Wee1-dependent mechanisms required for coordination of cell growth and cell division

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Summary

Wee1-related kinases function in a highly conserved mechanism that controls the timing of entry into mitosis. Loss of Wee1 function causes fission yeast and budding yeast cells to enter mitosis before sufficient growth has occurred, leading to formation of daughter cells that are smaller than normal. Early work in fission yeast suggested that Wee1 is part of a cell-size checkpoint that prevents entry into mitosis before cells have reached a critical size. Recent experiments in fission yeast and budding yeast have provided new support for this idea. In addition, studies in budding yeast have revealed the existence of highly intricate signaling networks that are required for regulation of Swe1, the budding yeast homolog of Wee1. Further understanding of these signaling networks may provide important clues to how cell growth and cell division are coordinated.

Key words: Wee1, Swe1, Cell growth, Cell division, Mitosis, Yeast

Introduction

To maintain a specific size, cells must coordinate their growth and division. Yeast cells are thought to use cell-size checkpoints to coordinate these two processes (Fantes and Nurse, 1977; Hartwell and Unger, 1977; Johnston et al., 1977; Nurse, 1975; Rupes, 2002). Cell-size checkpoints prevent passage through key cell-cycle transitions until cells have reached a critical size. In fission yeast, the critical size requirement is exerted primarily at the G2/M transition, whereas in budding yeast it is exerted primarily at the G1/S transition (Rupes, 2002). Although the existence of cell-size checkpoints was proposed over 20 years ago, the underlying molecular mechanisms have remained elusive, and how cells monitor cell size or cell growth is unknown. Genetic analysis of cell-size checkpoints is difficult because mutations that accelerate or delay cell-cycle progression may have indirect effects on cell size. For example, if cell growth is continuous during the cell cycle, then mutations that accelerate or delay the cell cycle will cause changes in cell size simply by changing the amount of time that cells have to grow. Despite these difficulties, early work in fission yeast suggested that the Wee1 kinase plays an important role in a checkpoint that coordinates cell growth and cell division at the G2/M transition (Fantes and Nurse, 1978; Nurse, 1975; Thuriaux et al., 1978).

Wee1-related kinases delay entry into mitosis and are required for cell-size control in yeasts

In fission yeast, Wee1 delays entry into mitosis by inhibiting the activity of Cdk1, the cyclin-dependent kinase that promotes entry into mitosis (Cdk1 is encoded by the *cdc2*$^{+}$ gene in fission yeast and the *CDC28* gene in budding yeast) (Russell and Nurse, 1987a). Wee1 inhibits Cdk1 by phosphorylating a highly conserved tyrosine residue at the N-terminus (Featherstone and Russell, 1991; Gould and Nurse, 1989; Lundgren et al., 1991; Parker et al., 1992; Parker and Piwnica-Worms, 1992). The phosphatase Cdc25 promotes entry into mitosis by removing the inhibitory phosphorylation (Dunphy and Kumagai, 1991; Gautier et al., 1991; Kumagai and Dunphy, 1991; Millar et al., 1991; Russell and Nurse, 1986; Strausfeld et al., 1991). Loss of Wee1 activity causes cells to enter mitosis before sufficient growth has occurred and cytokinesis therefore produces two abnormally small daughter cells (Fig. 1A) (Nurse, 1975). Conversely, increasing the gene dosage of *wee1* causes delayed entry into mitosis and an increase in cell size, indicating that the levels of Wee1 activity determine the timing of entry into mitosis and can have strong effects on cell size (Russell and Nurse, 1987a). Similarly, *cdc25*$^{-}$ mutants undergo delayed entry into mitosis, producing abnormally large cells, and an increase in the gene dosage of *cdc25* causes premature entry into mitosis and decreased cell size (Russell and Nurse, 1986). These observations show that Wee1 and Cdc25 are required for cell-size control, but they do not tell us whether these proteins play a direct role in controlling cell size. This issue is addressed further below.

Recent work has shown that Swe1, the budding yeast homolog of Wee1, also delays entry into mitosis and is required for cell-size control (Harvey and Kellogg, 2003; Jorgensen et al., 2002). *swe1*Δ cells undergo premature entry into mitosis before sufficient growth of the daughter bud has occurred, producing abnormally small cells (Fig. 1B) (Harvey and Kellogg, 2003). In addition, loss of function of Mih1, the budding yeast homolog of Cdc25, causes delayed entry into mitosis and produces abnormally large cells (Harvey and Kellogg, 2003; Russell et al., 1989) (G. Pal and D.K., unpublished). Finally, budding yeast Swe1 can rescue a temperature-sensitive *wee1*$^{-}$ mutant in fission yeast (Booher et al., 1993). These observations demonstrate that the basic functions of fission yeast Wee1 and Cdc25 have been conserved in budding yeast. Loss of function of Wee1-related kinases in *Xenopus* and *Drosophila* also causes premature entry into mitosis; however, a requirement for Wee1 in cell-

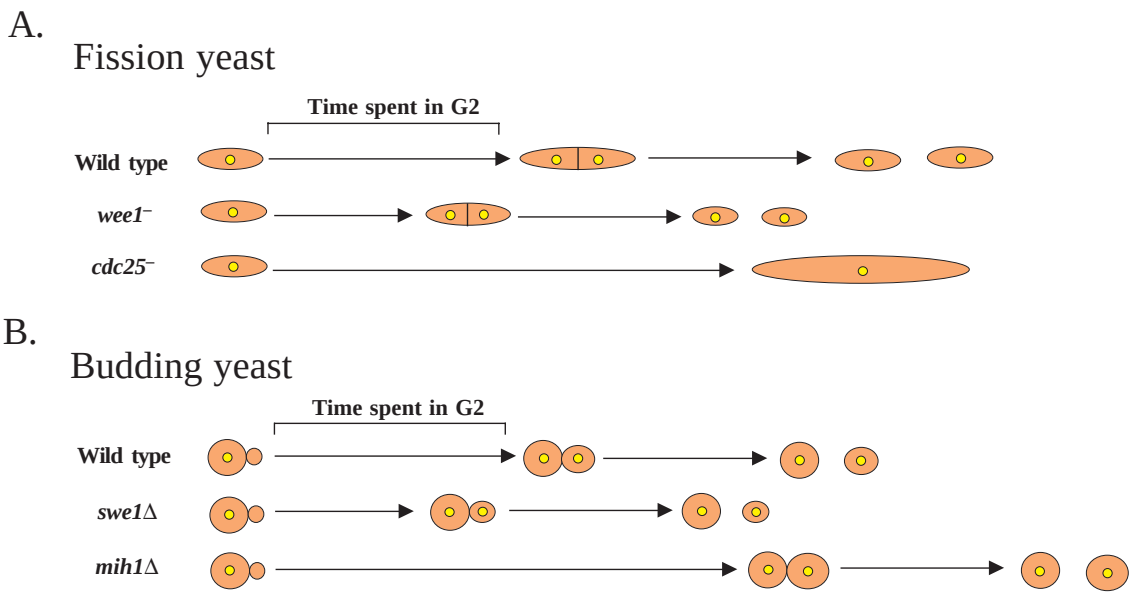

Fig. 1. Comparison of the effects of *wee1*⁻ and *cdc25*⁻ mutants in fission yeast and *swe1*Δ and *mih1*Δ in budding yeast. *cdc25*⁻ mutants arrest at G2/M in fission yeast, whereas *mih1*Δ cells undergo a delay but eventually enter mitosis. In wild-type budding yeast, the daughter cell is smaller than the mother cell, but undergoes additional growth during G1 (Hartwell and Unger, 1977).

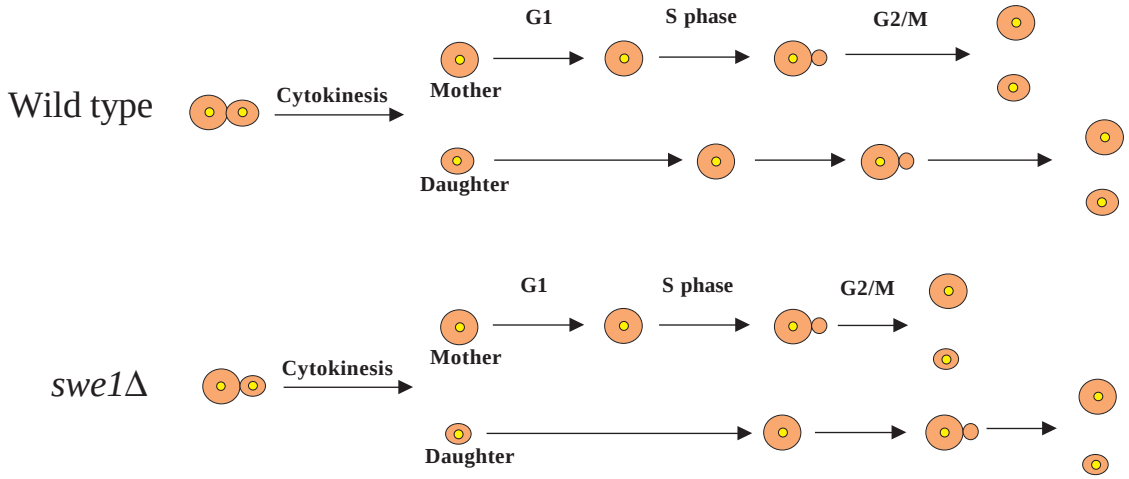

Fig. 2. A G1 cell-size checkpoint ensures that budding yeast daughter cells that are below a critical size spend more time in G1 undergoing growth before entering the cell cycle. As a result, a population of *swe1*Δ cells will consist of newly born daughter cells that are abnormally small, and mother cells that are of a normal size.

size control has not yet been demonstrated in animal cells (Walter et al., 2000) (S. Campbell, personal communication).

A requirement for Swe1 in coordination of budding yeast growth and division was missed in early studies because the cell-size phenotype of *swe1*Δ mutants is more subtle than that of fission yeast *wee1*⁻ mutants: a smaller proportion of *swe1*Δ cells show a cell-size phenotype (Harvey and Kellogg, 2003). This is probably owing to different growth and division strategies used by fission yeast and budding yeast. Fission yeast grow at their ends and undergo medial division to produce two daughter cells of equal size. Premature entry into mitosis therefore leads to birth of two equally sized daughter cells that are smaller than normal (Fig. 1A). Budding yeast grow by formation of a daughter bud, and all cell growth occurs in the daughter bud after G1 phase. *swe1*Δ cells enter mitosis prematurely before the daughter bud has finished growing, and cytokinesis therefore yields a mother cell of normal size and an abnormally small daughter cell (Fig. 1B) (Harvey and Kellogg, 2003). Mother cells are of a normal size because the G1 cell-size checkpoint delays cells in G1 until they have reached a critical cell size (Fig. 2) (Hartwell and Unger, 1977; Harvey and Kellogg, 2003; Johnston et al., 1977; Rupes, 2002). Furthermore, once a mother cell reaches this critical size, it can bud repeatedly to produce daughter cells. A population of *swe1*Δ cells should therefore be composed of mother cells that are of a normal size and newly born daughter cells that are abnormally small. Another difference between fission yeast and budding yeast is that *wee1*⁻ mutants are unviable at

elevated temperatures, whereas *swe1*Δ mutants are viable. This might be due to the existence of redundant mechanisms in budding yeast that can partially compensate for a loss of Swe1 function.

Physiological roles of Wee1-related kinases

Do Wee1-related kinases play a direct role in controlling cell size, or do they simply control the timing of entry into mitosis? Since cell growth is thought to be continuous during the cell cycle, the cell-size defects observed in *wee1*$^-$ mutants could be due simply to premature entry into mitosis, rather than to a loss of cell-size control. However, several observations argue against this interpretation. First, fission yeast cells decrease their size in response to nutrient limitation by lowering the critical size required for entry into mitosis (Fantes and Nurse, 1977). *wee1*$^-$ mutants fail to alter the critical size threshold at G2/M in response to nutrient deprivation, which suggests that Wee1 functions in the mechanism that sets the cell-size threshold in response to nutrient availability (Fantes and Nurse, 1978).

More recent experiments in both budding yeast and fission yeast provide further support for the idea that Wee1-related kinases play a direct role in coordinating growth and division at the G2/M transition. These experiments found that depolymerization of the actin cytoskeleton with latrunculin A causes a G2/M delay that is dependent upon tyrosine phosphorylation of Cdk1 (Lew and Reed, 1995; McMillan et al., 1998; Rupes et al., 2001; Sia et al., 1996). Wee1-related kinases are therefore part of a mechanism that monitors successful completion of an actin-dependent event. Since actin is required for cell growth, actin depolymerization might cause a Wee1-dependent G2/M delay because cells fail to reach a critical size. To test this idea, Rupes et al. used the *cdc25*$^-$ mutant to arrest fission yeast cells in G2 phase (Rupes et al., 2001). They kept the cells at the arrest point for varying periods to allow growth to different sizes and then released from the arrest in the presence of latrunculin A and assayed entry into mitosis. These experiments demonstrated that, once cells reach a critical size, they no longer undergo Wee1-dependent arrest in response to actin depolymerization (Rupes et al., 2001). Similarly, experiments in budding yeast have shown that, once daughter buds pass a critical size, cells no longer arrest at G2/M in response to actin depolymerization (Harvey and Kellogg, 2003). Taken together, these experiments are consistent with the idea that Wee1-related kinases function in a conserved checkpoint that monitors cell size or growth.

An alternative model proposed for budding yeast is that Swe1 functions in a bud morphogenesis checkpoint (Lew, 2000; McMillan et al., 1998; Sia et al., 1996; Sia et al., 1998). According to this model, Swe1 monitors successful completion of bud emergence or the status of the actin cytoskeleton. The observation that actin depolymerization blocks bud emergence and induces a Swe1-dependent G2/M delay is consistent with this idea. Similarly, temperature-sensitive mutations that block bud emergence also induce a Swe1-dependent G2/M delay. However, several observations argue against the existence of a checkpoint that monitors bud morphogenesis or the status of the actin cytoskeleton. First, actin depolymerization induces a Swe1-dependent G2/M delay in cells that have already undergone bud emergence and formed a medium-sized bud (Harvey and Kellogg, 2003; McMillan et al., 1998). Thus, actin depolymerization induces a G2/M delay when morphogenesis of the bud is largely complete and the bud is simply increasing in size. Second, cells with daughter buds that have grown beyond a critical size no longer undergo a G2/M delay in response to actin depolymerization (Harvey and Kellogg, 2003; McMillan et al., 1998). These two observations argue against a checkpoint that simply monitors bud emergence or the status of the actin cytoskeleton.

The G2/M delay caused by mutants that fail to undergo bud emergence can also be explained by a G2/M cell-size checkpoint. Since cell growth after G1 phase occurs entirely in the daughter bud, one might predict that cell size at G2/M is monitored in the daughter bud, and that a signal is sent from the daughter bud once a critical size has been reached to trigger entry into mitosis (Hartwell and Unger, 1977; Karpova et al., 2000). In cells that do not form a bud, such a signal would never be sent and cells should arrest at G2/M. Recent work has shown that daughter bud size has a strong influence on when cells enter mitosis, whereas mother cell size has no influence (Harvey and Kellogg, 2003). These observations are consistent with the idea that cell size at G2/M is monitored specifically in the daughter bud. The idea that budding yeast monitor the size of the daughter bud at G2/M makes sense because cells must ensure that the daughter bud is large enough to accommodate the nucleus before nuclear division occurs. How might cells specifically monitor the size of the daughter bud? One possibility is that they measure the concentration of a molecule present only in the daughter bud. Such a molecule could be localized uniquely to the daughter bud through an association with actin patches, which are found predominantly in the daughter bud.

It is clear that we still have much to learn about the physiological functions of Wee1-related kinases in yeasts. An interesting model consistent with the data in both fission yeast and budding yeast is that Wee1-related kinases monitor the total amount of polar growth that occurs. Fission yeast are rod-shaped cells and all growth occurs at the ends of the cell (polar growth). By contrast, budding yeast undergo a brief period of polar growth during bud emergence, but then grow over the entire surface of the bud (isotropic growth). A role for Wee1-related kinases in monitoring polar growth could help explain why loss of function of Wee1 causes a much more severe phenotype in fission yeast, since fission yeast rely almost entirely on polar growth that occurs during G2, whereas budding yeast have only a brief period of polar growth and then switch to isotropic growth.

Fission yeast and animal cells have multiple Wee1-related kinases. *Xenopus* has a second Wee1 kinase called Wee2 (Leise and Mueller, 2002). Wee1 is expressed maternally, whereas Wee2 is expressed zygotically. Interestingly, Wee2 is expressed in non-dividing tissues and might therefore play a role in arresting the cell cycle at specific developmental stages (Leise and Mueller, 2002). Fission yeast and vertebrates also each have a Wee1-related kinase: Mik1 and Myt1, respectively (Lundgren et al., 1991; Mueller et al., 1995a). In addition to phosphorylating the conserved tyrosine residue at the N-terminus of Cdk1, Myt1 can phosphorylate a neighboring threonine (Mueller et al., 1995a). Loss of Mik1 alone has no phenotype in fission yeast, but *mik1*Δ strongly enhances *wee1*$^-$ mutants, which suggests that Mik1 has related functions

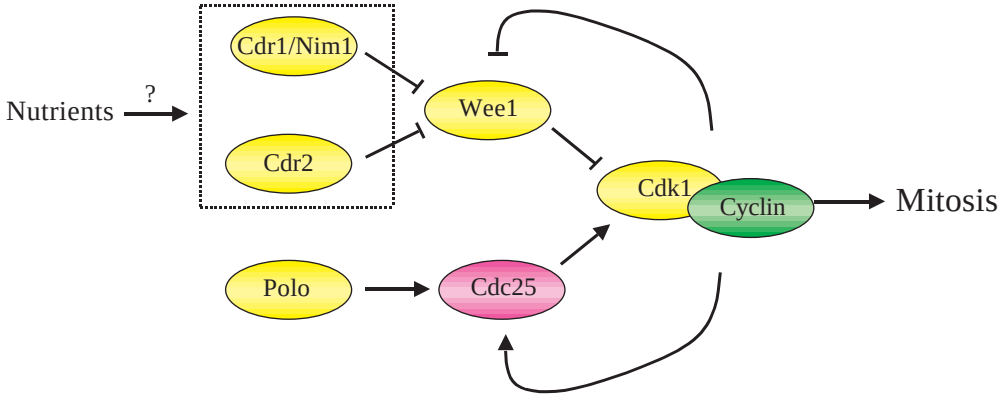

Fig. 3. A model for entry into mitosis based on work in fission yeast and *Xenopus*. Work in *Xenopus* suggests that Polo kinase might be required for activation of Cdc5 and entry into mitosis; however, work in fission yeast suggests that Polo kinase might not be required for entry into mitosis (see text). Kinases are shown in yellow and phosphatases are shown in pink.

(Lundgren et al., 1991). Mik1 is also required in fission yeast for mediating a DNA damage checkpoint at G2/M (Furuya and Carr, 2003; Rhind and Russell, 2001). Since it is unclear whether these additional Wee1-related kinases are required for coordination of growth and division, their functions and regulation are not reviewed extensively here.

Regulation of Wee1-related kinases

The signaling mechanisms that regulate Wee1 and Cdc25 are particularly interesting because they might provide important clues to how cells monitor cell size or cell growth. Wee1-related kinases from *Xenopus*, fission yeast and budding yeast undergo extensive hyperphosphorylation during mitosis, and the hyperphosphorylated form of *Xenopus* Wee1 isolated from mitotic extracts has reduced kinase activity (Harvey and Kellogg, 2003; Mueller et al., 1995b; Sreenivasan and Kellogg, 1999; Tang et al., 1993). Hyperphosphorylation of Wee1 in *Xenopus* extracts is dependent upon mitotic Cdk1 activity, and mitotic Cdk1 can bind and phosphorylate Wee1 in vitro (Mueller et al., 1995b; Tang et al., 1993). Similarly, recent work in budding yeast has shown that a Cdk complex composed of the Clb2 mitotic cyclin and Cdk1 can bind and phosphorylate Swe1 (S. Harvey, A. Sreenivasan, and D.K., unpublished). These observations have led to the idea of a positive-feedback loop in which a small amount of mitotic Cdk1 inactivates Wee1-related kinases, thereby triggering a rapid rise in mitotic Cdk1 activity and entry into mitosis (Fig. 3). Although this model is appealing, it has never been rigorously tested. Ideally, one would like to create a mutant version of Wee1 that cannot be hyperphosphorylated and then test whether it prevents entry into mitosis. If the model is correct, it raises a number of interesting questions. How might a small amount of mitotic Cdk1 trigger the feedback loop? Also, how can mitotic Cdk1 phosphorylate Wee1 if Wee1 phosphorylates and inhibits Cdk1? *Xenopus* Wee1 can undergo hyperphosphorylation in extracts that appear to lack mitotic Cdk1 activity, which suggests that at least one other kinase can directly phosphorylate Wee1 (Mueller et al., 1995b). Such kinases might play a role in triggering the positive-feedback loop.

Experiments in fission yeast and budding yeast have identified several kinases required for regulation of Wee1-related kinases in vivo. The fission yeast kinases Cdr1/Nim1 and Cdr2 were identified in a screen for mutants that fail to adjust cell size properly in response to nitrogen limitation (Young and Fantes, 1987). Cdr1/Nim1 was independently identified in a screen for high-copy suppressors of a *cdc25-ts* allele (Russell and Nurse, 1987b). Both *cdr* mutants undergo a prolonged G2/M delay that is eliminated in *cdr wee1* double mutants, and overexpression of Cdr1 drives premature entry into mitosis (Breeding et al., 1998; Feilotter et al., 1991; Kanoh and Russell, 1998; Russell and Nurse, 1987b). These observations suggest that the Cdr kinases promote entry into mitosis by inhibiting Wee1 activity (Fig. 3). The Cdr kinases can directly phosphorylate Wee1 in vitro; however, it is not clear whether they phosphorylate Wee1 in vivo (Coleman et al., 1993; Kanoh and Russell, 1998; Parker et al., 1993; Wu and Russell, 1993).

In budding yeast, an intricate signaling network is required for regulation of Swe1 and for coordination of cell growth and cell division at G2/M (Fig. 4). This network includes the kinases Gin4, Hsl1, Cla4 and Elm1. In addition, a number of proteins that are required for regulation of these kinases have been identified, including Nap1, Hsl7, Cdc42 and a family of proteins called the septins (Altman and Kellogg, 1997; Barral et al., 1999; Carroll et al., 1998; Edgington et al., 1999; Kellogg and Murray, 1995; Longtine et al., 2000; Ma et al., 1996; Shulewitz et al., 1999; Sreenivasan and Kellogg, 1999; Tjandra et al., 1998). Inactivation of this signaling network causes cells to undergo continuous polar growth during a prolonged G2/M delay, producing highly elongated cells that are abnormally large. The G2/M delay and cell elongation caused by inactivation of the network are reversed by deletion of the *SWE1* gene. Furthermore, inactivation of the network leads to a failure to fully hyperphosphorylate Swe1 (Barral et al., 1999; Longtine et al., 2000; Ma et al., 1996; Sreenivasan and Kellogg, 1999). Finally, overexpression of Swe1 causes a G2/M arrest and a continuous polar growth phenotype that is similar to the phenotype caused by inactivation of the signaling network. Taken together, these observations argue that the signaling network is required for hyperphosphorylation and inactivation of Swe1 to allow entry into mitosis. However, it is unclear how the kinases in the signaling network regulate Swe1 because none of them has been found to phosphorylate Swe1 directly. In addition, the physiological signals that regulate the network are poorly understood, although there is some evidence that components of the network respond to nutritional cues (Cullen and Sprague, 2000; Garrett, 1997; La Valle and Wittenberg, 2001). Many of the proteins that function in the network are highly conserved, suggesting that similar networks work in all

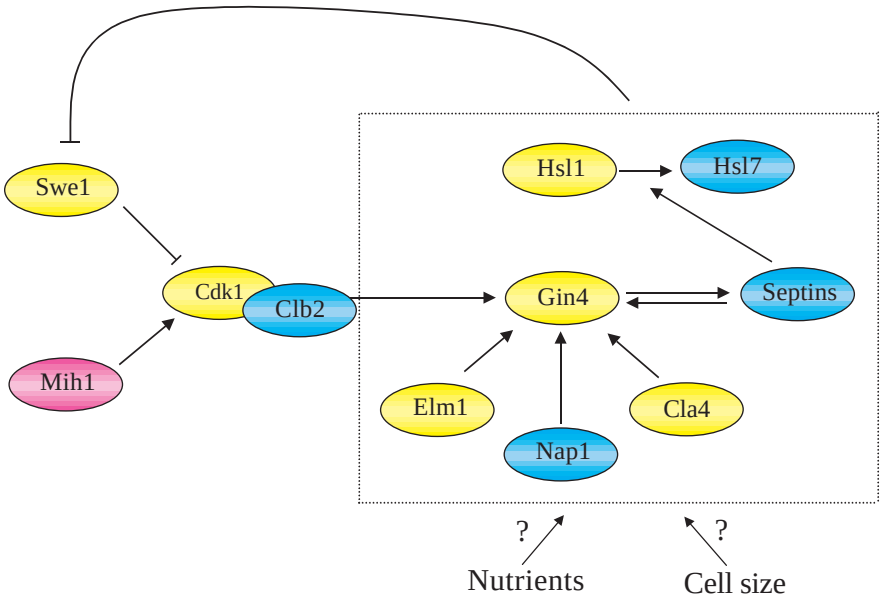

Fig. 4. Dependency relationships in the signaling network required for Swe1 regulation in budding yeast. Kinases are shown in yellow and phosphatases are shown in pink.

eukaryotic cells. Gin4 and Hsl1, for example, are related to fission yeast Cdr1/Nim1 and Cdr2.

Biochemical and genetic experiments have identified physical interactions and functional relationships that connect the proteins in the signaling network that regulates Swe1 (Figs 4, 5). For example, Hsl7 associates with both Swe1 and Hsl1, although in separate complexes (McMillan et al., 1999; Shulewitz et al., 1999). Gin4, Nap1 and the septins assemble into a complex during mitosis, which leads to hyperphosphorylation and activation of Gin4 (Mortensen et al., 2002). Hsl1 also forms a complex with the septins and is activated in a septin-dependent manner (Barral et al., 1999). Although the septins are required for Gin4 activation, Gin4 is required for septin organization, and Gin4 appears to control septin organization at least in part by direct phosphorylation of the Shs1 septin (Longtine et al., 1998; Longtine et al., 2000; Mortensen et al., 2002). Interestingly, Gin4 and Elm1 were identified in a screen for substrates of a mitotic CDK complex composed of the Clb2 cyclin and Cdk1 (Ubersax and Morgan, 2003). In addition, hyperphosphorylation of both Gin4 and Cla4 appears to be dependent upon Cdk1 (Mortensen et al., 2002; Tjandra et al., 1998). These results suggest that the signaling network may be part of a positive-feedback loop initiated by mitotic Cdk1 to regulate Swe1. However, regulation of these kinases appears to be more complex than simple direct phosphorylation by Cdk1. For example, although Gin4 appears to be a direct substrate of Clb2/Cdk1, hyperphosphorylation of Gin4 in vivo is also dependent upon Elm1, Cla4, Nap1 and the septins (Altman and Kellogg, 1997; Carroll et al., 1998; Sreenivasan and Kellogg, 1999; Tjandra et al., 1998). The molecular mechanisms underlying these dependency relationships are largely unknown.

One can imagine two models for the functions of the signaling network. First, the network might directly regulate Swe1. For example, kinases in the network may directly phosphorylate Swe1 or inactivate phosphatases that act on Swe1. Alternatively, the network may be required for the successful completion of events that are monitored by Swe1 (Barral et al., 1999; Longtine et al., 2000; Shulewitz et al., 1999). According to this model, failure to complete these events would block full hyperphosphorylation of Swe1, thereby triggering a Swe1-dependent delay in cell-cycle progression until the events are successfully completed. At this point, however, it is difficult to distinguish which of these models applies without knowing more about the molecular mechanisms that regulate Swe1 or the physiological signals that trigger Swe1-dependent G2/M delays.

In vertebrate cells, 14-3-3 proteins have been found to bind and positively regulate Wee1-related kinases (Lee et al., 2001; Rothblum-Oviatt et al., 2001; Wang et al., 2000). However, there is no evidence yet that yeast homologs of 14-3-3 proteins play a role in regulation of Wee1-related kinases or in mechanisms required for coordination of cell growth and division at G2/M.

Regulation of Wee1-related kinase protein levels

One series of studies concluded that the Swe1 protein is destroyed at the G2/M transition, and that Swe1-dependent checkpoint arrests are mediated by stabilization of the Swe1 protein (McMillan et al., 1999; McMillan et al., 2002; Sia et al., 1998). These studies also concluded that Swe1 is targeted for destruction by the SCF ubiquitin ligase complex. These studies followed the behavior of a 13-Myc-tagged Swe1 and determined cell-cycle stage by assaying bud emergence. However, more-recent experiments have failed to find a role for the SCF complex in Swe1 destruction (McMillan et al., 2002). Furthermore, experiments following the behavior of the endogenous Swe1 protein concluded that Swe1 is stable during G2/M and is not degraded until exit from mitosis (Harvey and Kellogg, 2003; Sreenivasan and Kellogg, 1999). Wee1-related kinases from *Xenopus* and fission yeast are also present throughout mitosis (Aligue et al., 1997; Mueller et al., 1995b; Tang et al., 1993). Several recent observations are consistent with the idea that Swe1 is normally degraded upon exit from mitosis. First, Swe1 is present in cells arrested in late mitosis by expression of a nondegradable form of the Clb2 mitotic cyclin (S. Harvey and D.K., unpublished). Second, the endogenous Swe1 protein is strongly stabilized in cells lacking the anaphase-promoting complex, which targets proteins for destruction during exit from mitosis and early G1 phase (Thornton and Toczyski, 2003). Wee1-related kinases are therefore probably present throughout G2 and early mitosis, and protein destruction is unlikely to play an important role in regulating Wee1-related kinases at the G2/M transition.

Proteins required for regulation of Wee1-related kinases are likely to have additional functions

Several observations indicate that proteins required for

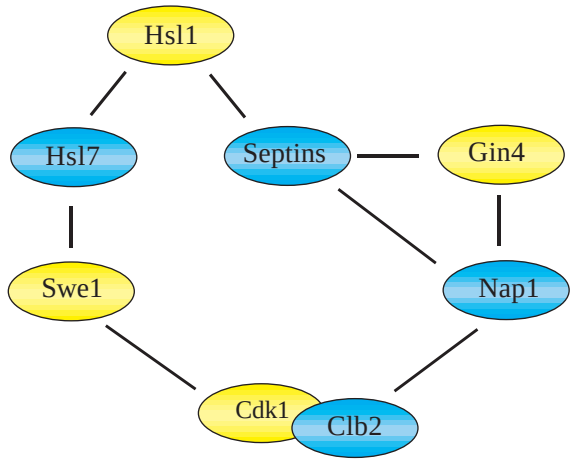

Fig. 5. Protein-protein interactions that have been detected thus far in the signaling network required for Swe1 regulation in budding yeast. The interaction between Swe1 and Clb2/Cdc28 is unpublished data (S. Harvey, A. Sreenivasan and D.K.). Kinases are shown in yellow.

regulation of Swe1 at G2/M are likely to have additional functions. First, although *swe1*Δ eliminates the G2/M delay and growth defects associated with *elm1*Δ, the *elm1*Δ *swe1*Δ cells show morphological defects, failure to localize the septins, severe cytokinesis defects and lethality at 37°C (Bouquin, 2000; Sreenivasan and Kellogg, 1999). Similar genetic interactions have been observed between fission yeast Cdr1/Nim1 and Wee1 (Breeding et al., 1998). Second, *HSL1* and *HSL7* were first isolated in a screen for mutations that cause synthetic lethality in cells lacking the N-termini of histones H3 and H4 (hence the name *HSL*, for histone synthetic lethal) (Ma et al., 1996). The functional significance of these genetic interactions remains unknown. Finally, Elm1, Cla4, Nap1, Gin4 and the septins are synthetically lethal with deletions of the redundant G1 cyclins *CLN1* and *CLN2* (Benton et al., 1993; Cvrckova et al., 1995; Cvrckova and Nasmyth, 1993; Sreenivasan et al., 2003; Zimmerman and Kellogg, 2001) Furthermore, experiments using an analog-sensitive allele of Elm1 have shown that inhibition of Elm1 kinase activity in early G1 phase causes a prolonged delay in bud emergence and in transcription of Cln2 (Sreenivasan et al., 2003). Inhibition of Elm1 later in the cell cycle causes a G2/M delay, which demonstrates that Elm1 has separable functions at G1 and G2/M. These observations suggest that proteins required for regulation of Swe1 at G2/M may also have functions during G1 phase.

Physiological roles of Cdc25-related phosphatases

Cdc25-related phosphatases reverse the inhibitory phosphorylation of Cdk1 by Wee1-related kinases, thereby promoting entry into mitosis. Entry into mitosis appears then to be determined by a balance of the activities of Cdc25 and Wee1 (Dunphy and Kumagai, 1991; Gautier et al., 1991; Lee et al., 1992; Russell and Nurse, 1986). In fission yeast, Cdc25 is required for entry into mitosis (Russell and Nurse, 1986). By contrast, the budding yeast homolog of Cdc25 (Mih1) is not required for entry into mitosis or for viability (Russell et al., 1989). However, *mih1*Δ cells show delayed entry into mitosis and are larger than wild-type cells, which suggests that the basic functions of fission yeast Cdc25 have been conserved in Mih1 (Harvey and Kellogg, 2003) (G. Pal and D.K., unpublished).

Dephosphorylation of Cdk1 by Cdc25 appears to be a crucial rate-limiting step for entry into mitosis in at least some cells. In the *Drosophila* embryo, for example, entry into mitosis during the first few divisions that occur after cellularization requires transcription of Cdc25, and ectopic expression of Cdc25 can drive entry into mitosis (Edgar and O'Farrell, 1990). Furthermore, fission yeast cells in which Cdc25 has been replaced by a constitutively active tyrosine phosphatase enter mitosis prematurely when growth is inhibited by actin depolymerization (Rupes et al., 2001). By contrast, inactivation of Wee1 and Mik1 does not cause premature entry into mitosis in cells that have been arrested in G2 phase by actin depolymerization. These results suggest that dephosphorylation of Cdk1 can be rate limiting for entry into mitosis in fission yeast, and that regulation of Cdc25 plays an important role in coordinating cell growth and cell division. In vertebrates and fission yeast, inhibition of Cdc25 in response to DNA damage plays an important role in enforcing the DNA damage checkpoint, which again demonstrates that regulation of Cdc25 can be limiting for entry into mitosis (Donzelli and Draetta, 2003; Furnari et al., 1999; Furnari et al., 1997; Kumagai et al., 1998a; Kumagai et al., 1998b; Peng et al., 1997; Rhind and Russell, 1998; Sanchez et al., 1997).

Regulation of Cdc25-related phosphatases

Xenopus Cdc25 undergoes hyperphosphorylation upon entry into mitosis, which stimulates its phosphatase activity (Izumi et al., 1992; Kumagai and Dunphy, 1992). Polo kinase binds to and phosphorylates Cdc25, and immunodepletion of Polo kinase from oocyte extracts blocks both Cdc25 hyperphosphorylation and activation of Cdk1 activity during entry into meiosis II (Donaldson et al., 2001; Kumagai and Dunphy, 1996; Qian et al., 2001). Cdc25 can also be directly phosphorylated by Cdk1 complexes that contain cyclin A or cyclin B (Izumi and Maller, 1993). These findings suggest the existence of another feedback loop leading to activation of mitotic Cdk activity (Fig. 3). In this loop, initial activation of a small amount of Cdc25 appears to be triggered by Polo kinase. This would lead to activation of a small amount of mitotic Cdk, which could then activate more Cdc25 to trigger a rapid rise in mitotic Cdk activity. However, in fission yeast, Polo kinase appears to be downstream of mitotic Cdk activity, and there is no evidence that budding yeast Polo kinase (encoded by *CDC5*) regulates entry into mitosis (Tanaka et al., 2001). It is therefore unclear whether regulation of Cdc25 by Polo kinase is part of a conserved mechanism that regulates entry into mitosis. The DNA damage and replication checkpoints in fission yeast and vertebrates regulate Cdc25 by activating the kinases Chk1 or Chk2, which phosphorylate Cdc25 (Blasina et al., 1999; Boddy et al., 1998; Furnari et al., 1999; Furnari et al., 1997; Peng et al., 1997; Sanchez et al., 1997). Phosphorylation of Cdc25 by these kinases inhibits Cdc25 phosphatase activity, thereby preventing entry into mitosis in the presence of damaged or unreplicated DNA. The mechanisms that might regulate Cdc25 or Mih1 activity in the context of a cell-size or cell-growth checkpoint are unknown.

Future directions

Studies of both fission yeast and budding yeast demonstrate that conserved Wee1-dependent mechanisms are required for coordination of growth and division at G2/M. Recent work has also revealed the existence of highly intricate signaling networks that are required for regulation of Wee1-related kinases and for coordination of cell growth and cell division at G2/M. Yet our understanding of Wee1-related kinases and their regulation remains limited. It is still controversial as to whether Wee1-related kinases play a direct role in controlling cell size, or whether they simply control the timing of entry into mitosis. A key to resolving this controversy may come from analysis of the signaling networks that regulate Wee1-related kinases, since this might lead to the identification of physiological signals that regulate Wee1-related kinase activity. Do these networks directly regulate Wee1-related kinases and, if so, do they respond to cell volume, cell growth, plasma membrane surface area, macromolecular content or nutrient availability? Future work needs to focus on identifying the physiological signals that the networks respond to and the molecular mechanisms by which they are relayed to the cell-cycle control machinery. An understanding of these mechanisms might lead to a better understanding of how the diverse cell sizes and morphologies observed in nature are generated.

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