homologous to demonstrated protein-arginine methyltransferases (Pollack *et al*., 1999; Ma, 2000; McBride & Silver, 2001). However, the function of Hsl7 in inactivating Swe1 at G_2-M does not seem to require this putative catalytic activity because several different site-directed mutants, in which the residues most highly conserved between Hsl7 and its orthologues have been altered, still display an intact checkpoint (M. Shulewitz, V. J. Cid, S. E. Crown & J. Thorner, unpublished results). Therefore, the primary role of Hsl7 at the bud neck seems to be as an adaptor or bridge between Hsl1 and Swe1 to permit the formation of Hsl1–Hsl7–Swe1 ternary complexes (Shulewitz *et al*., 1999). A reported interaction between Swe1 and the actin cytoskeleton protein Bem1, recently detected in a comprehensive two-hybrid screen (Drees *et al*., 2001), suggests that both septin- and actin-based structures may be monitored through this pathway during polarized growth. However, neither Hsl1 nor Hsl7 seems to associate with the actin cytoskeleton, so yet unknown Swe1 regulators may account for this function. Interestingly, the Slt2 MAP kinase pathway (also known as the cell integrity pathway), which controls multiple aspects related to morphogenesis, actin polarization and cell wall biosynthesis (see Heinisch *et al*., 1999, for a review), seems

Table 1. Cell-cycle regulators that localize to the bud neck and/or the SPB in the budding yeast

Gene	Network	Function	Reference	Fission yeast orthologue
HSL1	MCP	Neck-associated Ser/Thr protein kinase, negative regulator of Swe1	McMillan *et al.* (1999); Shulewitz *et al.* (1999); Barral *et al.* (1999)	*nim1*
HSL7	MCP	Putative methyltransferase which complexes with Hsl1, negative regulator of Swe1	Cid *et al.* (2001b); McMillan *et al.* (1999); Shulewitz *et al.* (1999); Ma (2000)	*skb1*
SWE1	MCP	Tyr protein kinase, negative regulator of the mitotic CDK	McMillan *et al.* (1998); Booher *et al.* (1993)	*wee1*
CDC5	MCP/SOC/MEN	Ser/Thr protein kinase of the polo family with multiple roles in mitosis	Shou *et al.* (2002); Hu *et al.* (2001); Bartholomew *et al.* (2001); Song & Lee (2001); Charles *et al.* (1998); Kitada *et al.* (1993)	*plo1*
BFA1	SOC	Component of a GAP for Tem1	Ro *et al.* (2002); Wang *et al.* (2000); Pereira *et al.* (2001)	*byr4*
BUB2	SOC	Component of a GAP for Tem1	Lee *et al.* (2001b); Pereira *et al.* (2000); Fesquet *et al.* (1999)	*cdc16*
TEM1	MEN	Rho family GTPase	Lippincott *et al.* (2001); Pereira *et al.* (2000); Jaspersen *et al.* (1998); Shirayama *et al.* (1994)	*spg1*
CDC15	MEN	Ser/Thr protein kinase	Mah *et al.* (2001); Visintin & Amon (2001); Menssen *et al.* (2001); Asakawa *et al.* (2001); Xu *et al.* (2000); Cenamor *et al.* (1999); Schweitzer & Philippsen (1991)	*cdc7*
DBF2	MEN	Ser/Thr protein kinase	Yoshida & Toh-e (2001); Lee *et al.* (2001a); Frenz *et al.* (2000); Toyn & Johnston (1994); Johnston *et al.* (1990)	*sid2*
MOB1	MEN	Protein that binds tightly to Dbf2	Luca *et al.* (2001); Komarnitsky *et al.* (1998)	*mob1*
CDC14	MEN	Protein phosphatase	Pereira *et al.* (2002); Stegmeier *et al.* (2002); Jaspersen & Morgan (2000); Bardin *et al.* (2000); Shou *et al.* (1999); Visintin *et al.* (1998)	*clp1/flp1*

to play a role in the MCP (Harrison *et al.*, 2001). However, genetic evidence indicates that the cell integrity pathway might target Mih1, the tyrosine phosphatase that relieves inhibitory phosphorylation by the Wee1-like kinase Swe1 on the mitotic CDK, constituting a different branch of the MCP (Harrison *et al.*, 2001; see Fig. 1). Careful dynamic localization experiments on components of this pathway might shed light on its relationship with actin-polymerization-dependent signalling.

A stunning observation is that, besides its association with Hsl1 and Swe1 at the bud side of the septins through budding, Hsl7 concentrates at the outer plaque of the SPB in G_1 unbudded cells (Cid *et al.*, 2001b). A C-terminally truncated version of Hsl7, which cannot be phosphorylated by Hsl1 *in vitro*, seems to remain longer at the premitotic SPBs, but eventually the protein is excluded from this structure during mitosis, returning only at the time of mitotic exit (Cid *et al.*, 2001b; a summary of cell-cycle-dependent localization of MCP proteins is given in Fig. 3a). We are unaware of the role of such localization and cell-cycle-regulated transition, but, remarkably, several other mitotic regulators switch between the bud neck and the SPB at different stages, as we comment below.

The spindle orientation checkpoint (SOC) operates from the SPB

A conserved checkpoint mechanism ensures correct spindle assembly and function, arresting mitosis in metaphase when the microtubular structure is damaged. In *S. cerevisiae*, the spindle assembly checkpoint (SAC) induces this arrest in metaphase (Hoyt *et al.*, 1991; Li & Murray, 1991; Wang & Burke, 1995; see Rudner & Murray, 1996, and Burke, 2000, for reviews). Although the Bub2 protein has been considered a component of the SAC, recent evidence points to a role of Bub2 in

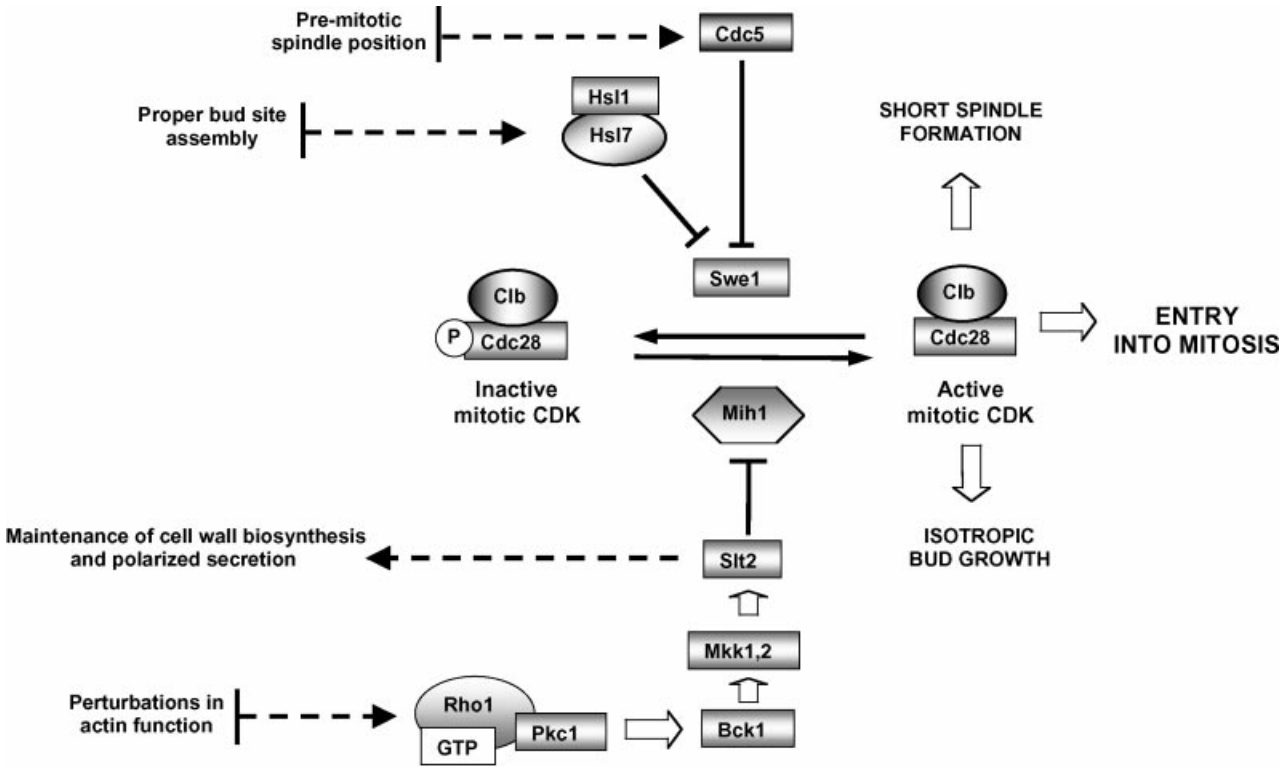

Fig. 1. Morphogenetic checkpoint (MCP) in *S. cerevisiae*. The mitotic CDK (the B cyclin–Cdc28 complex) is required for two events before the onset of anaphase: the formation of a short pre-mitotic spindle and the loss of apical bud growth for the shaping of an ellipsoidal bud. The mitotic CDK undergoes inhibitory tyrosine phosphorylation by the Swe1 kinase. The septin-associated proteins Hsl1 and Hsl7 translate morphogenetic signals from the bud neck that allow the timely inactivation of Swe1. The polo kinase Cdc5 may also be involved in Swe1 negative regulation, perhaps by sensing nuclear position in the pre-mitotic stage. The tyrosine phosphatase that counteracts Swe1 function, Mih1, senses alterations in the actin cortical cytoskeleton via the Rho1-led cell integrity MAP kinase pathway, which maintains polarized secretion through budding.

blocking the cell cycle further, namely at mitotic exit (Alexandru *et al*., 1999; Fesquet *et al*., 1999; Fraschini *et al*., 1999; Li, 1999; Krishnan *et al*., 2000; Daum *et al*., 2000; Wang *et al*., 2000; Lee *et al*., 2001b). It is now accepted that the principal role of Bub2 is to ensure that cytokinesis is only committed when the spindle pole destined to the daughter has moved through the neck (Hoyt, 2000; Adames *et al*., 2001), a function which is commonly referred to as the spindle position or spindle orientation checkpoint (SOC). To accomplish this function, Bub2 permanently associates with Bfa1 (Pereira *et al*., 2000; Lee *et al*., 2001b), and this binary complex is known to interact with components of the so-called MEN (Table 1; Fig. 2, see below). In particular, these proteins are thought to act as a two-component GTPase activating protein (GAP) for the small GTPase Tem1 (Shirayama *et al*., 1994; Bardin *et al*., 2000; Pereira *et al*., 2000; Shou *et al*., 1999). Activation of Tem1 by stabilization of its GTP-bound form is mandatory for mitotic exit. Interestingly, Bub2, Bfa1 and Tem1 are all bound to the outer plaque of the SPB, probably through their interaction with the SPB permanent component Nud1 (Bardin *et al*., 2000; Bloecher *et al*., 2000; Daum *et al*., 2000; Pereira *et al*., 2000; Gruneberg *et al*., 2000). Thus passage of one SPB through the neck into the daughter cell compartment becomes a prerequisite for the activation of Tem1. Reinforcing this hypothesis a specific activator for Tem1 – the GDP/GTP exchange factor (GEF) Lte1 – is asymmetrically localized only to the daughter cell (Bardin *et al*., 2000; Pereira *et al*., 2000). However, it is not clear how Bub2 is able to monitor such spatial signals. Clues to this might well be the reported interaction between Bub2 and the Cdc3 septin (Krishnan *et al*., 2000) or between a septin-interacting protein, Nfi1, and a protein related to mitotic spindle positioning, Nip100 (Drees *et al*., 2001). Still, there is no evidence that a transient interaction between the migrating SPB and the neck takes place at the G_2/M transition. Finely tuned localization experiments should reveal whether such transient interaction takes place. It has actually been reported that interactions between cytoplasmic microtubules and the neck affect the timing of mitotic exit (Segal *et al*., 2000; Adames *et al*., 2001). Also, recent evidence demonstrates that the specific association of the Bub2–Bfa1 complex with the SPB destined to the bud depends on the previous establishment of bud cortex–SPB connections via microtubular bundles rather than

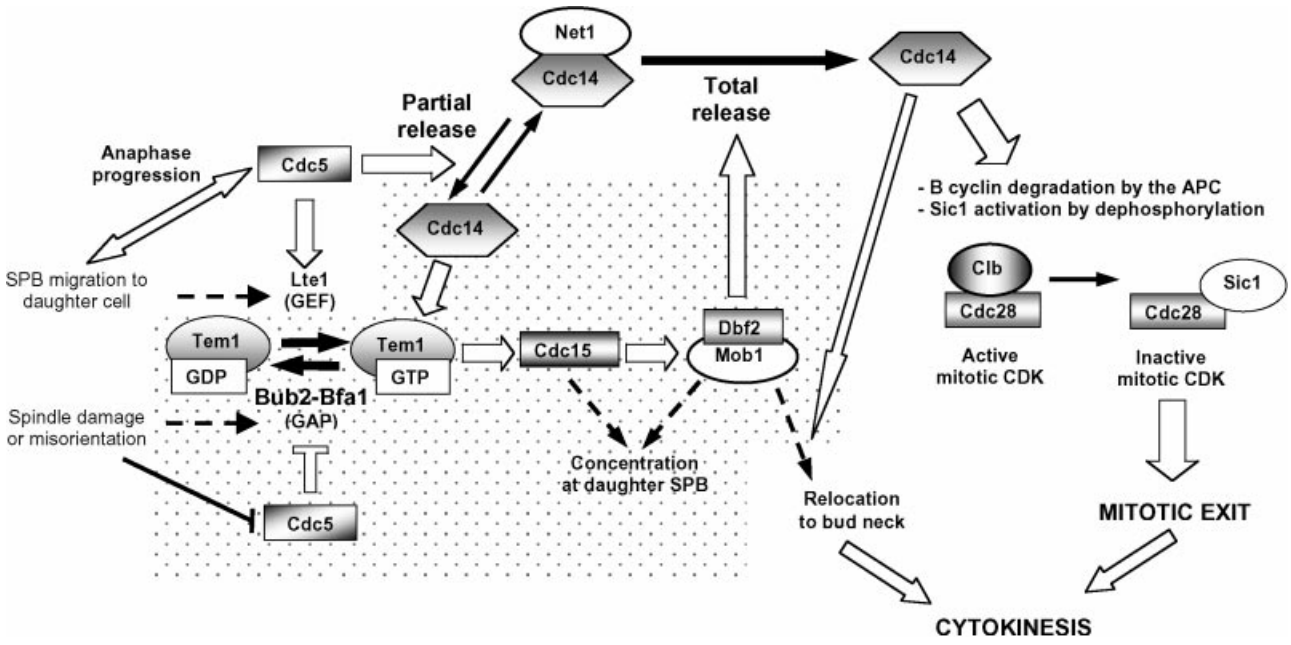

Fig. 2. Mitotic exit network (MEN) and its connection with the spindle orientation checkpoint (SOC). Activation of Tem1 by stabilization of its GTP-bound form is the trigger for MEN activation. This is inhibited by the Bub2–Bfa1 GAP complex, but activated by the daughter-cell-specific Lte1 GEF. The onset of anaphase allows the SPB, which contains the Bub2–Bfa1–Tem1 complex, to pass through the neck into the daughter cell. Cdc5, the polo kinase, involved in the regulation of multiple mitotic events, may transduce spatial signals to these proteins at this stage. Cdc5 regulation of the MEN occurs at three levels: by activation of the Lte1 GEF, by inhibition of the Bfa1–Bub2 GAP and by partial release of the Cdc14 protein from its nucleolar confinement. By the end of anaphase, GTP-bound Tem1 has successfully recruited the Cdc15 kinase to the daughter SPB, which in turn allows activation of the Mob1–Dbf2 kinase. As a consequence, the Cdc14 phosphatase is totally released, leading to APC activation and Sic1 expression for the eventual inhibition of the mitotic CDK, as well as relocation of some MEN components to the neck, including Cdc5, Cdc15 and especially Mob1–Dbf2, to promote cytokinesis. The relationship between CDK inactivation and the onset of cytokinesis is still poorly understood. The dotted area marks the regulatory events that take place at the SPB.

on putative permanent marks (Pereira *et al*., 2001). Molecular hints are only starting to appear of how checkpoint mechanisms subordinate cell cycle progression to cytoskeletal dynamics at the SPB and the cortex.

Mitotic control exerted by the polo/Cdc5 protein kinase

As in other eukaryotes, the budding yeast polo kinase homologue, Cdc5, plays multiple key roles in the coordination of mitosis (Table 1), including chromatid separation by phosphorylation of cohesin (Alexandru *et al*., 2001). Interestingly, Cdc5 is also localized to the SPB in the premitotic stage, such localization decaying at the time of APC activation and eventually disappearing prior to mitotic exit (Shirayama *et al*., 1998; Song *et al*., 2000; see Fig. 3). Also, although less conspicuously, the polo kinase localizes to the bud neck filaments, interacting with the septins directly via its polo domain (Song & Lee, 2001; Fig. 3). Consistent with this, its deregulation leads to the formation of ectopic cytokinetic rings (Song *et al*., 2000). However, the timing of Cdc5 association with the neck in the cell cycle has not been studied in depth.

Remarkably, several clues indicate that the *S. cerevisiae* polo kinase orthologue, Cdc5, may also participate in the MCP (Fig. 1). First, Cdc5 is able to interact with Swe1 *in vivo* (Bartholomew *et al*., 2001); second, overproduction of Cdc5 leads to an ectopic localization of Swe1 to the SPB, instead of to the bud neck, regardless of its kinase activity (Bartholomew *et al*., 2001); third, certain *cdc5* alleles display an elongated bud phenotype that is alleviated by *swe1*Δ (Song & Lee, 2001); and fourth, Cdc5 (but not Hsl1) is able to phosphorylate Swe1 *in vitro* (Cid *et al*., 2001b; M. Shulewitz, K. S. Lee & J. Thorner, unpublished results).

Moreover, a participation of the polo kinase in the SOC/MEN networks has been detected as well. Cell-cycle-dependent phosphorylation of Bfa1 is at least partially dependent on Cdc5 (Fig. 2; Lee *et al*., 2001b; Hu *et al*., 2001). Since the Bfa1–Bub2 complex seems to be bound to Tem1 permanently throughout budding (Pereira *et al*., 2000; Lee *et al*., 2001b), this phosphorylation event may be a major regulatory event for its GAP activity on Tem1 along mitosis. By these means, Cdc5 might negatively regulate Bfa1 GAP activity on Tem1 to promote mitotic exit (see below). Interestingly, activation of both the SAC and the SOC inhibits polo-

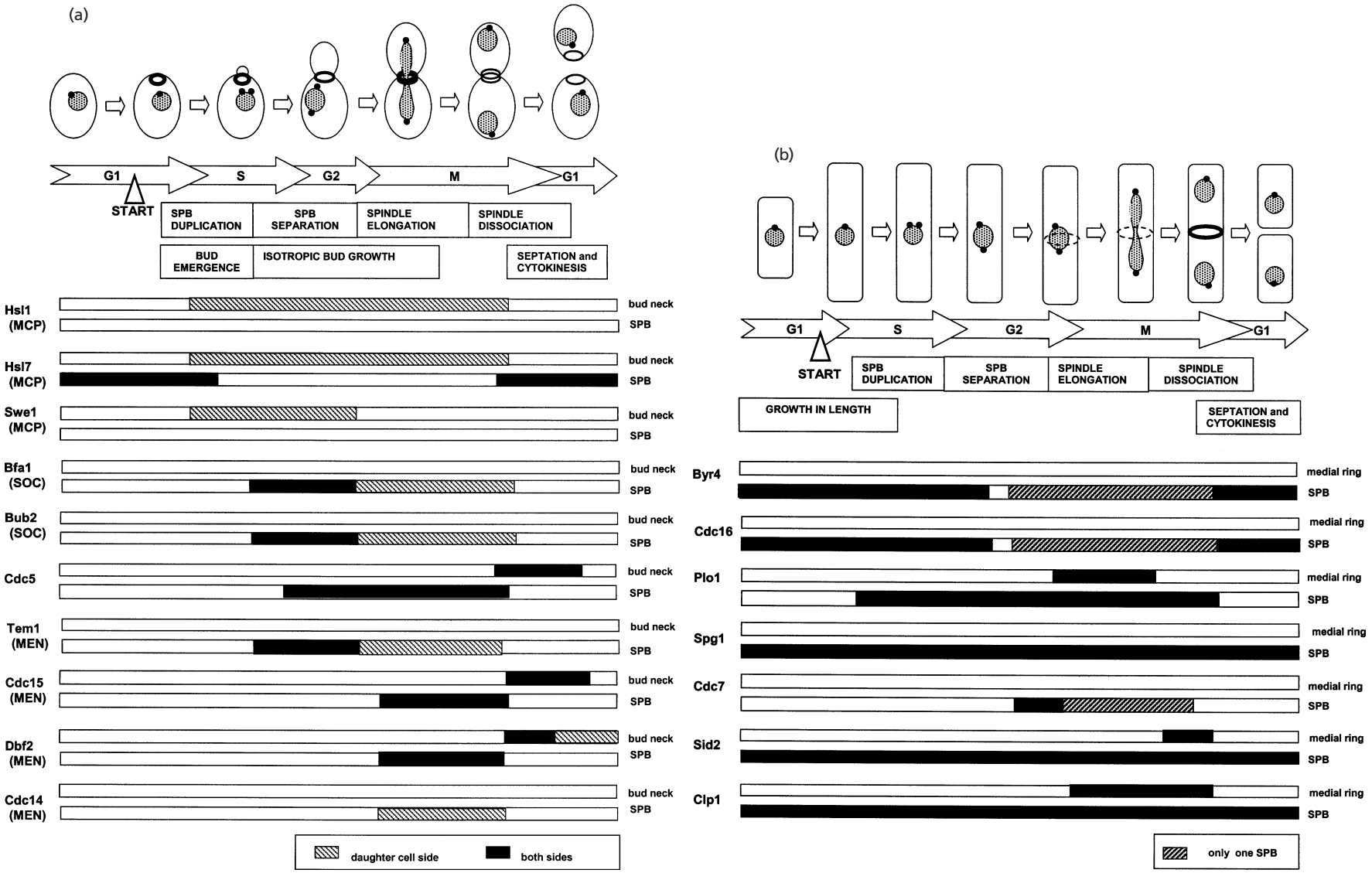

Fig. 3. (a) Dynamic localization of MCP, SOC and MEN components to the septin filaments at the bud neck and the SPBs during the budding cycle. The timing of each localization pattern is illustrated according to the cell cycle and morphogenetic stages shown in the picture at the top. In these drawings, the nucleus is dotted, SPBs are represented by solid dots and the septin ring by a circular line. Dashed lines in the bars indicate that localization to these structures is restricted within the daughter cell compartment. (b) Dynamic localization of SIN components in the fission yeast. As in (a), the timing of each localization pattern is illustrated according to the cell cycle and morphogenetic stages shown at the top. Again, the nucleus is the dotted area, SPBs are represented by solid dots, the medial ring by a dashed circular line, and the actomyosin cytokinetic ring by a bold circular line. Each line is paired to that of its corresponding *S. cerevisiae* orthologue in (a). Dashed lines indicate asymmetrical localization at the SPBs.

dependent Bfa1 phosphorylation (Hu *et al*., 2001). Concomitantly, the Tem1-GEF Lte1 is phosphorylated in mitosis as well in a Cdc5-dependent fashion (Fig. 2; Lee *et al*., 2001b).

In addition, Cdc5 is required for mitotic exit by modulation of MEN components (Lee *et al*., 2001a; Hu *et al*., 2001), namely by the controlled release of the Cdc14 protein phosphatase through anaphase (see below; Stegmeier *et al*., 2002; Pereira *et al*., 2002; Fig. 2). Thus, being required for the eventual activation of the APC that leads to the degradation of B cyclins, Cdc5 is a target for the APC itself (Cheng *et al*., 1998; Charles *et al*., 1998; Shirayama *et al*., 1998). The multifunctional role in mitotic control exerted by Cdc5 may be a key to our future understanding of how different cell-cycle-control mechanisms merge.

Temporally ordered association of components of the MEN at the SPB

Besides Tem1 and Cdc5, the MEN comprises a variety of regulatory proteins: the protein kinases Cdc15 and Dbf2 (which has a close homologue called Dbf20), the Mob1 protein, found tightly complexed with Dbf2, and the Cdc14 protein phosphatase (Table 1). All the components of the network are essential for the commitment and maintenance of B cyclin removal by the Cdh1–APC and for Sic1-dependent CDK inactivation after anaphase (Jaspersen *et al*., 1998; the MEN pathway has been recently reviewed by Pereira & Schiebel, 2001; McCollum & Gould, 2001; and Bardin & Amon, 2001). Genetic interactions among them are complex, but recent studies assessing the influence of MEN mutations in Dbf2 kinase activity suggest the following order of function (Lee *et al*., 2001a; Mah *et al*., 2001; Visintin & Amon, 2001; see Fig. 2): GTP-bound Tem1 binds to Cdc15 (Asakawa *et al*., 2001), which in turn activates – through phosphorylation of conserved residues – the Dbf2 kinase. Dbf2 must be complexed with Mob1 to undergo proper activation by Cdc15 (Mah *et al*., 2001). The eventual release of the Cdc14 phosphatase from the nucleolus (by dissociation from its sequestering factor Net1; Shou *et al*., 1999; Visintin *et al*., 1999) depends on all the components of the pathway and overproduction of this enzyme suppresses all the MEN mutations, suggesting that this phosphatase is the downstream effector of the pathway. However, although the total and maintained release of Cdc14 in late anaphase, determinant for mitotic exit, depends indeed on the MEN pathway, Stegmeier *et al*. (2002) and Pereira *et al*. (2002) recently reported that a partial release of Cdc14 from its nucleolar confinement is actually initiated in early anaphase. Such early release of Cdc14 depends on a novel pathway in which the polo kinase Cdc5 operates (Stegmeier *et al*., 2002; see Fig. 2 for an illustration on such biphasic release). Thus Cdc14 seems to constitute both an early mitotic activator and a late mitotic effector of the pathway, although its particular role at the early anaphase stage remains to be established.

The localization of the components of the MEN (Fig. 3a) should shed light on how these events are coordinated. Like Cdc5 and Tem1, Cdc14, Cdc15 and the Dbf2–Mob1 complex become attached to the SPB (Cenamor *et al*., 1999; Xu *et al*., 2000; Frenz *et al*., 2000; Menssen *et al*., 2001; Luca *et al*., 2001; Visintin & Amon, 2001; Pereira *et al*., 2002). However, Tem1 stains the daughter cell SPB from the premitotic stage (Bardin *et al*., 2000; Pereira *et al*., 2000), whereas Cdc14, Cdc15 and the Mob1–Dbf2 complex appear at the SPBs only in anaphase (Cenamor *et al*., 1999; Xu *et al*., 2000; Menssen *et al*., 2001; Yoshida & Toh-e, 2001; Pereira *et al*., 2002). At least in the case of Mob1–Dbf2, localization of these proteins to the SPB is a fine indicator of Tem1 activation by inhibition of the Bub2–Bfa1 GAP (Pereira *et al*., 2002). The early pool of Cdc14, released from the nucleolus by Cdc5 as cells commit anaphase, attaches to the SPB and binds Bfa1 and Tem1 (Pereira *et al*., 2002). Such binding is important for MEN activation, but such function has not been related to Cdc14 phosphatase activity (Pereira *et al*., 2002). However, in late anaphase, Cdc14 is involved in a dramatic dephosphorylation of Cdc15 (Xu *et al*., 2000; Jaspersen & Morgan, 2000) and Bfa1 (Pereira *et al*., 2002), as well as in the dephosphorylation of Cdh1 and Sic1 that lead to cyclin B depletion and CDK inactivation (Visintin *et al*., 1998). In summary (see Fig. 2), as the daughter SPB passes through the bud neck, Tem1 becomes activated by inhibition of the Bfa1 GAP, a phenomenon in which Cdc5 and, somehow, Cdc14 (released by Cdc5 from the nucleolus) participate. GTP-bound Tem1 recruits the Cdc15 and Dbf2 kinases to the SPB, resulting in a signal that, by the end of anaphase, triggers the total release of Cdc14, which is necessary for the eventual inactivation of the mitotic CDK. Remarkably, the players involved in such regulation have their headquarters at the outer plaque of the anaphase SPB (see the dotted area in Fig. 2).

Evidence is now gathering to suggest that some MEN components could play a direct role in cytokinesis. First, thermosensitive MEN mutants maintained at the restrictive temperature are able to eventually re-bud but are never able to perform septation (Jiménez *et al*., 1998); second, *tem1 net1* mutants, which cannot efficiently sequester Cdc14 at the nucleolus, exit mitosis but fail to separate (Shou *et al*., 1999); third, Tem1 is directly involved in triggering the dynamics of septin splitting and actomyosin contraction at cytokinesis, although it is not essential for actomyosin ring assembly (Lippincott *et al*., 2001); and, finally, Cdc15, Dbf2 and Mob1 relocate from the SPB to the neck at the time of cytokinesis, such behaviour depending on Cdc14 activity (Frenz *et al*., 2000; Xu *et al*., 2000; Yoshida & Toh-e, 2001). Also, the fact that a mutation that perturbs septin structure bypasses anaphase arrest in MEN mutants (Jiménez *et al*., 1998) suggests that relocation of the MEN kinases to the neck at the time of cytokinesis may constitute a feedback mechanism to monitor mitotic CDK inactivation at this point. Such a mechanism might work in a similar fashion to that of the MCP (also

dependent on neck-associated proteins) and might constitute a cytokinesis checkpoint.

Subcellular localization of mitotic regulators is similar in fission yeast

The medial ring in *S. pombe* does not seem to play a key role comparable to that of the bud neck in *S. cerevisiae*. Neither Nim1 nor Wee1, the fission yeast homologues of Hsl1 and Swe1, respectively (see Table 1 for nomenclature of the fission yeast orthologues for budding yeast genes), have been found associated with cytokinetic structures (Wu *et al*., 1996). Therefore, Wee1 has been related to general mitotic control (see Murakami & Nurse, 2000, for a review) rather than to coordination of the cell cycle and morphogenesis. However, based on genetic observations, it has been suggested that a cytokinesis checkpoint with a yet undetermined mechanism would subordinate nuclear division to the previous septation in a Wee1- and F-actin-dependent fashion (Le Goff *et al*., 1999; Liu *et al*., 2000). Future work will hopefully reveal if such a mechanism would be reminiscent of the budding yeast MCP. In any case, the current data make clear that the key role of the septin-based neck in cell cycle regulation in *S. cerevisiae* seems characteristic of the budding process, and will not be totally conserved in non-budding organisms.

S. pombe homologues to Bfa1 and Bub2, respectively Byr4 and Cdc16, are negative regulators of cytokinesis, since their elimination leads to uncontrolled septation (Minet *et al*., 1979; Fankhauser *et al*., 1993; Song *et al*., 1996; Jwa & Song, 1998) whereas their overproduction inhibits septation. However, the functional homologues of the small GTPase Tem1 and the protein kinase Cdc15 – respectively Spg1 and Cdc7 – exert the opposite effect, their inactivation leading to defects in the onset of septum formation and their overexpression to premature septation, indicating a positive role in the regulation of cytokinesis (Fankhauser & Simanis, 1994; Schmidt *et al*., 1997). Supporting the universality of the role of these pathways, the localization of Byr4, Cdc16, Spg1 and Cdc7 to the SPB is similar to that of their functional homologues in *Saccharomyces*, commented above (Fig. 3b). However, the timing of these localizations is different. The Ras GTPase Spg1, for instance, is permanently located at the spindle poles, and the associated kinase Cdc7 joins it through mitosis (Sohrmann *et al*., 1998). However, strikingly for a cell that divides symmetrically, as mitosis advances only one SPB is marked with Cdc7. Interestingly, Byr4 and Cdc16 also stain the SPB asymmetrically in mitotic cells, precisely bound to the Cdc7-free pole (Cerutti & Simanis, 1999). Also, *byr4* mutant cells show symmetric Cdc7 localization, supporting the notion that, as in the budding yeast, it is the active GTP-bound form of Spg1 that recruits Cdc7, whereas the GAP Byr4 favours a GDP-bound form that is unable to bind the kinase (Furge *et al*., 1999; Li *et al*., 2000). Other components of the SIN (for septation initiation network, which parallels the budding yeast MEN), such as Sid2 (the Dbf2 homologue) and Mob1, seem to play similar roles to those of their budding yeast homologues as well, localizing at the medial ring during cytokinesis and at the SPB along the cell cycle (Sparks *et al*., 1999; Hou *et al*., 2000). However, the association of the Sid2 complex with the SPB seems permanent rather than transient. Finally, recent characterization of the Cdc14 fission yeast homologue, Flp1/Clp1, has revealed a non-essential role in mitotic exit and cytokinesis (Cueille *et al*., 2001; Trautmann *et al*., 2001; reviewed by Oliferenko & Balasubramanian, 2001). Dispensability of Cdc14 for mitosis marks the main difference between the fission and budding models of mitotic regulation. Flp1/Clp1 is released from the nucleolus specifically in mitosis, as in *S. cerevisiae*, but, peculiarly, it was found to localize to the cytokinetic ring in mitosis, a phenomenon that has not been reported for Cdc14 (Cueille *et al*., 2001; Trautmann *et al*., 2001; Fig. 3b). The association of Flp1/Clp1 with the SPB, like that of Sid2, seems permanent through the cell cycle (Fig. 3b), although its release from the nucleolus in mitosis results in an enhancement of the spindle polar mark and an association with the whole spindle structure (Cueille *et al*., 2001; Trautmann *et al*., 2001; reviewed by Oliferenko & Balasubramanian, 2001).

The fission yeast polo kinase, namely Plo1, also seems to act at different levels of mitotic regulation. It is required for the assembly of the contractile actin ring at the cytokinetic plane, whereas its overexpression causes premature ring assembly and septation to occur (Ohkura *et al*., 1995). However, unlike the budding yeast Cdc5, Plo1 is essential for bipolar mitotic spindle assembly. Again, like Cdc5 in budding yeast, Plo1 temporarily decorates the SPB (Bahler *et al*., 1998; Mulvihill *et al*., 1999; Fig. 3) and its function in the SIN precedes that of Spg1 (Tanaka *et al*., 2001). Specifically, the polo kinase associates with the SPB as cells enter mitosis, such localization being dependent on activation of the mitotic CDK. Upon APC activation, in early anaphase B, Plo1 only weakly stains the spindle poles, partially decorating the spindle microtubules, like Clp1/Flp1 (a behaviour that has not been reported for either Cdc5 or Cdc14 in *Saccharomyces*), and eventually disappears from both structures at the time of cytokinesis. Plo1 is not associated with microtubular structures in interphase and forms a medial ring in metaphase (Bahler *et al*., 1998). A striking difference between budding and fission yeasts as regards their polo kinases is that, in the latter, protein levels do not seem to vary along the cell cycle, although its removal from the SPB does depend on the APC function (Cheng *et al*., 1998; Mulvihill *et al*., 1999).

In a nutshell, the fission yeast SIN seems to work essentially like the homologue budding yeast MEN. Not only are the sequential activation and SPB localization conserved, but correlation of APC-dependent B cyclin removal and cytokinesis, already known to exist in budding yeast (Jaspersen *et al*., 1998), also might be true for fission yeast (Chang *et al*., 2001). However, some aspects, related to the roles of their respective Cdc14 phosphatases and polo kinases, seem specific for each

model. Their study will help to elucidate universal as well as particular rules for the regulation of mitosis.

What sort of conversation arises between the bud neck and the SPB?

From the results reviewed here, the general idea arises that both the SPB and the budding yeast neck are key signalling 'hot spots' for cell-cycle regulation, enabling the coordination of cortical and nuclear events. The fact that CDK regulators are concentrated in these structures suggests that CDK targets operate from these points to orchestrate cytoskeletal rearrangements along cell division. The exchange of regulatory factors between the neck and the outer plaque of the SPB and phosphorylation–dephosphorylation events seem to be the key events in these processes, although we are only starting to learn how this regulation works. Regarding the latter point, antibodies against short phosphopeptide epitopes specifically recognize both SPBs and the bud neck in late anaphase cells, but not in other stages of the cell cycle (V. J. Cid & J. Thorner, unpublished results). Also in agreement with this view, the catalytic subunit of type I protein phosphatase in *S. cerevisiae*, Glc7, is also found at the bud neck, at the actomyosin contractile ring in cytokinesis and at the SPBs in late mitosis (Bloecher & Tatchell, 2000). Also, in the fission yeast, a regulatory subunit of protein phosphatase 2A that genetically interacts with the SIN components (Jiang & Hallberg, 2001) associates with the SPBs and medial ring (Le Goff *et al*., 2001). Although the targets of protein phosphatases in these spots are unknown, they may be counteracting or modulating the phosphorylation events discussed above. As a neat example of the SPB–neck interplay, it is worth remarking that Hsl7 is only localized to the SPB when mitotic regulators are absent and confined to the daughter-side neck along mitosis (Cid *et al*., 2001b). Consistent with this, Hsl7 is not found in a complex with either Tem1 or Bub2 (V. J. Cid, M. Molina & J. Thorner, unpublished results). Local Swe1 inhibition mediated by Hsl7 might account for a hypothetical local activation of B cyclin–CDK complexes at particular sites to allow SPB separation or loss of apical growth in pre-mitotic stages. The small GTPase Tem1 seems to appear at the SPB in opposite stages to Hsl7 (Bardin *et al*., 2000; Pereira *et al*., 2000). Since entry into mitosis involves passage of the Tem1-containing SPB through the neck and since both structures are in close proximity in the pre-mitotic stage, it is likely that important spatial signals are being transmitted. Recently, an actin-dependent checkpoint has been reported to monitor spindle orientation in fission yeast (Gachet *et al*., 2001). Like the MCP in *S. cerevisiae*, it is mediated by MAP kinase signalling. It might be general to fungal cells that a molecular cross-talk exists to coordinate spindle orientation and actin-based morphogenesis in stages prior to anaphase. Another as yet unexplored interplay may take place at the septation site, between components of the MEN and cytokinetic targets, signalling for APC-dependent inactivation of the CDK. Such connections will undoubtedly become challenges for yeast cell biologists in the near future.

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