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Regulation of the Cell Cycle by Protein Phosphatase 2A in *Saccharomyces cerevisiae*

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INTRODUCTION

The cell cycle comprises a series of sequential events that are orchestrated together for the production of two genetically identical progenies. The major mechanism that governs this complicated process is reversible protein phosphorylation catalyzed by many different types of kinases and phosphatases. Over the past two decades, the roles of protein kinases in cell cycle progression have been subjects of intensive studies. It is well established that activation of protein kinases, such as cyclin-dependent kinases (CDKs), is the driving force for transitions of the cell cycle from one stage to the next. However, recently, with a better understanding of the regulatory mechanisms of the cell cycle, there is an increasing appreciation for the roles of phosphatases in the process. It is believed that phosphatases, like their cognate kinases, provide both spatial and temporal regulation to many cell cycle events.

Many different types of phosphatases have been implicated in cell cycle regulation, among which is protein phosphatase 2A (PP2A). PP2A represents a group of highly abundant and ubiquitously expressed Ser/Thr phosphatases in eukaryotes; its activity is found in numerous cellular processes. It was first implicated in cell cycle control by findings showing that its inactivation promoted premature mitotic entry (31, 54, 98). Since then, its role in mitotic entry has been of a great interest (58, 122). Because PP2A is functionally and structurally conserved from yeast to human, the budding yeast (*Saccharomyces cerevisiae*), with its powerful genetic system, has served as an excellent model for studying the function of PP2A in cell cycle progression. This review focuses on many important findings concerning the roles of PP2A at different stages of the *S. cerevisiae* cell cycle, dating back to early 1990s, when PP2A was first identified in yeast. An excellent summary of the structure, regulation, and general functions of the yeast PP2A has been published previously (99), and the latest advances are nicely recapitulated in two recent review articles (25, 120).

PP2A and 2A-Like Phosphatases in Yeast

Subunit composition of the yeast PP2A. The yeast PP2A, like its counterparts in higher eukaryotes, is a multiprotein complex composed of three distinct subunits, namely, the A, B, and C subunits (Fig. 1). The A subunit is the structural subunit that serves as a scaffold to accommodate the other two subunits. The C subunit (PP2Ac) is the catalytic subunit, which, in complex with the A subunit, forms the dimeric core enzyme. The B subunit is the regulatory subunit that dictates the sub-

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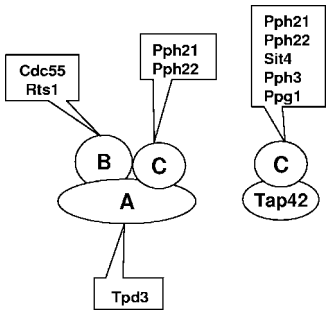

FIG. 1. Multiple forms of PP2A in yeast. See the text for details.

strate specificity and intracellular localization of the AC dimeric core enzyme (13). In yeast, the catalytic subunit is encoded by two nearly identical genes, *PPH21* and *PPH22* (86, 97). Each gene product contributes approximately half of the PP2A activity in the cell. Deletion of either gene is without notable effect on cell growth; however, deletion of both genes eliminates 80 to 90% of total PP2A activity in the cell and severely cripples cell growth (97). The residual PP2A activity in the absence of *PPH21* and *PPH22* is believed to be contributed by *PPH3*, which encodes a phosphatase structurally similar to PP2Ac (86). Deletion of *PPH3* does not affect cell growth but is lethal to *pph21 pph22* double deletion cells. Despite this, the Pph3 phosphatase displays enzymatic properties that differ significantly from those of PP2A (86). In addition, there is no indication that Pph3 is in complex with the A and B regulatory subunits. The A subunit in yeast is encoded by a single gene, *TPD3*. Deletion of *TPD3* is not lethal but renders yeast cells cold and temperature sensitive. The *tpd3* deletion cells are also sensitive to various stress conditions (107). The B subunits in yeast are encoded by two distinct genes, *CDC55* and *RTS1*, whose products are, respectively, the yeast homologues of the B and B′ subunits of PP2A in mammalian cells. None of the genes for the B subunits is essential for viability under normal growth conditions. However, their inactivation is lethal in some unfavorable conditions, such as reduced or elevated temperatures (28, 45, 92). Cells lacking both B subunits characteristically resemble *tpd3* cells, indicating that Cdc55 and Rts1 are the only B subunits in yeast (92). Formation of the PP2A holoenzyme in yeast is regulated by reversible methylation at the carboxyl terminus of the catalytic subunits (114, 116). Inhibition of the methylation greatly reduces the interaction of the catalytic subunits with the regulatory subunits (29, 53).

2A-like phosphatases. Yeast cells also contain a group of phosphatases called 2A-like phosphatases, so named because they are structurally more related to PP2A than to type 1 phosphatase. Like PP2A, 2A-like phosphatases, are also highly conserved from yeast to human (5, 71). Three different 2A-like phosphatases have been identified in yeast; these are Pph3, Sit4, and Ppg1, among which Sit4 is the only one that plays a critical role in cell growth and proliferation (85, 86, 102). Deletion of *SIT4* severely reduces cell growth and is lethal in certain strain backgrounds (103). Sit4 exists mainly in complex with the Sap proteins, upon which its function depends (69). Genetically, *SIT4* interacts extensively with the PP2A genes, suggesting that Sit4 may have partially overlapping functions with PP2A or may provide some PP2A activity in its absence (19, 81, 103).

Tap42-phosphatase complexes. In addition to the association with the A and B subunits, PP2Ac also exists in complex with another yeast protein termed Tap42 (Fig. 1) (19). Interaction of PP2Ac with Tap42 is independent of the A and B subunits, and thus the resulting Tap42-PP2Ac complex represents another form of PP2A (52). However, the amount of PP2Ac that associates with Tap42 accounts for only 5 to 10% of the phosphatase, indicating that the Tap42-PP2Ac complex is a minor form of PP2A (19).

The interaction of Tap42 with PP2Ac is a dynamic process that is regulated by the Tor signaling pathway in response to nutrient conditions (19). Inhibition of the Tor signaling pathway by rapamycin, an inhibitor of the Tor kinases, causes dissociation of Tap42 from PP2Ac and consequently inactivates the complex. Functionally, the Tap42-PP2Ac complex appears to be involved in cellular processes that differ from those requiring the PP2A holoenzyme (6, 26, 90). Tap42 also associates with Sit4 and other 2A-like phosphatases in a Tor-dependent manner (19, 111). Inactivation of Tor induces a rapid activation of the phosphatases, which is accompanied by their dissociation from Tap42. This correlation has led to the suggestion that Tap42 acts as a phosphatase inhibitor, which binds to and inhibits phosphatases in response to Tor signaling activity (51). Despite this, several lines of evidence suggest that Tap42 may play a positive role in phosphatase activity, at least to a subset substrate of the phosphatase (11, 26). Homologues of Tap42 have been found in many different organisms and in many cases have been demonstrated to be associated with 2A or 2A-like phosphatases, suggesting that the Tap42-containing phosphatase complexes are conserved (10, 16, 44, 66, 77, 78).

The study of PP2A in yeast has been greatly facilitated by the powerful genetic tools for the system. Genetic manipulations, such as mutational analysis, conditional inactivation, and gene deletion, are commonly used to address the roles of individual subunits of PP2A at different stages of the cell cycle. While inactivation of PP2Ac always results in loss of PP2A activity, inactivation of the regulatory subunits may not, depending on the individual subunits. Since the A and B regulatory subunits dictate the specificity of PP2Ac, inactivation of these subunits is generally believed to reduce PP2A activity toward its substrates. However, this may not be true for Tap42, which is believed to be a negative regulator of phosphatases (51, 88). In addition, there is genetic evidence indicating that in some cases inactivation of the regulatory subunits may cause runaway activity (112). Therefore, caution must be used when interpreting any genetic data.

PP2A AND 2A-LIKE PHOSPHATASES IN THE G_1/S TRANSITION

Requirement of Sit4 for Expression of the G_1 Cyclins

SIT4 was originally isolated by mutations in the gene that allowed the transcription of *HIS4* in the absence of *GCN*, *BAS1*, and *BAS2*, the transcription factors required for *HIS4* expression (2). Whether or not *SIT4* is essential for viability depends on another gene, *SSD1*, which is polymorphic in different strain backgrounds (103). Deletion of *SIT4* is lethal in

strains carrying an *ssd1-d* allele or an *ssd1* deletion but is not lethal in those containing an *SSD1-v* allele. In an *ssd1-d* background, inactivation of Sit4 causes cell cycle arrest at late G_1, suggesting that the Sit4 phosphatase is required for the G_1/S transition (103). A study using a temperature-sensitive (Ts^-) allele of *SIT4*, *sit4-102* revealed that in the arrested cells, the mRNA levels of the G_1 cyclins, including *CLN1*, *CLN2*, and *PCL1*/*HCS26*, were significantly lower than those in normal G_1 cells (32). Furthermore, it was found that expression of *CLN2* from a *SIT4*-independent promoter alleviated the G_1 block of the *sit4* mutant cells to a degree that DNA synthesis was permitted but the bud emergence was still blocked. This study establishes the role of Sit4 in controlling the expression of the G_1 cyclins. In addition, it also suggests a requirement of Sit4 for bud emergence.

Requirement of Sit4 for *SWI4* expression. The expression of the G_1 cyclins at late G_1 is controlled by the Swi4/6 cell cycle box binding factor (SBF) transcription factor, which is composed of Swi4 and Swi6, as well as by a Cln3-dependent positive-feedback activation of Cdc28 (15, 22, 80, 82). It was found that inactivation of Sit4 did not affect the mRNA level of *CLN3*, which was relatively constant throughout the cell cycle (32, 79). However, inactivation of Sit4 drastically reduced the expression level of *SWI4*, suggesting that Sit4 is required for *SWI4* expression. Consistent with this notion, expression of *SWI4* from a *SIT4*-independent promoter was found to enhance the level of the G_1 cyclins and cure the G_1 defect in the *sit4* cells (32). The requirement of Sit4 for *SWI4* expression, and hence for SBF activity, explains the role of Sit4 in the G_1/S transition. In addition to controlling G_1 cyclin expression, SBF is also required for expression of genes needed for bud emergence, which may explain the role of Sit4 in bud emergence (43, 49).

The genetic network of *SIT4* involved in G_1/S. *SWI4* expression is regulated mainly by the Mlu1 cell cycle box binding factor (MBF) transcription factor, which is composed of Mbp1 and Swi6 (35, 96). Whether and how Sit4 regulates MBF remains unclear. In efforts to elucidate the mechanism, many *SIT4*-interacting genes were isolated through various genetic screens (20, 21, 101, 102). Some of the genes are required for the G_1/S transition but act in a way independent of Sit4. One example is the *SSD1* gene, which encodes an RNA binding protein that is able to bind and stabilize mRNA (106). An *SSD1-v* allele may suppress the lethality of the *sit4* deletion by enhancing the mRNA levels of the G_1 cyclins.

Among the SIT4-interacting genes, *SIS2*/*HAL3* is epistatic to *SIT4* and is involved in regulation of Swi4 and/or Swi6. Overexpression of *SIS2*/*HAL3* was found to enhance the accumulation of the mRNAs of *CLN1*, *CLN2*, and *SWI4* at G_1 and to suppress the growth defects of *sit4* mutants (20). Since *SIS2*/*HAL3* encodes an inhibitor of Ppz1, a type 1 phosphatase-related phosphatase, its overproduction is expected to downregulate Ppz1 activity (18). Indeed, deletion of *PPZ1*, like *SIS2*/*HAL3* overexpression, was found to enhance G_1 cyclin expression and suppress the growth defect of *sit4* mutants. In contrast, overexpression of *PPZ1* or deletion of *SIS2*/*HAL3* reduced the mRNA levels of the G_1 cyclins and was lethal to cells lacking Sit4 (12, 20). These observations suggest that the Sit4 and Ppz1 phosphatases play opposite roles in regulation of cell cycle progression.

Another *SIT4*-interacting gene that is involved in regulating the expression of the G_1 cyclins is *BCK2*. Overexpression of *BCK2*, like that of *SIS2*/*HAL3*, was found to induce high-level expression of the G_1 cyclins and suppress the lethality of a *sis2 sit4* double deletion (21, 76). The target of Bck2 action in controlling G_1 cyclin expression appears to be the SBF transcription factor. Bck2 was shown to activate SBF collaboratively with Cln3. However, unlike the case for Cln3, the effect of Bck2 on SBF activation was not dependent upon Cdc28 (27, 115).

A role for Sit4 in the Pkc1-MAPK pathway. The molecular basis for the genetic interaction of *SIT4* with *PPZ1* and *BCK2* is still poorly understood. However, all three genes have been shown to be involved in a signaling pathway, called the Pkc1–mitogen-activated protein kinase (MAPK) pathway or the cell wall integrity pathway (1, 57). This pathway plays a major role in regulating expression of genes required at late G_1 and S phases under normal conditions and is also needed for transcriptional changes in response to stresses that alter cell wall integrity (60). At the center of the pathway lies Pkc1, which controls gene expression, actin cytoskeleton, and cell wall expansion through both MAPK-dependent and -independent mechanisms (46). The signaling activity of the pathway is often initiated at the cell surface and culminates at the activation of the MAPK Mpk1 (also known as Slt2). The targets of Mpk1 include two transcription factors, SBF and MBF (3, 49). Mpk1 has been shown to phosphorylate Swi6, the common component of SBF and MBF, which contributes to its regulation of both SBF and MBF (70). As such, the connection of Sit4 with the Pkc1-MAPK pathway provides a potential mechanism by which the phosphatase controls SBF and MBF activity.

Under normal conditions, the Mpk1 kinase retains a basal activity and is activated only at late G_1 before bud emergence (121). However, in the absence of Sit4, Mpk1 is hyperactivated, suggesting that Sit4 plays a negative role in regulating the signaling activity of the pathway (1). The notion that the G_1 delay in the *sit4* cells may be caused by an elevated signaling activity of the pathway is supported by the finding that inactivation of Pkc1 was able to cure the G_1 delay associated with the *sit4* cells. In addition, overexpression of Pkc1, like *sit4* deletion, causes a G_1 delay (1). Nevertheless, inactivation of Mpk1 does not suppress the growth defect, nor does it overcome the G_1 delay of the *sit4* cells, suggesting that Sit4 contributes to at least some of the Pkc1-dependent processes that are not regulated by Mpk1. Therefore, if Sit4 mediates the G_1 transition through its role in the Pkc1-MAPK pathway, the mechanism is more complicated than simply downregulating Mpk1 activity.

Saps versus Tap42. The Sit4 phosphatase exists in two distinct complexes, namely, the Sap-Sit4 and Tap42-Sit4 complexes (19), which raises the question of which complex is involved in the G_1/S transition. The function of Sit4 depends on the Sap proteins. Deletion of the *SAP* genes is like deletion of *SIT4*, causing a growth defect in an *SSD1-v* background and lethality in an *ssd1-d* background (69). Formation of the Sap-Sit4 complex appears to be cell cycle dependent. Two Sap members, Sap155 and Sap190, associate with Sit4 at G_1 but dissociate during mitotic phase. In addition, overexpression of *SAP155* is able to suppress the growth defect of *sit4-102* cells, which are defective for the expression of *CLN1*, *CLN2*, and

PCL1/*HCS26* (69). These observations seem to indicate that the Sap-Sit4 complex is the one responsible for the G_1 function of Sit4. On the other hand, Tap42 was isolated as a high-copy suppressor of the *sit4-102* mutant, in which the mutant Sit4 protein was specifically defective for its interaction with Tap42 (19, 111). In addition, inactivation of Tap42 induces a late G_1 arrest, as seen for *sit4-102* cells grown at the nonpermissive temperature (19). These seemingly conflicting observations may suggest an intrinsic functional connection between the two complexes. Because Sit4 activation accompanies its release from Tap42, one possibility is that upon release from Tap42, Sit4 becomes active by associating with Saps and subsequently acts on its targets involved in the regulation of G_1 cyclin expression.

The Ceramide-Activated Protein Phosphatase

The complicated subunit composition and regulatory mechanisms that control the formation of PP2A make it very difficult to upregulate PP2A activity by genetic approaches, and consequently, it is hard to assess the effect of PP2A activation on cell cycle progression. However, the existence of a ceramide-activated PP2A in yeast has allowed a glimpse into the effect of an activated PP2A on cell cycle progression. Ceramide is an intermediate metabolite of the sphingomyelin cycle and is produced by hydrolyzation of membrane sphingomyelin (42). It serves as a secondary messenger that eventually leads to growth inhibition and cell differentiation. The direct targets of ceramide include a ceramide-activated protein kinase and a ceramide-activated protein phosphatase (CAPP) (41). The latter has been demonstrated to be protein phosphatase 2A. In yeast, the cell-permeable ceramide analog C_2-ceramide induces a dose-dependent inhibition of cell growth and cell cycle arrest at G_1 (33). Inactivation of either Cdc55 or Tpd3, the two regulatory subunits of PP2A, renders yeast cells ceramide resistant, indicating that a PP2A holoenzyme containing Cdc55 and Tpd3 is the primary target of ceramide (81). However, the identity of the catalytic subunit of CAPP is less clear. The observation that inactivation of Sit4 reduces CAPP activity and renders yeast ceramide resistant appears to suggest that Sit4 is the catalytic subunit. However, direct evidence showing that Sit4 is in a complex with Cdc55 and Tpd3 is absent. In addition, CAPP activity seems to be partially dependent on Pph21 and Pph22. Therefore, the possibility that Pph21 and Pph22 contribute to CAPP activity cannot be excluded (81).

The mechanism by which an activated CAPP induces G_1 arrest is unclear. *SIT4* has been shown to genetically interact with genes involved in the RAS pathway and in G_1 cyclin expression (32, 103), both of which are required for the G_1/S transition. It is possible that the function of CAPP in these events contributes to its role in the G_1/S transition.

REGULATION OF MITOSIS BY PP2A

The Positive Role of PP2A at Mitotic Entry

In *Schizosaccharomyces pombe* as well as higher eukaryotes, PP2A plays a negative role in the regulation of mitotic entry. Inactivation of PP2A stimulates CDK activity and, consequently, premature mitotic entry (55, 59, 84). However, in *S. cerevisiae*, PP2A appears to have a positive role during the G_2/M transition. Inactivation of PP2Ac by conditional mutations leads to cell cycle arrest at G_2/M and accumulation of cells with a 2N DNA content, a short spindle, and small aberrant buds (30, 65). The mitotic entry in yeast is controlled mainly by two mechanisms, i.e., G_2 cyclin accumulation and the inhibitory phosphorylation of Cdc28 at tyrosine 19 (9, 36). An examination of the activity of Clb2-Cdc28, the major CDK at G_2, in a Ts^- allele of *PPH21*, *pph21-102* (in the *pph21 pph3* background), revealed a reduced CDK activity in comparison with that in wild-type cells at the permissive temperature, which was further diminished upon the shifting of the cells to the nonpermissive temperature. Removal of the inhibitory phosphorylation site, tyrosine 19, in Cdc28 drastically enhanced Clb2-Cdc28 activity in the mutant cells, allowing them to overcome the G_2 block. Despite this, the mutant cells were unable to sustain the enhanced Clb2-Cdc28 activity when incubated at the nonpermissive temperature and, consequently, were unable to complete the next round of mitosis (65). In addition to the reduced Clb2-Cdc28 activity, the expression level of *CLB2* in the mutant cells was lower than that in wild-type cells. However, overexpression of *CLB2* was unable to increase the Clb2-specific Cdc28 kinase activity. It was thus believed that the low expression level of *CLB2* in the mutant cells was caused by loss of the Clb2-Cdc28-dependent positive-feedback control on *CLB2* expression, owing to a low Cdc28 activity. Taken together, these findings suggest a role for PP2A in regulation of the phosphorylation of Cdc28 at tyrosine 19 but not in controlling the accumulation of the G_2 cyclins.

The phosphorylation at tyrosine 19 of Cdc28 is reciprocally regulated by the Swe1 kinase and Mih1 phosphatase, the counterparts of Wee1 and Cdc25, respectively, in *S. pombe* and higher eukaryotes (9, 87). In fission yeast as well as in mammals, Wee1/Cdc25-dependent phosphorylation plays a major role in controlling CDK activity and mitotic entry. Activation of Cdc25 promotes mitotic entry by removal of the inhibitory phosphorylation of Cdc2 at tyrosine 15, which is equivalent to tyrosine 19 of Cdc28 in yeast (8, 14, 73). PP2A downregulates Cdc25 by dephosphorylating it at a site that is required for its activation, thus imposing a negative effect on mitotic entry (83). In *S. cerevisiae*, inactivation of PP2A has been shown to stabilize Swe1 and alter the phosphorylation level of Mih1 (117), suggesting that the yeast PP2A, like its counterparts in other eukaryotes, regulates the G_2/M transition by a similar mechanism (Fig. 2). However, in yeast, the Swe1/Mih1-dependent mechanism is not essential for cell cycle progression under normal growth conditions and is utilized mainly as a means to coordinate the nuclear cycle with the process of bud formation (see below) (9, 61). Deletion of *MIH1* or overexpression of *SWE1* causes no catastrophic effect on cell cycle progression other than a delay at G_2 (9). Therefore, even though PP2A regulates the G_2/M transition by modulating Swe1 and Mih1 activity, the impact of this regulation on cell cycle progression is limited. Why, then, does inactivation of PP2Ac cause a G_2 block?

PP2Ac exists in three distinct forms of PP2A enzyme, including the Tap42-PP2Ac complex and the Cdc55- and Rts1-containing PP2A holoenzyme (Fig. 1). Conceivably, inactivation of PP2Ac would affect all three different enzymes, producing a pleiotropic effect. In addition to the G_2/M block,

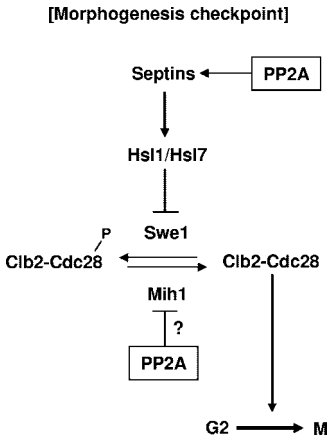

FIG. 2. PP2A positively regulates mitotic entry. PP2A is required for proper organization of the septin rings, which promotes mitotic entry by downregulating Swe1. PP2A may also inhibit the Mih1 phosphatase, the positive regulator of Clb2-Cdc28 kinase. However, the impact of this inhibition on Clb2-Cdc28 activity is likely to be inconsequential under normal growth conditions.

the cells lacking PP2A activity display other defects, such as aberrant bud morphology, random distribution of actin, and cell lysis defects (30, 65). As such, the terminal phenotypes associated with inactivation of PP2Ac are a synergistic effect caused by inactivation of the all three forms of PP2A. This notion raises the possibility that the G_2 block associated with PP2A inactivation is caused by multiple defects during G_2 phase. Indeed, study of two different *pph22* Ts^- alleles (in a *pph21 pph3* background) revealed a severely delayed and aberrant nuclear division when the mutant cells were incubated at the nonpermissive temperature, which was accompanied by a rapid loss of viability. Supplementing the growth medium with 1 M sorbitol, an osmotic stabilizer, was found to prevent the viability loss and restore cell growth to various degrees, depending on the severity of the mutant allele (30). Similarly, sorbitol was able to partially suppress the Ts^- phenotype of *pph21-102* (H. Wang and Y. Jiang, unpublished observation). These findings suggest that the cell lysis defects contributed to the lethality of the *pph* mutant cells at the nonpermissive temperature. Because the mutant cells were able to grow in the presence of sorbitol, it is conceivable that sorbitol cured the G_2 block of the mutant cells. Therefore, the direct effect of PP2Ac inactivation on cell cycle progression at G_2/M is not a block but a delay, which is likely to be caused by the low Clb2-Cdc28 activity in the affected cells.

The Negative Role of PP2A at Mitotic Exit

The onset of anaphase in yeast is triggered by activation of the Cdc20-dependent anaphase-promoting complex or cyclosome (APC/C), which results in ubiquitination and degradation of Pds1, the anaphase inhibitor (securin) that sequesters and inhibits the separase, Esp1 (64, 109). Once released, Esp1 promotes sister chromatid segregation by cleaving the cohesin protein complex that holds the sister chromatids together (50). Esp1 also initiates a signaling cascade called the FEAR pathway (for "Cdc fourteen early anaphase release") that leads to the early-anaphase release of the Cdc14 phosphatase from the nucleolus (7). Completion of chromatid segregation in late anaphase and telophase activates the mitosis exit network (MEN) pathway and triggers further release and accumulation of Cdc14, which in turn inactivates CDK by promoting the destruction of the G_2 cyclins and by stabilizing the CDK inhibitor Sic1, thus driving mitosis exit (24, 104).

Regulation of Cdc20. PP2A is implicated in mitotic exit by the findings that deletion of *CDC55* allows sister chromatid segregation in the absence of a fully assembled spindle, which normally arrests cell cycle progression at metaphase by activating the spindle checkpoint (74). Genetic analysis indicates that the Cdc55-containing PP2A is involved in at least two distinct steps during this stage of the cell cycle, the metaphase-anaphase transition and mitotic exit (112, 113, 118). Deletion of *CDC55* was found to partially suppress the temperature sensitivity of *cdc20-1*, a mutant allele that was defective in transition from metaphase to anaphase due to defects in APC/C activation (89, 112). Furthermore, Pds1, the target of Cdc20-dependent APC/C, was found to be degraded in *cdc55* cells during spindle checkpoint activation, which normally inhibits Cdc20 and prevents Pds1 degradation (118). These findings suggest that Cdc55 is involved in the regulation of Cdc20-dependent APC/C activation, the initial step leading to sister chromatid segregation and onset of anaphase. The lack of a full suppression of *cdc20-1* also indicates that deletion of *CDC55* does not bypass the requirement of Cdc20, thus supporting the possibility that the deletion enhances Cdc20 activity. Consistent with this notion, it was found that overexpression of *CDC20* allowed premature mitotic exit in the absence of a fully assembled spindle (48).

A negative role in the FEAR and MEN pathways. Deletion of *CDC55* has also been shown to suppress many mutations causing defects in mitotic exit, including *spo12*, *slk19*, *cdc5*, *lte1*, and *tem1* (113, 118). The *SPO12*, *SLK19*, and *CDC5* genes are part of the FEAR pathway that controls the early release of Cdc14 (24). *LTE1* encodes the guanine nucleotide exchange factor for Tem1, a GTP binding protein that is responsible for initiating the signal of the MEN pathway (91). The ability to suppress defects in the FEAR and MEN pathways by *cdc55* deletion suggests that the Cdc55-containing PP2A plays a negative role in these pathways, thus acting as an inhibitor of mitotic exit.

The mechanism by which the Cdc55-containing PP2A negatively regulates the FEAR and MEN pathways is not clear. However, a recent study has shown that deletion of *CDC55* causes premature release of Cdc14 from nucleolus during spindle checkpoint activation (118). This finding suggests that PP2A may act downstream of FEAR in the regulation of the early release of Cdc14. As Cdc14 released by the FEAR pathway has the ability to enhance the MEN pathway, a role for the Cdc55-containing PP2A in FEAR may contribute, at least in part, to its effect in MEN (17). In addition, it has been found that inactivation of Cdc55 alters the phosphorylation levels of Tem1, indicating that Cdc55 may directly or indirectly regulate Tem1 (113). Given the pleiotropic nature of PP2A in many different processes, it is plausible that the Cdc55-containing PP2A may target multiple components in the FEAR and MEN pathways (Fig. 3).

Even though the molecular details of PP2A action in controlling mitotic exit remain unclear, it is certain that the role of

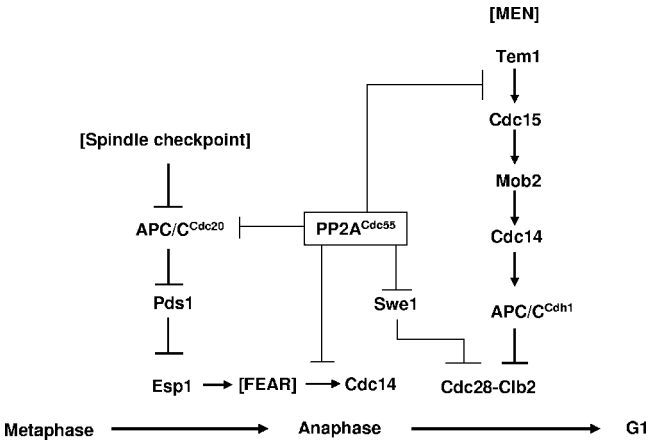

FIG. 3. The negative role of PP2A at mitotic exit. The Cdc55-containing PP2A ($PP2A^{Cdc55}$) is involved in controlling both the metaphase-anaphase transition and mitotic exit. It represses the onset of anaphase by inhibiting Cdc20-dependent activation of APC/C (APC/C^{Cdc20}) and prevents mitotic exit by downregulating the FEAR and MEN pathways. Swe1 is normally degraded at the onset of mitosis and does not play a role at mitotic exit. However, defects in PP2A result in Swe1 stabilization and, consequently, CDK downregulation at mitotic exit.

PP2A at this stage of the cell cycle is pleiotropic. A defect in Cdc55-containing PP2A activity is expected to affect at least three different processes that are critical in the regulation of mitotic exit. First, it downregulates Clb2-Cdc28 activity by enhancing the inhibitory phosphorylation of Cdc28 at tyrosine 19 (74, 117). Second, it stimulates APC/C activation through a Cdc20-dependent process (112). Third, it increases the signaling activity of the MEN pathway (113, 118). As such, a defect in PP2A activity may compromise mechanisms that secure an accurate separation of DNA and cellular components and may predispose the cell to an error-prone exit. Therefore, it is not surprising that cells lacking Cdc55-containing PP2A activity are unable to prevent sister chromatid segregation in the presence of the microtubule-depolymerizing drug nocodazole, which prevents assembly of the spindle (74, 117). The effect of PP2A in the activation of APC/C and MEN signaling may also explain why precluding the inhibitory phosphorylation of Cdc28 is not sufficient to cure the sensitivity of *cdc55* cells to microtubule-depolymerizing drugs.

PP2A IN CYTOKINESIS

Cytokinesis is the final stage of the cell cycle, during which a cleavage apparatus that leads to separation of the mother and daughter cells is assembled (40). One major step during the assembly of the cytokinetic machinery is formation of the septin rings at the bud neck, which in yeast are composed of five different septins, i.e., Cdc3, Cdc10, Cdc11, Cdc12, and Shs/Sep7 (39). The septins first appear as a single ring at the presumptive bud site at G_1 and split into two rings that partition to the mother and daughter sides of the bud neck during the early stage of cytokinesis. Once formed, the septin rings serve as a scaffolding structure for recruiting other components involved in the cleavage, such as Myo1, the component of the contractile ring (67). In addition, the septin rings also function as a signaling hub that mediates a complex signaling network to coordinate cytokinesis with segregation of sister chromatids. The infrastructures of both the spindle and morphogenesis checkpoints are built around the septin rings, and perturbations in both spindle assembly and bud formation can be sensed and processed for proper adjustments in cell cycle progression in a septin-dependent manner (62).

PP2A was initially implicated in cytokinesis by the observations that inactivation of the regulatory subunits of PP2A, including Tpd3 and Cdc55, often caused defects in formation of the septum, resulting in accumulation of multibudded and multinucleated cells (45, 107). At reduced temperatures, both *cdc55* and *tpd3* cells display elongated bud morphology similar to that of cells defective for septin ring formation, such as the *cdc3*, *cdc10*, *cdc11*, and *cdc12* mutants (34). A localization study has revealed a dynamic distribution of PP2A at the bud neck during cytokinesis, reinforcing the role of PP2A in this process (38). At posttelophase, Tpd3 initially accumulates at the bud neck as two rings on both sides of the juncture between the mother and bud. Just before cytokinesis, the two rings are condensed into a single band at the juncture. In postdivisional cells, Tpd3 is localized at the presumptive bud site. The distribution of Cdc55 at the bud neck mirrors that of Tpd3, suggesting that the Cdc55-containing PP2A is the form of the phosphatase that is involved in cytokinesis. The target of the Cdc55-containing PP2A in cytokinesis remains unknown. The ring-like distribution pattern of Cdc55 during cytokinesis is reminiscent of the septin rings, suggests that the Cdc55-containing PP2A may associate with the septins and regulate their phosphorylation levels (38). Interestingly, Rts1, the B′ subunit of PP2A, also accumulates at the bud neck, and its distribution during the cell cycle follows that of Tpd3. However, the accumulation of Rts1 at the bud neck is transient, and it disappears from the site before completion of cytokinesis. Rts1 appears to be required for maintaining a proper organization of the septin rings during cytokinesis and for their dissociation at G_1. In its absence, the septin rings dissolve into aberrantly shaped structures and fail to disassemble after cytokinesis (23). The localization of Rts1 at the bud neck requires activation of the MEN pathway, suggesting that execution of mitotic exit is a prerequisite for Rts1 function in cytokinesis. This notion is consistent with the finding that Rts1 localizes to kinetochores at S and G_2 and translocates to the bud neck at telophase, at the time when MEN signaling becomes active (23). Deletion of Rts1 was found to affect the phosphorylation levels of Shs1/Sep7, one of the five septins, suggesting that Rts1 may control the septin ring dynamics and stability by regulating the phosphorylation of Shs/Sep7 (23).

PP2A IN CELL CYCLE CHECKPOINT CONTROLS

PP2A in the Spindle Checkpoint Control

Accurate segregation of sister chromatids at the end of mitosis depends upon the integrity of the spindle to which each pair of sister chromatids is attached. The spindle checkpoint contributes to the accuracy by delaying the onset of anaphase until the successful completion of the assembly of the spindle (37). In wild-type cells, perturbations in the spindle activate the checkpoint and prevent sister chromatid segregation. However, cells defective for the checkpoint exit mitosis in the ab-

sence of a fully functional spindle, resulting in aneuploidy and cell death. Consequently, these cells are hypersensitive to microtubule-depolymerizing drugs that disassemble the spindle, such as benomyl and nocodazole (100). A genetic screen based on this criterion led to the identification of many components that were required for preventing cells from exiting mitosis before completion of the spindle assembly, such as *MAD1* to *-3* and *BUB1* to *-3* (47, 63). Interestingly, *CDC55* was also identified in the same screen (74). In addition, *CDC55* was isolated along with *MAD1* to *-3* and *BUB1* to *-3* from a different screen designated for identifying mutations that were synthetically lethal with a *ctf13* Ts^- allele, which caused a defect in the kinetochore (112). Like cells defective for the spindle checkpoint, *cdc55* cells are hypersensitive to benomyl and nocodazole (74, 112). In addition, deletion of *TPD3* also renders cells hypersensitive to nocodazole. These observations suggest a role for the Cdc55-containing PP2A in the spindle checkpoint (56, 112).

The spindle checkpoint inhibits APC/C, which is required at two transitions of the cell cycle, the metaphase-anaphase transition and mitotic exit (37). Inhibition of the Cdc20-dependent activation of APC/C prevents sister chromatid segregation, and inhibition of the Cdh1-dependent activation of APC/C prevents destruction of Clb2 (104). As a result, an activated spindle checkpoint blocks onset of anaphase and arrests cells in mitosis by sustaining a high Clb2-Cdc28 activity. However, when the spindle checkpoint is compromised, as in *mad2* and *bub3* cells, the affected cells are unable to restrict APC/C activation in the absence of a fully assembled spindle. Consequently, sister chromatids segregate and Clb2-Cdc28 activity falls (119). This is the scenario when the *cdc55* cells are treated with nocodazole, which prevents the assembly of the spindle, suggesting that the spindle checkpoint is defective in the *cdc55* cells (74). However, unlike the *mad* and *bub* mutants, in which the downregulation of Clb2-Cdc28 activity is caused by the destruction of Clb2, the *cdc55* cells contain a level of Clb2 that is comparable to that in wild-type cells, suggesting that Clb2-Cdc28 activity is downregulated by a different mechanism. Replacing tyrosine 19 in Cdc28 with the nonphosphorylatable residue phenylalanine (Cdc28 F19) prevents the downregulation of Clb2-Cdc28 activity and inhibits chromatid segregation in the *cdc55* cells treated with nocodazole, suggesting that the downregulation is caused by phosphorylation at tyrosine 19 (74). Indeed, in both asynchronized and mitosis-arrested *cdc55* cells, the phosphorylation level at tyrosine 19 of Cdc28 is much higher than that in wild-type cells (74, 117).

As mentioned above, the phosphorylation of Cdc28 at tyrosine 19 is controlled by the Swe1 kinase and Mih1 phosphatases. Swe1, which is normally destroyed during mitosis, is stabilized in nocodazole-treated *cdc55* cells (117). Regulation of Swe1 degradation, and consequently Clb2-Cdc28 activity at late G_2, involves a complex signaling network that plays a vital role in coordination of cell cycle progression with bud morphogenesis (62) (see below). It is thus possible that the Cdc55-containing PP2A is part of the signaling network and that inactivation of Cdc55 may alter the signaling activity, leading to Swe1 stabilization and Clb2-Cdc28 downregulation. However, downregulation of Clb2-Cdc28 activity cannot be the only mechanism that contributes to the spindle checkpoint defect in *cdc55* cells. If Cdc55 exerts its checkpoint function exclusively through regulation of Clb2-Cdc28, then other components involved in the morphogenetic signaling network may also be qualified as checkpoint proteins. The fact that no other component of the signaling network was recovered from two independent genetic screens designed for isolating checkpoint genes appears to suggest involvement of Cdc55 in other aspects of the checkpoint (74, 112). Consistent with this notion, it was found that removal of tyrosine 19 in Cdc28 was unable to cure the sensitivity of *cdc55* cells to depolymerizing drugs, suggesting that abnormal mitotic exit persists in the mutant cells despite the enhanced Clb2-Cdc28 activity (74).

As discussed above, the Cdc55-containing PP2A has been shown to play a negative role in regulation of Cdc20, which is required for targeting Pds1 to APC/C for degradation, and of MEN signaling, which promotes mitotic exit (112, 113, 118). Therefore, it is conceivable that in the absence of Cdc55, an enhanced Cdc20 and MEN signaling activity may predispose the cell to error-prone sister chromatid segregation and mitotic exit, which in combination with a reduced Clb2-Cdc28 activity contribute the defects in the spindle checkpoint. In this regard, the requirement of Cdc55 for proper function of the spindle checkpoint reflects the pleiotropic role of the Cdc55-containing PP2A in regulating Clb2-Cdc28 activity, sister chromatid segregation, and mitotic exit (Fig. 3).

PP2A in the Morphogenesis Checkpoint Control

The budding yeast, as its name implies, proliferates through a distinctive process called budding. During the cell cycle, bud formation occurs concomitantly with nuclear division. To ensure the viability of the progenies, yeast cells have developed a unique cell cycle checkpoint control to coordinate bud formation with nuclear division. This checkpoint, termed the morphogenesis checkpoint, monitors the budding process and delays nuclear division in the event that bud formation is disrupted (61). How the perturbations in bud formation are sensed is currently unclear. However, the signaling mechanisms that lead to delays in cell cycle progression have been well established, and PP2A appears to be involved in the signaling process.

Activation of the morphogenesis checkpoint has been shown to stabilize the Swe1 kinase, which in turn downregulates Clb2-Cdc28 activity by phosphorylation of Cdc28 at tyrosine 19. Under normal conditions, Swe1 accumulates during late G_1 and S phases but is mostly degraded at the onset of mitosis (94, 95). However, in *cdc55* cells, Swe1 is stabilized in late G_2 (117). Concomitantly, the mutant cells display a reduced Clb2-Cdc28 activity and a G_2 delay (74).

How does inactivation of Cdc55 prevent Swe1 degradation? Degradation of Swe1 occurs at the bud neck and requires the assembly of septin rings, which serve as scaffolding structures to recruit the Hsl1 kinase and Hsl7. Hsl7 acts as an adapter protein to target Swe1 for Hsl1-dependent phosphorylation and degradation (4, 93). Perturbations in septin rings as well as deletion of *HSL1* disrupt the signaling process, resulting in Swe1 stabilization and, consequently, downregulation of Clb2-Cdc28 activity (68, 72). Cdc55 has been found to accumulate at the bud neck, where the septin rings are assembled, and its inactivation has been shown to perturb the septin rings (23, 45). As such, inactivation of Cdc55 is expected to interfere with

the process that leads to Swe1 degradation (Fig. 2). How Cdc55 affects the septin rings is not clear. Since the septins, including Cdc3, Cdc10, Cdc11, and Cdc12, are phosphoproteins, it is possible that the Cdc55-containing PP2A may regulate phosphorylation levels of one or multiple septins (23, 75, 105, 108).

In addition to roles in septin ring formation, PP2A has been implicated in bud emergence, actin polarization, and cell wall synthesis, all of which are essential for bud formation (30, 65, 110). Defects in any one of the processes are expected to disrupt bud growth and, consequently, activate the morphogenesis checkpoint. It is thus conceivable that PP2A inactivation may lead to the activation of the morphogenesis checkpoint and, consequently, the downregulation of Clb2-Cdc28 activity. This notion is consistent with observations that defects in PP2A often result in a delay at the G_2/M transition.

The involvement of PP2A in the morphogenesis checkpoint makes it difficult to assess the direct role of PP2A, if any, in the regulation of Clb2-Cdc28 activity at the G_2/M transition. Although PP2A has been implicated in the regulation of Mih1, the impact of this regulation on cell cycle progression is expected to be insignificant, as inactivation of Mih1 itself does not block the G_2/M transition (9, 117). It is likely that the G_2 defects associated with PP2A inactivation are caused by activated checkpoints in combination with other defects in bud formation and cell wall synthesis.

CONCLUSION

Since the identification of the genes for yeast PP2A in the early 1990s, yeast has served as an excellent model to study the function of this enzyme in cell cycle progression. The relatively simple subunit composition of PP2A, in combination with the powerful genetic tools of the yeast system, has allowed study of the roles of various forms of PP2A in different stages of the cell cycle, a task that is nearly impossible in other genetically less sophisticated systems. A wealth of information has since been obtained. As expected, the effect of PP2A on the yeast cell cycle is pleiotropic, reflecting its role, either direct or indirect, at virtually every stage of the cell cycle. However, while genetic analysis is effective in mapping the extensive connections of PP2A with many cell cycle control mechanisms, it often falls short in providing the molecular basis for these connections. To fully understand the role of PP2A in cell cycle regulation, one needs to identify the targets of PP2A at different stages of the cell cycle and look into the mechanisms by which PP2A is regulated spatially and temporally. So far, these tasks have been proven to be difficult even with the yeast system. The major obstacles are the pleiotropic role and the multilevel regulation of PP2A, which make it hard to distinguish the direct targets of PP2A from those affected indirectly. However, the realization that PP2A is often compartmentalized with its targets makes it possible to dissect the role of PP2A in different processes by isolating and analyzing the PP2A-containing complexes. In this regard, the genetic system of yeast is still a premium tool, which, in combination with biochemical and proteomic approaches, will allow further definition of the role of PP2A in cell cycle regulation.

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