THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 290, NO. 1, pp. 1–12, January 2, 2015


# Budding Yeast Swe1 Is Involved in the Control of Mitotic Spindle Elongation and Is Regulated by Cdc14 Phosphatase during Mitosis*[S]

Received for publication, July 2, 2014, and in revised form, November 17, 2014 Published, JBC Papers in Press, November 18, 2014, DOI 10.1074/jbc.M114.590984

**Erica Raspelli**[‡1], **Corinne Cassani**[‡2], **Elena Chiroli**[§3], **and Roberta Fraschini**[‡4]

*From the* [‡]*Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, 20126 Milano, Italy and the* [§]*IFOM, the FIRC (Fondazione Italiana per la Ricerca sul Cancro) Institute of Molecular Oncology, 20139 Milano, Italy*

**Background:** Timely mitotic spindle elongation ensures correct chromosome segregation, which is crucial for genetic stability.
**Results:** The protein kinase Swe1 contributes to restraining anaphase progression and is regulated by the protein phosphatase Cdc14.
**Conclusion:** Swe1 plays a role in the control of mitotic spindle elongation.
**Significance:** Swe1 regulation by Cdc14 allows proper coordination of spindle elongation with mitotic progression.

**Cyclin-dependent kinase (Cdk1) activity is required for mitotic entry, and this event is restrained by an inhibitory phosphorylation of the catalytic subunit Cdc28 on a conserved tyrosine (Tyr$^{19}$). This modification is brought about by the protein kinase Swe1 that inhibits Cdk1 activation thus blocking mitotic entry. Swe1 levels are regulated during the cell cycle, and they decrease during $G_2$/M concomitantly to Cdk1 activation, which drives entry into mitosis. However, after mitotic entry, a pool of Swe1 persists, and we collected evidence that it is involved in controlling mitotic spindle elongation. We also describe that the protein phosphatase Cdc14 is implicated in Swe1 regulation; in fact, we observed that Swe1 dephosphorylation *in vivo* depends on Cdc14 that, in turn, is able to control its subcellular localization. In addition we show that the lack of Swe1 causes premature mitotic spindle elongation and that high levels of Swe1 block mitotic spindle elongation, indicating that Swe1 inhibits this process. Importantly, these effects are not dependent upon the role of in Cdk1 inhibition. These data fit into a model in which Cdc14 binds and inhibits Swe1 to allow timely mitotic spindle elongation.**

In all eukaryotic cells mitotic entry is allowed only in the presence of high Cdk1[5] activity. In budding yeast an important regulator of this process is the protein kinase Swe1 that inhibits entry into mitosis by phosphorylating the Tyr$^{19}$ residue of the catalytic subunit of Cdk1, Cdc28 (1). Cdk1 activation and entry into mitosis are allowed by the action of the protein phosphatase Mih1 that removes this phosphorylation from Cdc28, thus promoting its activation (2). However, Tyr$^{19}$ phosphorylation and its subsequent dephosphorylation are not essential for M phase initiation in budding yeast (3); indeed the lack of Swe1 or Mih1 does not impair normal cell cycle progression in unperturbed condition. On the other hand, Cdc28 phosphorylation becomes essential for cell viability in response to perturbation in bud morphology or actin cytoskeleton (4). In these conditions, Cdc28 activation is prevented by the morphogenesis checkpoint, a pathway that inhibits Swe1 inactivation thus blocking entry into mitosis (5–7). Swe1 has therefore a critical role in coordinating cell morphogenesis with nuclear division, and it is subjected to multiple regulations that change its phosphorylation state (8), subcellular localization, and protein levels (9). During the S phase, Swe1 accumulates in the nucleus where it is phosphorylated by Clb-Cdc28 before being exported to the cytoplasm and then to the daughter side of the bud neck. Bud neck-localized Swe1 is phosphorylated by several protein kinases (10), and hyperphosphorylated Swe1 species are ubiquitylated (9, 11). Subsequently, Swe1 is degraded via the proteasome, and this event allows mitotic entry (9). Little is known about the role of Swe1 after this point of the cell cycle, although it is reported that a pool of Swe1 persists in the cells after mitotic entry (11, 12), suggesting that it might also play a role in mitotic progression.

The mitotic spindle is formed by sets of microtubules that are composed by heterodimers of $\alpha$- and $\beta$-tubulin assembled together in a head to tail fashion and that are nucleated by the spindle pole bodies. By the end of S phase, cells complete DNA synthesis and assemble a short bipolar spindle. During mitosis, chromosome segregation occurs in two steps: after cohesin removal, shortening of the kinetochore microtubules that link each sister chromatid to the correct spindle pole leads to chromosomes movement toward spindle poles (anaphase A). Afterward, pushing forces generated at the spindle midzone thanks to motor proteins cause the sliding of microtubules emanated from opposite poles, thus driving mitotic spindle elongation (anaphase B) (13). Spindle elongation is completed by the pull-

* This work was supported by grants from PRIN (Progetti di Ricerca di Interesse Nazionale) to R. F.
[S] This article contains supplemental Movies S1 and S2.
[1] Supported by a fellowship from Fondazione Buzzati-Traverso.
[2] Supported by a fellowship from Fondazione Confalonieri.
[3] Supported by a Young Investigator Programme 2013 fellowship from Fondazione Veronesi.
[4] To whom correspondence should be addressed: Dipt. di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy. Tel.: 390264483540; Fax: 390264483565; E-mail: roberta.fraschini@unimib.it.
[5] The abbreviation used is: Cdk, cyclin-dependent kinase.

**TABLE 1**
**Yeast strains used in this study**

| Strain | Description |
|---|---|
| yRF1 | *MATa, ade2-1, trp1-1, can1-100, leu2-3,112, his3-11,15, ura3, GAL, psi+* |
| yRF7 | *MATa, cdc14-3* |
| yRF33 | *MATa, swe1::LEU2* |
| yRF84 | *MATa, his3::TUB1-GFP::HIS3* |
| yRF119 | *MATa, SWE1HA3::klURA3* |
| yRF324 | *MATa, CEN5::tetO2X112::HIS3 (1,4Kb), LEU2::tetR-GFP* |
| yRF442 | *MATa, cdc14-1* |
| yRF702 | *MATa, ura3::URA3::GAL1-SWE1* (single integration) |
| yRF1041 | *MATa, cdc28:hisG, cdc28-Y19F::TRP1* |
| yRF1045 | *MATa, cdc28:hisG, cdc28-Y19F::TRP1, ura3::URA3::GAL1-SWE1* (single integration) |
| yRF1107 | *MATa, cdc28:hisG, cdc28-Y19F::TRP1, CEN5::tetO2X112::HIS3 (1,4Kb), LEU2::tetR-GFP* |
| yRF1135 | *MATa, ura3::URA3::GAL1-SWE1* (single integration), *CEN5::tetO2X112::HIS3 (1,4Kb), LEU2::tetR-GFP, cdc28:hisG, cdc28-Y19F::TRP1* |
| yRF1153 | *MATa, ura3::URA3::GAL1-SWE1* (single integration), *CEN5::tetO2X112::HIS3 (1,4Kb), LEU2::tetR-GFP* |
| yRF1393 | *MATa, ura3::GAL-SWE1-PK3 (SpHIS)* (single integration) |
| yRF1815 | *MATa, trp1::TRP1::1X CDC14-TAB6-1, ura3::URA3::GAL1-SWE1 (single integration)* |
| yRF1835 | *MATa, swe1::LEU2, his3::TUB1-GFP::HIS3* |
| yRF1846 | *MATa, GAL1-CDC14::URA3, SWE1-HA3::klURA3, cdc20::MET3-HA3-CDC20::TRP1* |
| yRF1848 | *MATa, GAL1-CDC14-C283A::LEU2, SWE1-HA3::klURA3, cdc20::MET3-HA3-CDC20::TRP1* |
| yRF1852 | *MATα, cdc14-1, swe1::kanMX4* |
| yRF1853 | *MATα, cdc14-3, swe1::LEU2* |
| yRF1854 | *MATa, cdc20::MET3-HA3-CDC20::TRP1, SWE1-HA3::klURA3* |
| yRF1868 | *MATα, cdc14-3, SWE1-HA3::klURA3* |
| yRF1872 | *MATa, cdc20::MET3-HA3-CDC20::TRP1, SWE1-HA3::klURA3, PDS1-myc18::LEU2* |
| yRF1877 | *MATa, ura3::GAL-SWE1-PK3 (SpHIS)* (single integration), *trp1::TRP1::1X CDC14-TAB6-1* |
| yRF1889 | *MATa, SWE1-HA3::klURA3, cdc14-3, cdc28::cdc28-as1::URA3* |
| yRF1893 | *MATa, ura3::MET3-CDC20::URA3, cdc20::LEU2, SWE1-HA3::klURA3* |
| yRF1896 | *MATa, cdc15::cdc15-as1(L99G)::URA3, CDC14-HA3, SWE1-PK3 (SpHIS)* |
| yRF1897 | *MATa, cdc15::cdc15-as1 (L99G)::URA3, CDC14-HA3* |

ing forces generated by motor proteins associated with astral microtubules that link the spindle to the cell cortex (14).

Whereas metaphase is characterized by high Cdk1 activity, anaphase is marked by a reduction of Cdk1 activity also because of the activation of the phosphatase Cdc14. During most of the cell cycle, Cdc14 is kept inactive inside the nucleolus, and at anaphase onset, it is released and activated in two steps. First, the FEAR Cdc-Fourteen Early Anaphase Release pathway triggers a partial and transient release of Cdc14 from the nucleolus to the nucleus (15). However, the FEAR-controlled Cdc14 is not sufficient to promote mitotic exit; only the full release of Cdc14 allows it, by counteracting and inactivating Cdk1 activity. This full release is controlled by another regulatory pathway, the mitotic exit network that both promotes mitotic exit and induces cytokinesis (16).

Cdc14 release regulates several factors associated with the spindle, thus providing a link between anaphase onset and the changes in microtubule dynamics required for the completion of mitotic spindle elongation. FEAR-activated Cdc14 is involved in the control of microtubule dynamics by targeting several spindle-associated proteins that form the spindle midzone and/or that are required for the stabilization of microtubules in anaphase cells (17, 18). In late anaphase, Cdc14 is able to dephosphorylate other proteins, thus helping mitotic spindle elongation (19).

In this study, we reveal that Swe1 plays a role during mitosis and is regulated by Cdc14; in particular, we demonstrate that Cdc14 is involved in Swe1 dephosphorylation during anaphase, which in turn controls its subcellular localization. This control might be important to ensure timely mitotic spindle elongation because we show an uncovered role for Swe1 in the inhibition of this process, which is correlated with its phosphorylation. Importantly, these effects are not dependent upon Swe1 role on Cdk1 inhibition.

## EXPERIMENTAL PROCEDURES

*Strains, Media, Reagents, and Genetic Manipulations*—All yeast strains (Table 1) were derivatives of W303 (*ade2-1, trp1-1, leu2-3,112, his3-11,15, ura3, ssd1*). α-Factor was used at 2 or 10 μg/ml, hydroxyurea was used at 150 mM, nocodazole was used at 15 μg/ml, and, unless differently stated, cells were grown at 25 °C in either synthetic medium supplemented with the appropriate nutrients (20) or YEP (1% yeast extract, 2% bactopeptone, 50 mg/liter adenine) medium supplemented with 2% glucose (YEPD) or 2% raffinose (YEPR) and 2% raffinose + 1% galactose (YEPRG). YEPR and YPD + methionine medium contained 2 mM methionine.

Inhibition of Cdc15 kinase activity was performed as described (21). *SWE1-PK3 cdc15-as1* and *SWE1-PK3 CDC14-HA3 cdc15-as1* cells were grown at 25 °C, prearrested with 2 μg/ml α-factor, and released in the presence of 5 μM 1NM-PP1 for 2 h to arrest them in late mitosis (88% budded cells with separated nuclei). The inhibition of Cdc28 kinase activity was performed as described (22). *cdc14-3 cdc28-as1* cells were grown at 23 °C and then shifted at 30 °C for 3 h to arrest them in telophase (90% budded cells with divided nuclei); then the culture was split in two and treated with 1% DMSO or with 25 μM 1NM-PP1 for 15 min.

Standard techniques were used for genetic manipulations (20, 23). Gene deletions were generated by one-step gene replacement (24). One-step tagging techniques were used to create 3PK-tagged Swe1 variant and HA3-tagged Swe1 (25). Cdc14-HA3 expressing strain and *cdc15-as1* mutant were a kind gift from Rosella Visintin. *GAL1-CDC14* and *GAL1-cdc14-C283A* expressing strains were a kind gift from Elmar Schiebel. *cdc28-E12K* mutant was a kind gift from Daniel J. Lew. *cdc28-as1* mutant was a kind gift from Gislene Pereira. All gene

replacements and tagging were controlled by PCR based methods or Southern blot analysis.

*Fluorescence Microscopy and Live Imaging*—*In situ* immunofluorescence was performed on formaldehyde-fixed cells and carried out as previously described (26). The nuclei were visualized by staining with 0.05 $\mu$g/ml DAPI. To detect spindle formation and elongation, $\alpha$-tubulin immunostaining was performed with the YOL34 monoclonal antibody (1:100, Serotec) followed by indirect immunofluorescence using rhodamine-conjugated anti-rat antibody (1:500; Pierce). Swe1–3HA localization was observed in formaldehyde-fixed cells as described in Ref. 11. Digital images were taken with a Leica DC350F charge-coupled device camera mounted on a Nikon Eclipse 600 and controlled by the Leica FW4000 software or with the MetaMorph imaging system software on a fluorescent microscope (Eclipse 90i; Nikon), equipped with a charge-coupled device camera (Coolsnap, Photometrics) with an oil 100× 0.5–1.3 PlanFluor oil objective (Nikon).

For time lapse movies, cells were grown in synthetic complete medium + 2% glucose then collected and imaged on agar in synthetic complete medium + 2% glucose using a Delta Vision Elite imaging system (Applied Precision) based on an IX71 inverted microscope (Olympus) with a camera CoolSNAP HQ2 from Photometrics, and a UPlanApo 60× (1.4 NA) oil immersion objective (Olympus). Every 3 min, four Z-stacks at 1.2-$\mu$m intervals were taken for each fluorescent channel and projected onto a single image per channel using Fiji software (27). The timing of mitotic spindle elongation was scored in 62 wild type cells and 63 *swe1*Δ cells. In Fig. 4*B*, time 0 is the time in which the spindle is correctly positioned and aligned at the bud neck.

*Protein Extracts and Analysis*—For monitoring Swe1, Cdc14, Clb2, Pds1, and Cdc20 levels total protein extracts were prepared by TCA precipitation as previously described (28). Co-immunoprecipitations were performed as previously described (29), except that cells were lysed in 50 mM Tris, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Nonidet P-40, 1 mM sodium orthovanadate, and 60 mM $\beta$-glycerophosphate, supplemented with a mixture of protease inhibitors (Complete mini; Roche). For Western blot analysis, proteins were transferred to Protran membranes (Schleicher & Schuell) and probed with monoclonal anti-HA (1:3000), anti-Myc (1:5000), anti-PK (1:3000), and anti-Pgk1 antibodies (1:40,000) or with polyclonal anti-Cdc14 (1:2000) and anti-Clb2 (1:3000) antibodies. Secondary antibodies were purchased from Amersham Biosciences, and proteins were detected by an enhanced chemioluminescence system according to the manufacturer.

*Statistical Analyses and Other Techniques*—The experiments shown in Figs. 4*A*, 5, and 6 (*A* and *B*) were repeated three times, and for the graphs, at least 200 cells were counted for each time point for each strain. Standard deviations were calculated using Microsoft Excel software. The significance of the differences in mitotic spindle elongation was statistically tested by the Wilcoxon signed rank sum test using MATLAB software; samples are significantly different if $p < 0.05$. Flow cytometric DNA quantification was performed according to Ref. 30 on a Becton-Dickinson FACScan. Visualization and quantification of sister chromatid separation with Tet operators using GFP were performed as described in Ref. 30. Quantification of Swe1 localization shown in Fig. 1*C* was performed with Fiji software.

## RESULTS

*Characterization of Swe1 Protein Levels and Modifications after $G_2$/M Transition*—We previously characterized Swe1 levels and phosphorylation in all phases of an unperturbed cell cycle, and we observed that, after mitotic entry, Swe1 is not completely degraded, and it is present in its dephosphorylated form in the following $G_1$ phase (11). However, little is known about Swe1 levels, modifications, and functions after $G_2$/M transition. We therefore decided to investigate these issues and started by checking Swe1 levels in metaphase-arrested cells. The metaphase to anaphase transition is marked by the proteolitic cleavage of the cohesin subunit Scc1 by the separase Esp1, which during the previous phases of the cell cycle is kept inactive by the securin Pds1 (31); at this cell cycle point, Pds1 is ubiquitylated by the anaphase-promoting complex bound to its regulatory subunit Cdc20, to be degraded via the proteasome. Therefore, yeast cells can be arrested in metaphase by the conditional depletion of Cdc20. *cdc20*Δ *MET3-CDC20-HA3 SWE1-HA3* cells were grown in synthetic medium lacking methionine and shifted in YEPD medium containing methionine, to turn off Cdc20 expression. After 2 h, all the cells were arrested with a large bud, undivided nuclei, and short bipolar spindle, and importantly, Western blot analysis indicates the absence of Cdc20 and the accumulation of the mitotic cyclin Clb2 (Fig. 1*A*) consistent with a metaphase arrest. As expected, in these conditions Swe1 is still present in the cells (Fig. 1*A*) and appears as multiple bands, indicative of its phosphorylated state (7).

To carefully analyze the kinetics of Swe1 levels and modifications from metaphase to the following $G_1$ phase, we arrested *cdc20*Δ *MET3-CDC20-HA3 SWE1-HA3 PDS1–18myc* cells in metaphase as described above and released them in synthetic medium lacking methionine to allow cell cycle progression and containing $\alpha$-factor to arrest cells in the next $G_1$ phase. We monitored the securin Pds1 and the mitotic cyclin Clb2 levels to follow metaphase to anaphase transition and mitotic exit, respectively. After release, cells progress correctly into mitosis and, as expected, as soon as Cdc20 is produced, Pds1 is degraded, and Clb2 disappears after nuclear division (Fig. 1*B*). We observed that Swe1 is phosphorylated both in metaphase and in anaphase; its dephosphorylation starts concomitantly with nuclear division and Clb2 degradation, and as expected, the protein remains dephosphorylated in the following $G_1$ phase (Fig. 1*B*, *30*, *45*, and *60 min*). Because Swe1 localization correlates with its modifications (10), we performed a similar experiment with *cdc20*::*MET3-CDC20 SWE1-HA3* cells to analyze Swe1 localization, and we observed that it is mostly present in the cytoplasm of the mother cell during metaphase, and it spreads into the bud concomitantly with nuclear division (Fig. 1*C*). Indirect immunofluorescence quantification shows that in metaphase-arrested cells, the Swe1 levels in the mother are double compared with those found in the bud, whereas they are almost equal in early and late anaphase (Fig. 1*C*, *right panel*). Together, these data confirm that Swe1 is still present in the

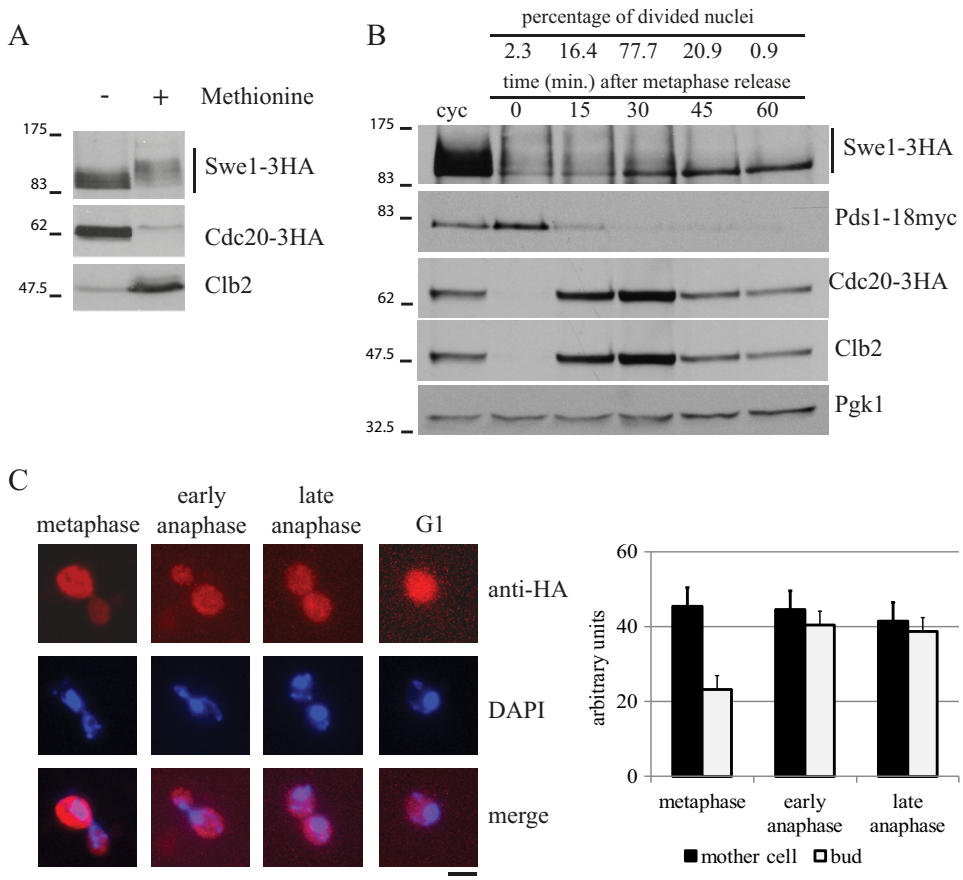



FIGURE 1. **Swe1 levels and localization during mitosis.** *A*, *cdc20Δ MET3-CDC20-HA3 SWE1-HA3* cells were grown to log phase in synthetic medium without methionine (−) and arrested in metaphase in methionine-containing medium for 3 h (+). Samples were taken in the indicated conditions to analyze Swe1 levels and phosphorylation and Cdc20 and Clb2 levels by Western blot using anti-HA and anti-Clb2 antibodies, respectively. *B* and *C*, metaphase-arrested *cdc20Δ MET3-CDC20-HA3 SWE1-HA3 PDS1–18myc* cells were released in synthetic medium without methionine containing 10 $\mu$g/ml $\alpha$-factor. *B*, at different time points after release, samples were taken to score nuclear division and for Western blot analysis with the indicated antibodies. Pgk1 was used as loading control. Size markers on the side of the gel blots indicate kilodaltons. *C*, immunostaining of Swe1-HA using anti HA antibodies and DAPI staining of DNA in *cdc20::MET3-CDC20 SWE1-HA3* cells in metaphase, early anaphase, late anaphase, and $G_1$. *Bar*, 5 $\mu$m. Mean fluorescence intensity was quantified in 30 cells for metaphase, early anaphase, and late anaphase, and values were plotted in the graph.

cells after $G_2$/M transition and show that it is phosphorylated in metaphase and dephosphorylated concomitantly with nuclear division and that these modifications correspond to a change in its localization.

*Cdc14 Controls Swe1 Dephosphorylation and Localization in Late Mitosis*—Because our data indicated that Swe1 is dephosphorylated concomitantly with nuclear division, we hypothesize that Swe1 could be regulated by a protein phosphatase that is active during mitosis. Interestingly, a Cdc14 allele defective in its nuclear export was reported to allow mitotic exit but to accumulate a fraction of cells with elongated buds (32). This phenotype could be due to high Swe1 levels or unscheduled Swe1 activity, conditions that interfere with normal isotropic bud growth and cause hyperpolarization of the cells (33). Moreover, Cdc14 and Swe1 were found to physically interact in an high throughput screening (34). Among all the other candidates, we therefore decided to gain insight into the role of this phosphatase on Swe1 regulation. At first, we asked whether other conditional *cdc14* mutants showed the accumulation of elongated cells and whether it was dependent upon Swe1. To this end, cells carrying two different *CDC14* thermosensitive alleles, *cdc14-1* and *cdc14-3*, with or without the deletion of *SWE1*, were grown at 25 °C to mid log phase and then shifted to the restrictive temperature to analyze the morphology of the cells. As shown in Fig. 2*A*, after shift at 37 °C, a fraction of *cdc14-3* and *cdc14-1* cells showed elongated buds, and this phenotype was at least partially reversed by deleting *SWE1* (Table 2), indicating that it was due to high Swe1 levels or activity (1). These data suggest that Cdc14 might be directly or indirectly involved in Swe1 inactivation.

To better understand whether Cdc14 could be directly involved in Swe1 regulation, we performed an immunoprecipitation experiment preparing total protein extracts from cells expressing Swe1-PK3 and Cdc14-HA3 and arrested in late mitosis because of *cdc15-as1* allele, an ATP analog-sensitive allele of *CDC15* (21). Importantly, Swe1 could specifically immunoprecipitate Cdc14, suggesting that Cdc14 might be the protein phosphatase that dephosphorylates Swe1 in late mitosis (Fig. 2*B*).

Swe1 levels and activity are regulated by phosphorylation (10), and we asked whether Cdc14 inactivation could cause changes in Swe1 phosphorylation state. For this purpose, we analyzed Swe1–3HA phosphorylation in wild type and in *cdc14-3* mutant cells after a shift at 34 °C, a temperature that is sufficient to inactivate Cdc14 as indicated by the fact that, after 2 h of incubation, 88% of *cdc14-3* cells were arrested in

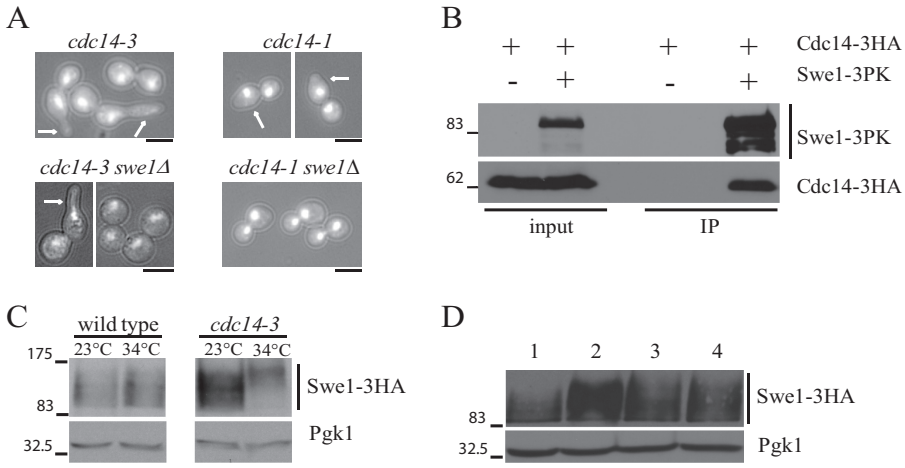



FIGURE 2. **Cdc14 is involved in Swe1 regulation.** *A*, exponentially growing cultures of *cdc14-3*, *cdc14-3 swe1*Δ, *cdc14-1*, and *cdc14-1 swe1*Δ cells were shifted at 37 °C for 4.5 h. After that time, cell samples were taken to visualize cell shape and nuclei by propidium iodide staining. *Arrows* indicate elongated buds. *Bar*, 5 μm. *B*, protein A beads bound to anti-PK antibodies were incubated with native protein extracts prepared from either *cdc15-as1 CDC14-HA3* or *cdc15-as1 CDC14-HA3 SWE1-PK3* cells arrested in late mitosis. Washed and boiled beads were subjected to SDS-PAGE followed by immunoblotting with anti-PK and anti-HA antibodies. *C*, exponentially growing cultures of *SWE1-HA3* and *cdc14-3 SWE1-HA3* cells were grown at 23 °C and then shifted at 34 °C for 2 h. Cell samples were taken to analyze Swe1 levels and phosphorylation using anti-HA antibodies. *D*, Swe1 phosphorylation was analyzed in exponentially growing cultures of *cdc28-as1 cdc14-3 SWE1-HA3* cells that were grown at 23 °C (*lane 1*), then shifted at 30 °C for 3 h (*lane 2*), and treated with DMSO (*lane 3*) or with the cdc28-as inhibitor 1NM-PP1 (*lane 4*). In *C* and *D*, Pgk1 was used as a loading control. Size markers on the side of the gel blots indicate kilodaltons. *IP*, immunoprecipitation.

**TABLE 2**
**Summary of the cellular morphology of the indicated mutants after shift at the restrictive temperature**

| Genotype | Elongated buds after 4.5 h at 37 °C |
|---|---|
| | % |
| *cdc14-1* | 24.12 |
| *cdc14-1 swe1* | 0 |
| *cdc14-3* | 38.26 |
| *cdc14-3 swe1* | 11.04 |

telophase as large budded cells with divided nuclei. In wild type cells, Swe1 phosphorylation state did not change after the shift at the restrictive temperature (Fig. 2*C*, *left panel*), whereas *cdc14-3* cells accumulated hyperphosphorylated Swe1 in the same experimental conditions (Fig. 2*C*, *right panel*). The same results were obtained by using *cdc14-1* cells (our observation), suggesting that Cdc14 could be involved in Swe1 dephosphorylation.

Swe1 is phosphorylated by different kinases, including Cdc28 (22). Because Cdc14 is involved in Cdc28 inactivation (35), the accumulation of Swe1 phosphorylated forms in the absence of Cdc14 activity could be due to hyperactivation of Cdc28 rather than to a direct role of Cdc14 in Swe1 dephosphorylation. To investigate this possibility, we took advantage of the Cdc28-as1 variant, whose kinase activity is blocked by the ATP analog 1NM-PP1 (36). *SWE1-HA3 cdc14-3 cdc28-as1* cells were shifted at the restrictive temperature, and then we added 1NM-PP1 to inactivate Cdc28, or DMSO, as a control. We used 30 °C as restrictive temperature because in these experimental conditions 1NM-PP1 is able to inhibit Cdc28 (22); this temperature is also sufficient to inactivate cdc14-3 because after 3 h 90% of *SWE1-HA3 cdc14-3 cdc28-as1* cells were arrested in telophase. As indicated in Fig. 2*D*, Swe1 phosphorylated forms do not disappear after inactivation of Cdc28 kinase activity (*lane 4*), indicating that they are likely due to the lack of Cdc14 phosphatase activity rather than to the lack of Cdc28 inhibition. In this experiment, the electrophoretic mobility of Swe1 is slightly different from the one shown in Fig. 2*C*, because of the different experimental conditions.

To further analyze Cdc14 role on Swe1 phosphorylation, we analyzed the changes in Swe1 electrophoretic mobility after overproduction of wild type Cdc14 or of a phosphatase-dead Cdc14 variant (Cdc14-C283A). We performed this experiment in metaphase-arrested cells because in this phase Swe1 is present in its phosphorylated form (Fig. 1*A*). We arrested *SWE1-HA3 MET3-CDC20-HA3 GAL1-CDC14* and *SWE1-HA3 MET3-CDC20-HA3 GAL1-cdc14-C283A* cells in metaphase by the depletion of Cdc20, and then we added galactose to induce Cdc14 overproduction. Samples were taken to check cell morphology and to analyze Cdc20, Cdc14 and Swe1 levels and modifications by Western blot. As shown in Fig. 3*A*, cycling cells contain Cdc20 and Swe1 in its phosphorylated forms (*lanes 1* and 3), whereas in metaphase-arrested cells Cdc20 is undetectable and Swe1 is still present and phosphorylated (*lanes 2* and *4*). Importantly, when wild type Cdc14 is overproduced, Swe1 phosphorylated forms disappear (*lane 5*); conversely, when the phosphatase-dead version of Cdc14 is overproduced, Swe1 phosphorylated forms persist (*lane 6*). These data indicate that Cdc14 phosphatase activity is required for Swe1 dephosphorylation.

Because Swe1 localization is coupled with its regulation (37), in the same experiment described above we also analyzed Swe1 subcellular localization. In metaphase-arrested wild type cells, Swe1 was present and mostly confined in the mother, but upon wild type Cdc14 overproduction, Swe1 spread also in the daughter cytoplasm (Fig. 3*B*, *left panel*). It is noteworthy that this change in Swe1 subcellular localization did not occur if the Cdc14 phosphatase-dead version was overproduced, indicating that it requires Cdc14 activity (Fig. 3*B*, *right panel*). Collectively, our data indicate that Cdc14 interacts with Swe1 and is involved in its dephosphorylation and localization, suggesting that Swe1 could be a target of Cdc14.

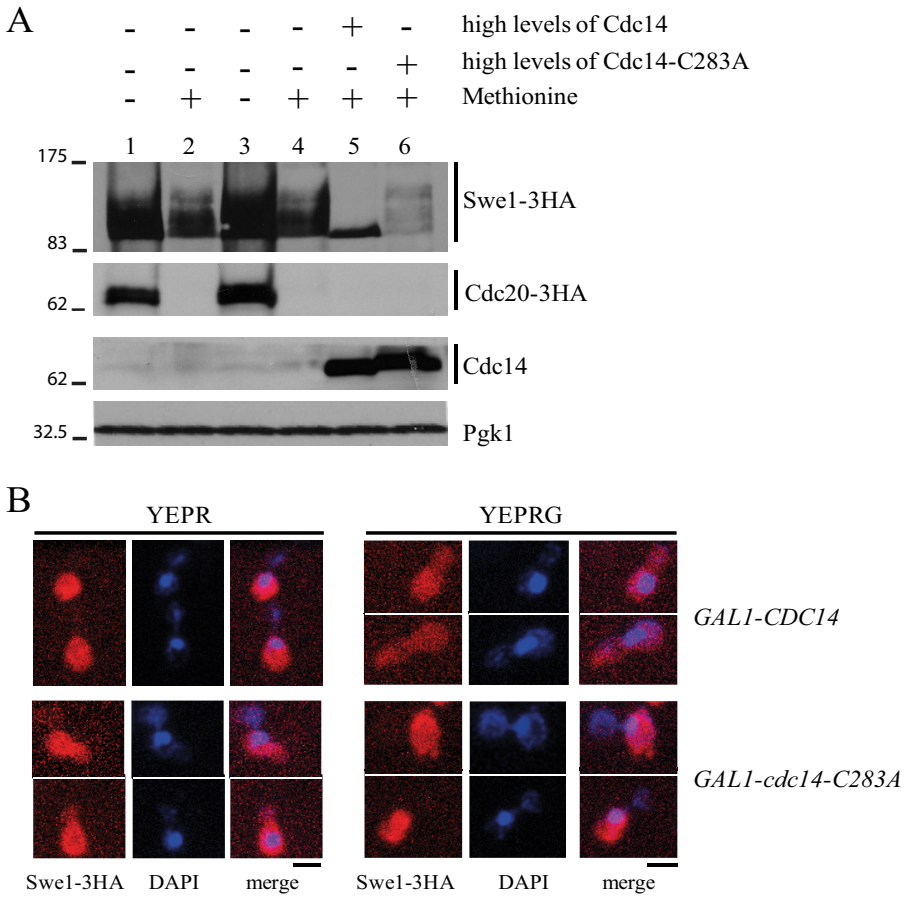



FIGURE 3. **Cdc14 regulates Swe1 phosphorylation and localization.** *A* and *B*, *GAL1-CDC14* and *GAL1-cdc14-C283A* cells, all carrying both *MET3-CDC20-HA3* and *SWE1-HA3*, were grown to log phase in raffinose synthetic medium without methionine and arrested in metaphase in YEPR + methionine medium for 3 h, and then galactose was added for an additional 2 h. Cell samples were taken in the indicated conditions to analyze Swe1 levels and phosphorylation and Cdc20 and Cdc14 levels by Western blot using anti-HA and anti-Cdc14 antibodies, respectively (*A*), and Swe1 localization by immunofluorescence using anti-HA antibodies and nuclei by staining with DAPI (*B*). Pgk1 was used as a loading control. Size markers on the side of the gel blots indicate kilodaltons. *Bar*, 5 $\mu$m.

*The Lack of Swe1 Induces Precocious Mitotic Spindle Elongation*—Our data led us to hypothesize a role for Swe1 after $G_2$-M transition. To shed light on this issue, we decided to carefully analyze cell cycle progression of *swe1*Δ cells released from a metaphase arrest. Samples were taken at different time points after release to monitor nuclear division and mitotic spindle dynamics by immunofluorescence. We observed that wild type and *swe1*Δ cells assemble a bipolar spindle with similar kinetics (Fig. 4*A*, *left panel*), but then *swe1*Δ cells elongate the mitotic spindle 10 min earlier than wild type cells, indicating that the lack of Swe1 caused precocious spindle elongation (Fig. 4*A*, *right panel*). These data suggest that Swe1 could act as an inhibitor of mitotic spindle elongation.

Because single cell analysis allows to point out even subtle differences, to confirm our population data, we performed time lapse analysis of mitotic spindle dynamics in wild type and *swe1*Δ cells expressing Tub1-GFP variant. Consistent with previous findings, we observed that the rate of bipolar spindle assembly and the metaphase spindle length are almost identical in wild type and *swe1*Δ cells (Refs. 38 and 39 and supplemental Movies S1 and S2). Interestingly, after the mitotic spindle was correctly positioned at the bud neck, in *swe1*Δ cells it reached the anaphase morphology faster than in wild type cells (6.8 ± 1.5 min *versus* 9.7 ± 2.9 min) (Fig. 4*B* and supplemental Movies S1 and S2). The difference between the two strains is significant ($p$ value obtained by Wilcoxon rank sum test $p = 2.6 \times 10^{-12}$) and indicates that Swe1 act as an inhibitor of mitotic spindle elongation, in line with the result of the experiment above. Swe1 inhibits Cdk1 activity by phosphorylating the $Tyr^{19}$ residue of its catalytic subunit, Cdc28 (1). To exclude that the effect that we observed in *swe1*Δ cells was due to precocious Cdc28 activation, we performed the same experiment in cells carrying the nonphosphorylatable version of Cdc28, cdc28-Y19F (3). If the effect of *SWE1* deletion was due to a precocious activation of Cdc28, we would expect to see a speed up of spindle elongation in a strain expressing a Cdc28 version lacking $Tyr^{19}$ phosphorylation site. Interestingly, we observed that not only the time of spindle elongation is not shorter (11.8 min ± 3.2, 59 cells), but it is also significantly longer than in wild type cells ($p$ value obtained by Wilcoxon rank sum test $p = 1.3 \times 10^{-5}$), according with the notion that Cdk1 controls the phosphorylation of several microtubule binding proteins and therefore the lack of Cdc28 inhibition might interfere with correct spindle dynamics. These data indicate that the precocious mitotic spindle elongation that we observed in *swe1*Δ cells is not due to precocious Cdc28 activation but likely other regulator(s) of

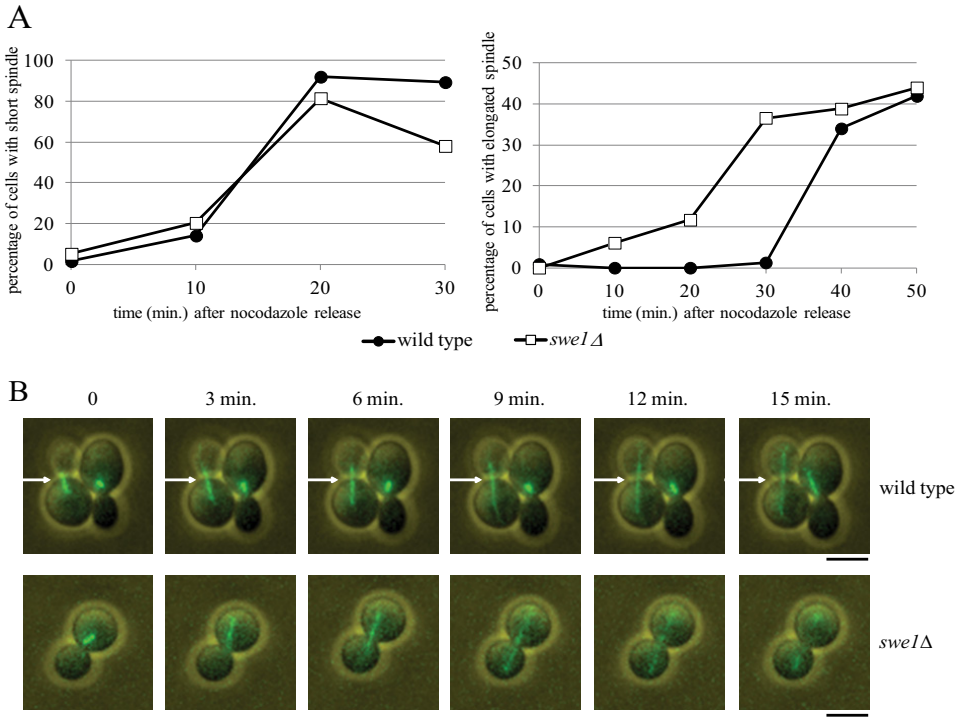



FIGURE 4. **The lack of Swe1 induces premature mitotic spindle elongation.** *A*, exponentially growing cultures of wild type and *swe1*Δ cells were arrested in metaphase with nocodazole and released from the block in YEPD fresh medium. Cell samples were taken at the indicated time points after release for scoring mitotic spindle formation and elongation by immunofluorescence using anti-Tub1 antibodies. *B*, exponentially growing cultures of wild type and *swe1*Δ cells, all expressing Tub1-GFP, were plated on glucose synthetic medium plates for time lapse analysis. Pictures were taken every 3 min for 276 min, and time-lapsed images were assembled to show mitotic spindle dynamics (supplemental Videos S1 and S2). The *arrows* indicate the wild type cells to be considered. Time 0 is the time in which the spindle is correctly positioned and aligned at the bud neck. *Bar*, 5 μm.

spindle elongation acting in a parallel pathway with respect to Cdc28 are misregulated in the absence of Swe1.

*The Nonphosphorylatable cdc28-Y19F Allele Does Not Restore Proper Spindle Elongation in SWE1-overexpressing Cells*—To confirm Swe1 role in mitotic spindle elongation, we decided to carefully analyze the phenotype of cells overexpressing *SWE1*. Swe1 overexpression from the *GAL1* promoter (*GAL1-SWE1*) is lethal for cells because it totally inhibits Cdc28, thus blocking both switch from polar to isotropic bud growth and spindle pole body separation; as a result, cells arrest with elongated buds, undivided nuclei, and without a mitotic spindle (39). As already reported, we observed that cell lethality induced by *GAL1-SWE1* is suppressed by the presence of a non-phosphorylatable version of Cdc28 (cdc28-Y19F) (3) or by the presence of cdc28-E12K, a variant that could not physically interact with Swe1 and therefore could not be phosphorylated and inhibited by Swe1 (40) (our observation). However, cdc28-Y19F and cdc28-E12K alleles are not able to restore normal cell cycle progression in Swe1-overexpressing cells (see below and our observation), further indicating that Swe1 could have other targets besides Cdc28 in controlling mitotic progression. Therefore we carefully characterized the phenotype of *GAL1-SWE1 cdc28-Y19F* cells in galactose containing medium. For this purpose, wild type, *GAL1-SWE1*, *cdc28-Y19F*, and *GAL1-SWE1 cdc28-Y19F* cells were inoculated in YEPR, arrested in $G_1$ with α-factor and released in galactose-containing medium (YEPRG) to induce Swe1 overproduction. Samples were taken at different time points after release to monitor the kinetics of DNA replication by FACS analysis, budding, nuclear division, and spindle assembly and elongation. Wild type and *cdc28-Y19F* cells showed very similar profiles of DNA replication, budding, and nuclear division (Fig. 5, *A–C*); *GAL1-SWE1* cells, as expected, replicated their DNA but failed to divide it, and, after 240 min, accumulated with 2C DNA content, undivided nuclei, and elongated buds and were unable to assemble a mitotic spindle (Fig. 5). Interestingly, *GAL1-SWE1 cdc28-Y19F* cells assembled a bipolar spindle with kinetics similar to *cdc28-Y19F* cells, but then spindle elongation was defective, because cells accumulated with the spindle stretched at the bud neck (Fig. 5, *C* and *D*). Accordingly, *GAL1-SWE1 cdc28-Y19F* cells were about 30–45 min delayed with respect to wild type cells in reaccumulating the 1C DNA peak (Fig. 5*A*); this difference was always observed in different *GAL1-SWE1 cdc28-Y19F* segregants during independent repeats of the same experiment (our observations). Interestingly, we also obtained the same results using the *cdc28-E12K* allele (data not shown). Therefore, the presence of Cdc28 variants that cannot be inhibited by Swe1 does not restore proper mitotic spindle elongation in Swe1 overproducing cells, further suggesting that the protein kinase Swe1 likely affects this process by acting on other target(s) besides Cdc28.

*The Spindle Elongation Defect of GAL1-SWE1 cdc28-Y19F Cells Is Not Due to a Defect in Sister Chromatids Cohesion Removal*—During anaphase, to allow sister chromatid separation, cohesin has to be removed because it is a structural barrier that opposes the pulling forces of the microtubules. After cohesin removal, shortening of the kinethocore microtubules that link each sister chromatid to the correct spindle pole leads to

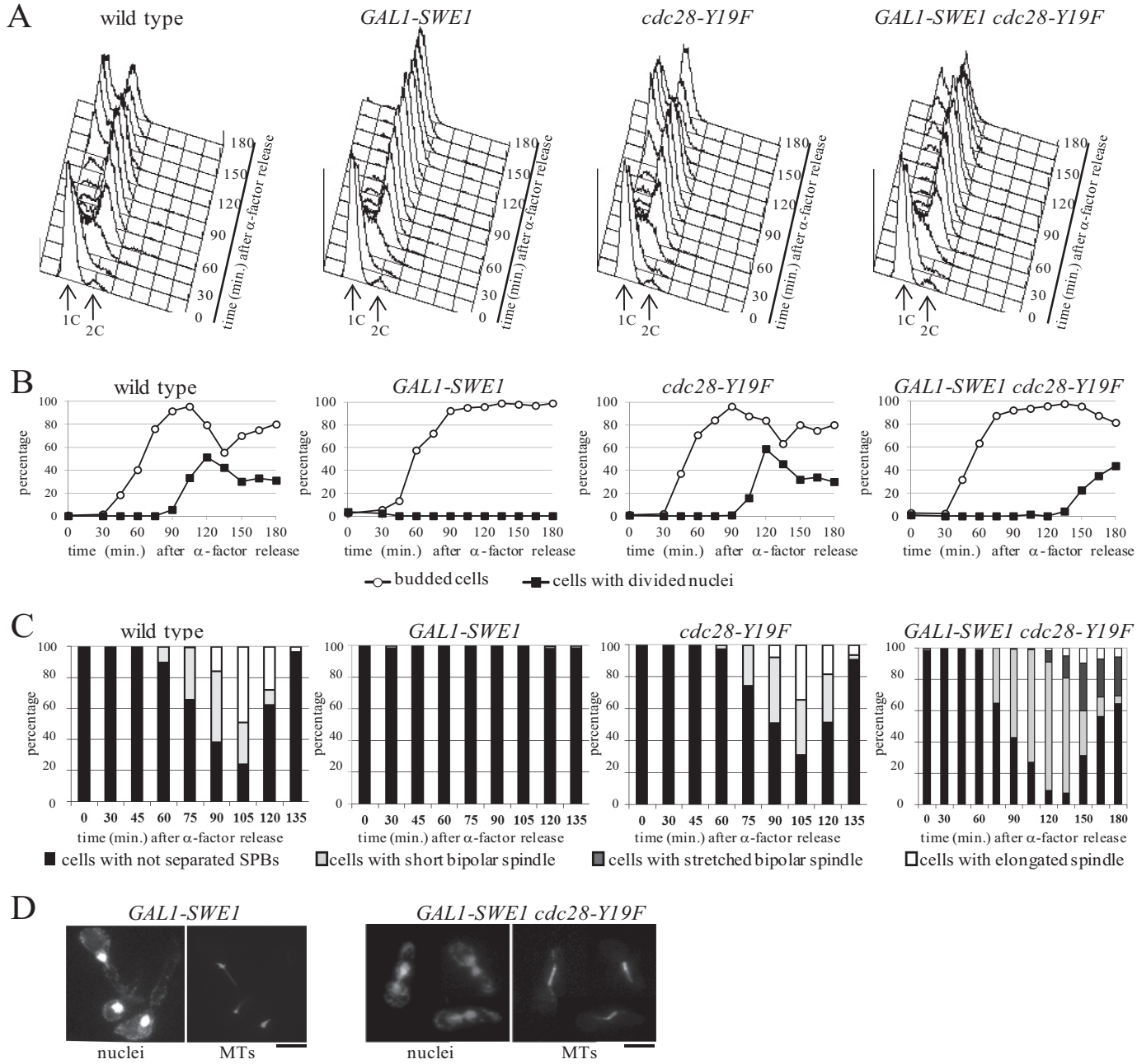



FIGURE 5. **cdc28-Y19F allele does not restore proper spindle elongation in *SWE1*-overexpressing cells.** Exponentially growing cultures of wild type, *GAL1-SWE1*, *cdc28-Y19F*, and *GAL1-SWE1 cdc28-Y19F* cells were arrested in $G_1$ by α-factor in YEPR and released in YEPRG at 25 °C (time 0) to induce *SWE1* overexpression. At the indicated times after release, cell samples were taken for FACS analysis of DNA content (*A*) and for scoring budding, nuclear division (*B*) and mitotic spindle formation and elongation by immunofluorescence using anti-tubulin antibodies (*C*). 180 min after release, pictures were taken to show nuclei and mitotic spindle (*MTs*) morphology of *GAL1-SWE1* and *GAL1-SWE1 cdc28-Y19F* cells (*D*). *Bar*, 5 μm.

chromosomes movement to spindle poles and afterward pushing forces drive mitotic spindle elongation (13). Therefore, for proper spindle elongation, a timely removal of the cohesion between sister chromatids is required. We therefore wondered whether the spindle elongation defect of *GAL1-SWE1 cdc28-Y19F* cells could be due to a delay in cohesin removal, rather than to a delay in spindle elongation. To address this point, wild type, *GAL1-SWE1*, *cdc28-Y19F*, and *GAL1-SWE1 cdc28-Y19F* cells, all carrying the TetO/TetR system to visualize sister chromatid separation (31), were inoculated in YEPR, arrested in $G_1$ with α-factor, and released in YEPRG to induce Swe1 overproduction. Samples were taken at different time points after release to monitor the kinetics of budding, nuclear division, and sister chromatid separation (Fig. 6*A*), visualized as fluorescent GFP dots. All the strains progressed through the cell cycle as described in Fig. 5. Interestingly, *GAL1-SWE1 cdc28-Y19F* cells started to separate sister chromatids with a kinetic similar to wild type and *cdc28-Y19F* cells (Fig. 6*A*), indicating that the spindle elongation defects of these cells are not due to a persistence in sister chromatids cohesion but to a defect in spindle dynamics, further supporting the idea that Swe1 plays a role in anaphase progression. In agreement with our previous experi-

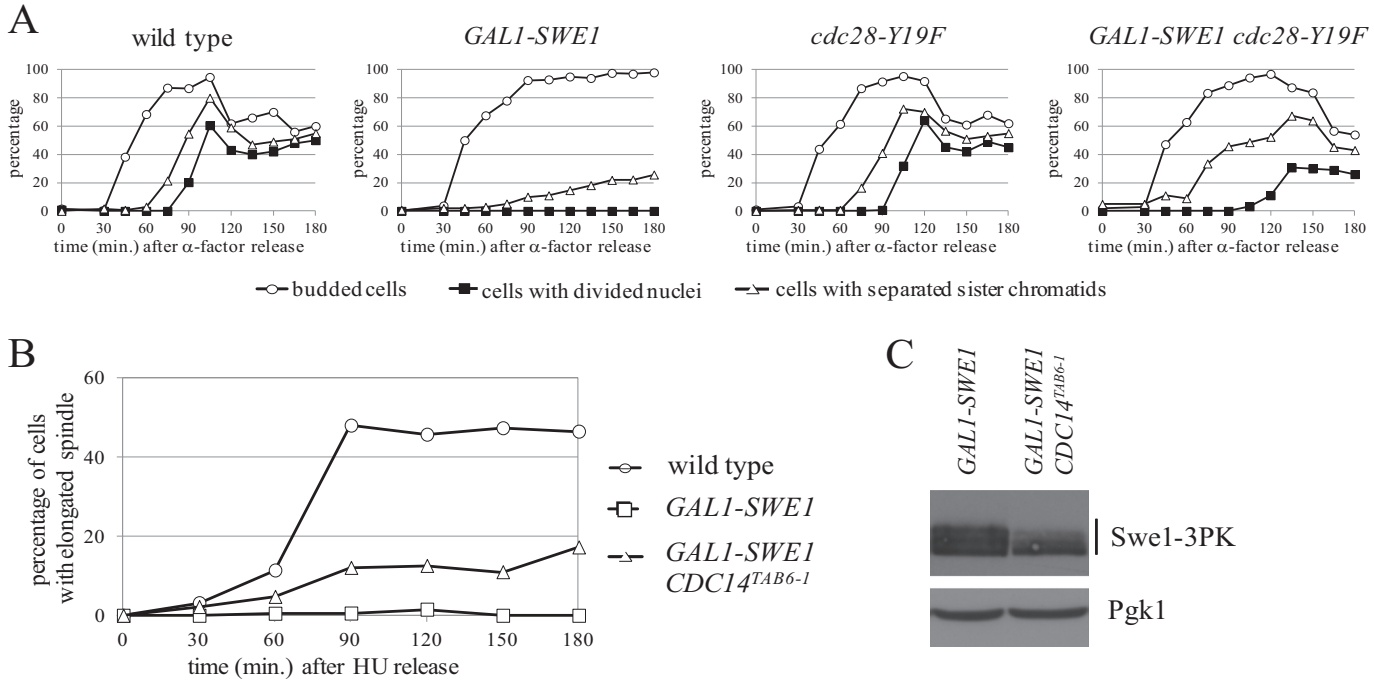



FIGURE 6. **Swe1-overexpressing cells are able to separate sister chromatids but cannot complete anaphase, and this defect is partially suppressed by a hyperactive Cdc14 variant.** *A*, exponentially growing cultures of wild type, *GAL1-SWE1*, *cdc28-Y19F*, and *GAL1-SWE1 cdc28-Y19F* cells were arrested in $G_1$ by α-factor in YEPR and released in YEPRG at 25 °C (time 0) to induce *SWE1* overexpression. At the indicated times after release, cell samples were taken for scoring budding, nuclear division, and sister chromatid separation. *B*, exponentially growing cultures of wild type, *GAL1-SWE1* and *GAL1-SWE1 CDC14$^{TAB6-1}$* cells were arrested in S phase in YEPR by hydroxyurea (*HU*) and released in the cell cycle in YEPRG medium at 25 °C to induce *SWE1* overexpression. Cell samples were taken at the indicated time points for scoring mitotic spindle elongation by immunofluorescence using anti-tubulin antibodies. *C*, *GAL1-SWE1-PK3* and *GAL1-SWE1-PK3 CDC14$^{TAB6-1}$* cells were treated as in *B*. 180 min after release, samples were taken to analyze Swe1 phosphorylation using anti-PK antibodies. Pgk1 was used as a loading control. Size markers on the side of the gel blots indicate kilodaltons.

ments, we also observed that *SWE1* overexpression from the *GAL1* promoter completely impairs spindle elongation in cells released both from a metaphase arrest and from S phase arrest (our observation and see below).

*Cdc14-dependent Swe1 Dephosphorylation Induces Spindle Elongation in SWE1-overexpressing Cells*—We showed that the protein phosphatase Cdc14, which is involved in anaphase progression, is likely involved in Swe1 inactivation (Figs. 1–3). Because Swe1 seems to act as an inhibitor of spindle elongation, a hyperactive Cdc14 variant might allow spindle elongation in Swe1-overexpressing cells. To test this hypothesis, we took advantage of *CDC14$^{TAB6-1}$* dominant allele (41), and we analyzed mitotic spindle dynamics in wild type, *GAL1-SWE1*, and *GAL1-SWE1 CDC14$^{TAB6-1}$* cells released from an hydroxyurea block, a condition in which cells arrest with separated spindle pole bodies and short bipolar spindle. Wild type cells elongate the spindle 90 min after the release, whereas Swe1 overproduction completely blocks this process (Fig. 6*B*), consistent with our previous data (Fig. 5). Interestingly, the introduction of *CDC14$^{TAB6-1}$* allele allows spindle elongation in a significant fraction of *SWE1*-overexpressing cells (Fig. 6*B*), confirming an important role of Cdc14 in Swe1 regulation. However, the presence of the *CDC14$^{TAB6-1}$* allele cannot restore wild type kinetics of spindle elongation, but this is not surprising because the phosphatase activity of the mutant enzyme is reduced to 75% of wild type levels (41) and because Swe1 is overexpressed in these cells. To test whether spindle elongation in the presence of *CDC14$^{TAB6-1}$* cells is related to Swe1 dephosphorylation, we analyzed mitotic spindle dynamics and Swe1 modifications in a similar experiment. *GAL1-SWE1-PK3* and *GAL1-SWE1-PK3 CDC14$^{TAB6-1}$* cells were released from an hydroxyurea block and after 180-min samples were taken to prepare protein extracts. As shown in Fig. 6*C*, Swe1 phosphorylated forms are reduced in *GAL1-SWE1-PK3 CDC14$^{TAB6-1}$* mutant with respect to *GAL1-SWE1-PK3*, supporting the hypothesis that Swe1 dephosphorylation correlates with mitotic spindle elongation.

## DISCUSSION

Even if the regulation of *Saccharomyces cerevisiae* Swe1 and its role in controlling mitotic entry have been very well described by previous studies, much of the evidence collected in this research indicates that other undiscovered Swe1 targets might exist and that it also might play a role during mitosis. Swe1 protein levels and activity are finely controlled during the cell cycle, because several protein kinases phosphorylate it, thus regulating its activity and the association with its substrates (10, 22). To allow mitotic entry, Swe1 is partially degraded during $G_2$ phase in unstressed cells (9); however, the molecular mechanisms that inactivate Swe1 are not fully uncovered. Accordingly, the isolation of Swe1 mutant variants that are more stable but do not show a defect in their interaction with any of the known Swe1 regulators (9) indicates the existence of other proteins that control Swe1 activity and abundance. In accordance with other studies (7, 12), we previously reported that Swe1 is not completely degraded after mitotic entry during an unperturbed cell cycle (11), and in this paper we confirm that Swe1 is present in metaphase-arrested cells, consistent with a physiological role for Swe1 after mitotic entry.

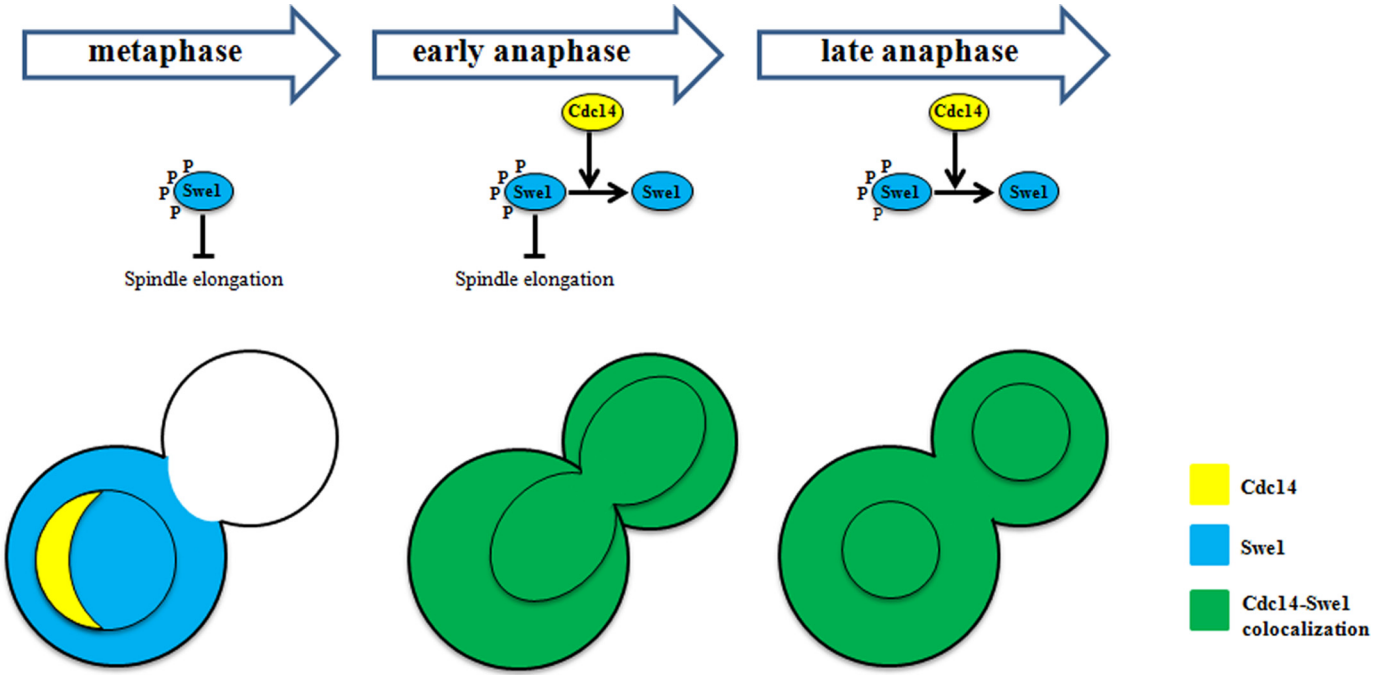



FIGURE 7. **Model of Swe1 role and regulation during mitosis.** Our data support a model in which Cdc14 regulates Swe1 dephosphorylation and localization, thus controlling mitotic spindle elongation and Swe1 distribution between mother and daughter cell (see text for details).

In addition, here we show that Swe1 is phosphorylated and mostly localized in the nucleus and in the cytoplasm of the mother cell during metaphase, and then, during anaphase and concomitantly with nuclear division, it is dephosphorylated and spread also into the bud. Interestingly, we observed that inactivation of the protein phosphatase Cdc14 leads to the formation of cells with elongated buds and that this phenotype is reversed by *SWE1* deletion, indicating that it is likely due to altered Swe1 regulation, which is known to cause hyperpolarized bud growth (9). In agreement with published data (34), our further analysis showed that Cdc14 can bind Swe1 *in vivo*, and we collected evidence that it is involved in its dephosphorylation. Indeed, Swe1 phosphorylated forms accumulate in the absence of Cdc14 function and disappear upon *CDC14* overexpression. The accumulation and disappearance of phosphorylated proteins is driven by a balance of kinase and phosphatase activities. It is known that Cdc14 is involved in Cdk1 inactivation (35), and Cdk1 is implicated in Swe1 phosphorylation (22); however, we demonstrated that the accumulation of Swe1 phosphorylated bands upon Cdc14 inactivation is not caused by high Cdk1 activity. In addition, we showed that Swe1 phosphorylated forms completely disappear upon overproduction of Cdc14 but persist in the presence of high levels of a phosphatase-dead Cdc14 variant. Our data suggest that the complete disappearance of Swe1 phosphorylated forms upon Cdc14 overexpression is more likely due to a direct Cdc14-dependent dephosphorylation rather than to Cdk1 inactivation that also occurs in these experimental conditions (35) and that has been reported to only partially decrease Swe1 hyperphosphorylated forms in mitosis (22). Consonant with the correlation between Swe1 phosphorylation and localization, we also observed that Cdc14-dependent dephosphorylation induces changes in Swe1 subcellular localization. Altogether, our data led us to envisage a role for Cdc14 on Swe1 inhibitory regulation during mitosis.

What could be the physiological role for Swe1 after mitotic entry? Meticulous analyses of *swe1*Δ phenotype revealed that the lack of Swe1 induces premature mitotic spindle elongation with respect to wild type cells, indicating that Swe1 might play a physiological role in this process. Interestingly, this phenotype is not due to unscheduled Cdc28 activation but to misregulation of regulator(s) of spindle elongation that act in parallel with respect to Cdc28. Premature mitotic spindle elongation is tolerated by otherwise wild type cells likely because, in normal conditions, a slight advance in anaphase progression does not affect cell viability. Instead, we observed that the lack of Swe1 increases the amount of binucleate cell bodies, arising from anaphase occurring in the mother cell, in spindle positioning mutants such as *kar9*Δ and *dyn1*Δ (our observation and Ref. 42). We also observed that the lack of Swe1 causes a slight sensitivity to benomyl (our observation), a compound that interferes with microtubules polymerization, further supporting the notion that Swe1 is involved in spindle dynamics.

In addition and in agreement with previous studies, we show that Cdc28 variants that cannot be inhibited by Swe1 are not able to completely revert the phenotype caused by a large amount of Swe1 (39). Indeed, *SWE1*-overexpressing cells accumulate with elongated buds and undivided nuclei and are unable to assemble a mitotic spindle; the presence of cdc28-Y19F and cdc28-E12K mutant proteins allows mitotic spindle formation but does not restore proper kinetics of spindle elongation and nuclear division in these cells. These results indicate that some players of anaphase progression are not active in these conditions and that Swe1 could inhibit this process by acting on other protein(s) besides Cdc28. We also show that the spindle elongation defect of *GAL1-SWE1 cdc28-Y19F* cells is not due to faulty sister chromatid separation, thus supporting the notion that Swe1 plays a role in anaphase progression after cohesin removal likely by playing a role in mitotic spindle dynamics. The idea that

Swe1 might phosphorylate protein(s) important for this process is also supported by the finding that its homolog in *Drosophila melanogaster* (dwee1) is involved in preserving mitotic spindle integrity (43). Importantly, we showed that an hyperactive Cdc14 variant ($Cdc14^{TAB6-1}$) could allow Swe1 dephosphorylation and spindle elongation in a considerable fraction of Swe1-overexpressing cells, thus confirming that Cdc14 controls Swe1 to allow timely anaphase progression.

Altogether, our results fit into the model depicted in Fig. 7. In metaphase, Swe1 is fully phosphorylated, and Cdc14 is sequestered in the nucleolus; in early anaphase, the FEAR pathway allows Cdc14 release from the nucleolus into the nucleus and to the spindle midzone to dephosphorylate specific targets to drive mitotic spindle elongation. After mitotic exit, the mitotic exit network pathway allows complete activation of Cdc14 that in turn localizes in the cytoplasm and at the bud neck to induce cytokinesis completion (16). In this scenario, we propose that Cdc14 binds and dephosphorylates Swe1, thus causing its inactivation and its distribution in the cytoplasm of the daughter cell in anaphase (Fig. 7). Because we could never visualize Swe1 at the spindle, we hypothesize that its dephosphorylation likely occurs in the nucleoplasm after Cdc14 has been released from the nucleolus. This regulation contributes to release the block of mitotic spindle elongation so that this process can take place. During early anaphase, Swe1 dephosphorylation could either inhibit Swe1 kinase activity or destroy the complex of Swe1 with its target(s). Both mechanisms are plausible because a nonphosphorylatable Swe1 variant was described to display reduced kinase activity *in vitro* (22), indicating that Swe1 dephosphorylation may lead to its inactivation. However, overproduction of a kinase-dead Swe1 version causes the same effects on spindle dynamics as the overproduction of the wild type protein (our observation), indicating that Swe1 might play its inhibitory role independently of its kinase activity. In anaphase, released Cdc14 could dephosphorylate the remaining Swe1, thus ensuring that a pool of Swe1 is translocated to the daughter cell and is present during the following $G_1$ phase. Our study discloses new aspects of the control of anaphase progression that reveal Swe1 as a new player in this process that is regulated in a Cdc14-dependent manner and that allows proper coordination of important cellular processes such as nuclear division and mitotic spindle elongation. We hypothesize that Swe1 contributes to restraining mitotic spindle elongation until anaphase and that its inactivation by dephosphorylation is required to allow timely mitotic spindle elongation, thus ensuring the maintenance of genomic stability.

An interesting question that remains unanswered is which protein is kept inactive or activated by Swe1 to block spindle elongation, and further studies will be required to shed light on this issue. We hypothesize that Swe1 could bind a factor that is localized at the mitotic spindle; however, we could never observe Swe1 localization at the spindle, suggesting that it might form a complex with a diffusible protein that binds microtubule-associated proteins or the spindle only after Swe1 inactivation.

*Acknowledgments—We are grateful to Daniel J. Lew, Elmar Schiebel, Gislene Pereira, Simonetta Piatti, and Rosella Visintin for strains and plasmids.*

## REFERENCES


1. Booher, R. N., Deshaies, R. J., and Kirschner, M. W. (1993) Properties of *Saccharomyces cerevisiae* wee1 and its differential regulation of p34CDC28 in response to $G_1$ and $G_2$ cyclins. *EMBO J.* **12,** 3417–3426
2. Russell, P., Moreno, S., and Reed, S. I. (1989) Conservation of mitotic controls in fission and budding yeasts. *Cell* **57,** 295–303
3. Amon, A., Surana, U., Muroff, I., and Nasmyth, K. (1992) Regulation of p34CDC28 tyrosine phosphorylation is not required for entry into mitosis in *S. cerevisiae*. *Nature* **355,** 368–371
4. McMillan, J. N., Sia, R. A., and Lew, D. J. (1998) A morphogenesis checkpoint monitors the actin cytoskeleton in yeast. *J. Cell Biol.* **142,** 1487–1499
5. Keaton, M. A., and Lew, D. J. (2006) Eavesdropping on the cytoskeleton: progress and controversy in the yeast morphogenesis checkpoint. *Curr. Opin. Microbiol.* **9,** 540–546
6. Sia, R. A., Herald, H. A., and Lew, D. J. (1996) Cdc28 tyrosine phosphorylation and the morphogenesis checkpoint in budding yeast. *Mol. Biol. Cell* **7,** 1657–1666
7. Sia, R. A., Bardes, E. S., and Lew, D. J. (1998) Control of Swe1p degradation by the morphogenesis checkpoint. *EMBO J.* **17,** 6678–6688
8. Kellogg, D. R. (2003) Wee1-dependent mechanisms required for coordination of cell growth and cell division. *J. Cell Sci.* **116,** 4883–4890
9. McMillan, J. N., Theesfeld, C. L., Harrison, J. C., Bardes, E. S., and Lew, D. J. (2002) Determinants of Swe1p degradation in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **13,** 3560–3575
10. Asano, S., Park, J. E., Sakchaisri, K., Yu, L. R., Song, S., Supavilai, P., Veenstra, T. D., and Lee, K. S. (2005) Concerted mechanism of Swe1/Wee1 regulation by multiple kinases in budding yeast. *EMBO J.* **24,** 2194–2204
11. Raspelli, E., Cassani, C., Lucchini, G., and Fraschini, R. (2011) Budding yeast Dma1 and Dma2 participate in regulation of Swe1 levels and localization. *Mol. Biol. Cell* **22,** 2185–2197
12. King, K., Kang, H., Jin, M., and Lew, D. J. (2013) Feedback control of Swe1p degradation in the yeast morphogenesis checkpoint. *Mol. Biol. Cell* **24,** 914–922
13. de Gramont, A., and Cohen-Fix, O. (2005) The many phases of anaphase. *Trends Biochem. Sci.* **30,** 559–568
14. Yeh, E., Skibbens, R. V., Cheng, J. W., Salmon, E. D., and Bloom, K. (1995) Spindle dynamics and cell cycle regulation of dynein in the budding yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.* **130,** 687–700
15. Stegmeier, F., Visintin, R., and Amon, A. (2002) Separase, polo kinase, the kinetochore protein Slk19, and Spo12 function in a network that controls Cdc14 localization during early anaphase. *Cell* **108,** 207–220
16. Stegmeier, F., and Amon, A. (2004) Closing mitosis: the functions of the Cdc14 phosphatase and its regulation. *Annu. Rev. Genet.* **38,** 203–232
17. Higuchi, T., and Uhlmann, F. (2005) Stabilization of microtubule dynamics at anaphase onset promotes chromosome segregation. *Nature* **433,** 171–176
18. Woodbury, E. L., and Morgan D. O. (2007) Cdk and APC activities limit the spindle-stabilizing function of Fin1 to anaphase. *Nat. Cell Biol.* **9,** 106–112
19. Pereira, G., and Schiebel, E. (2003) Separase regulates INCENP-Aurora B anaphase spindle function through Cdc14. *Science.* **302,** 2120–2124
20. Sherman, F. (1991) Getting started with yeast. *Methods Enzymol.* **194,** 3–21
21. Visintin, C., Tomson, B. N., Rahal, R., Paulson, J., Cohen, M., Taunton, J., Amon, A., and Visintin, R. (2008) APC/C-Cdh1-mediated degradation of the Polo kinase Cdc5 promotes the return of Cdc14 into the nucleolus. *Genes Dev.* **22,** 79–90
22. Harvey, S. L., Charlet, A., Haas, W., Gygi, S. P., and Kellogg, D. R. (2005) Cdk1-dependent regulation of the mitotic inhibitor Wee1. *Cell* **122,** 407–420
23. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1992) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
24. Wach, A., Brachat, A., Pöhlmann, R., and Philippsen, P. (1994) New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. *Yeast* **10,** 1793–1808
25. Janke, C., Magiera, M. M., Rathfelder, N., Taxis, C., Reber, S., Maekawa,

H., Moreno-Borchart, A., Doenges, G., Schwob, E., and Schiebel, E. (2004) A versatile toolbox for PCR-based tagging of yeast genes: new fluorescent proteins, more markers and promoter substitution cassettes. *Yeast* **21,** 947–962
26. Fraschini, R., D'Ambrosio, C., Venturetti, M., Lucchini, G., and Piatti, S. (2006) Disappearance of the budding yeast Bub2-Bfa1 complex from the mother-bound spindle pole contributes to mitotic exit. *J. Cell Biol.* **172,** 335–346
27. Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., and Schmid, B. (2012) Fiji: an open-source platform for biological-image analysis. *Nat. Methods* **9,** 676–682
28. Piatti, S., Böhm, T., Cocker, J. H., Diffley, J. F., and Nasmyth, K. (1996) Activation of S-phase-promoting CDKs in late $G_1$ defines a "point of no return" after which Cdc6 synthesis cannot promote DNA replication in yeast. *Genes Dev.* **10,** 1516–1531
29. Fraschini, R., Beretta, A., Sironi, L., Musacchio, A., Lucchini, G., and Piatti, S. (2001) Bub3 interaction with Mad2, Mad3 and Cdc20 is mediated by WD40 repeats and does not require intact kinetochores. *EMBO J.* **20,** 6648–6659
30. Fraschini, R., Formenti, E., Lucchini, G., and Piatti, S. (1999) Budding yeast Bub2 is localized at spindle pole bodies and activates the mitotic checkpoint via a different pathway from Mad2. *J. Cell Biol.* **145,** 979–991
31. Michaelis, C., Ciosk, R., and Nasmyth, K. (1997) Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell* **91,** 35–45
32. Bembenek, J., Kang, J., Kurischko, C., Li, B., Raab, J. R., Belanger, K. D., Luca, F. C., and Yu, H. (2005) Crm1-mediated nuclear export of Cdc14 is required for the completion of cytokinesis in budding yeast. *Cell Cycle* **4,** 961–971
33. Howell, A. S., and Lew, D. J. (2012) Morphogenesis and the cell cycle. *Genetics* **190,** 51–77
34. Breitkreutz, A., Choi, H., Sharom, J. R., Boucher, L., Neduva, V., Larsen, B., Lin, Z. Y., Breitkreutz, B. J., Stark, C., Liu, G., Ahn, J., Dewar-Darch, D., Reguly, T., Tang, X., Almeida, R., Qin, Z. S., Pawson, T., Gingras, A. C., Nesvizhskii, A. I., and Tyers, M. (2010) A global protein kinase and phosphatase interaction network in yeast. *Science* **328,** 1043–1046
35. Visintin, R., Craig, K., Hwang, E. S., Prinz, S., Tyers, M., and Amon, A. (1998) The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. *Mol Cell* **2,** 709–718
36. Bishop, A. C., Ubersax, J. A., Petsch, D. T., Matheos, D. P., Gray, N. S., Blethrow, J., Shimizu, E., Tsien, J. Z., Schultz, P. G., Rose, M. D., Wood, J. L., Morgan, D. O., and Shokat, K. M. (2000) A chemical switch for inhibitor-sensitive alleles of any protein kinase. *Nature* **407,** 395–401
37. Lew, D. J. (2003) The morphogenesis checkpoint: how yeast cells watch their figures. *Curr. Opin. Cell Biol.* **15,** 648–653
38. Harvey, S. L., and Kellogg, D. R. (2003) Conservation of mechanisms controlling entry into mitosis: budding yeast wee1 delays entry into mitosis and is required for cell size control. *Curr. Biol.* **13,** 264–275
39. Lim, H. H., Goh, P. Y., and Surana, U. (1996) Spindle pole body separation in *Saccharomyces cerevisiae* requires dephosphorylation of the tyrosine 19 residue of Cdc28. *Mol. Cell. Biol.* **16,** 6385–6397
40. McMillan, J. N., Sia, R. A., Bardes, E. S., and Lew, D. J. (1999) Phosphorylation-independent inhibition of Cdc28p by the tyrosine kinase Swe1p in the morphogenesis checkpoint. *Mol. Cell. Biol.* **19,** 5981–5990
41. Shou, W., Sakamoto, K. M., Keener, J., Morimoto, K. W., Traverso, E. E., Azzam, R., Hoppe, G. J., Feldman, R. M., DeModena, J., Moazed, D., Charbonneau, H., Nomura, M., and Deshaies, R. J. (2001) Net1 stimulates RNA polymerase I transcription and regulates nucleolar structure independently of controlling mitotic exit. *Mol. Cell* **8,** 45–55
42. Merlini, L., Fraschini, R., Boettcher, B., Barral, Y., Lucchini, G., and Piatti, S. (2012) Budding yeast Dma proteins control septin dynamics and the spindle position checkpoint by promoting the recruitment of the Elm1 kinase to the bud neck. *PLoS Genet.* **8,** e1002670
43. Garcia, K., Stumpff, J., Duncan, T., and Su T. T. (2009) Tyrosines in the kinesin-5 head domain are necessary for phosphorylation by Wee1 and for mitotic spindle integrity. *Curr. Biol.* **19,** 1670–1676

# Budding Yeast Swe1 Is Involved in the Control of Mitotic Spindle Elongation and Is Regulated by Cdc14 Phosphatase during Mitosis

Erica Raspelli, Corinne Cassani, Elena Chiroli and Roberta Fraschini

*J. Biol. Chem. 2015, 290:1-12.*
*doi: 10.1074/jbc.M114.590984 originally published online November 18, 2014*

# ADDITIONS AND CORRECTIONS

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 290, NO. 10, p. 6006, March 6, 2015

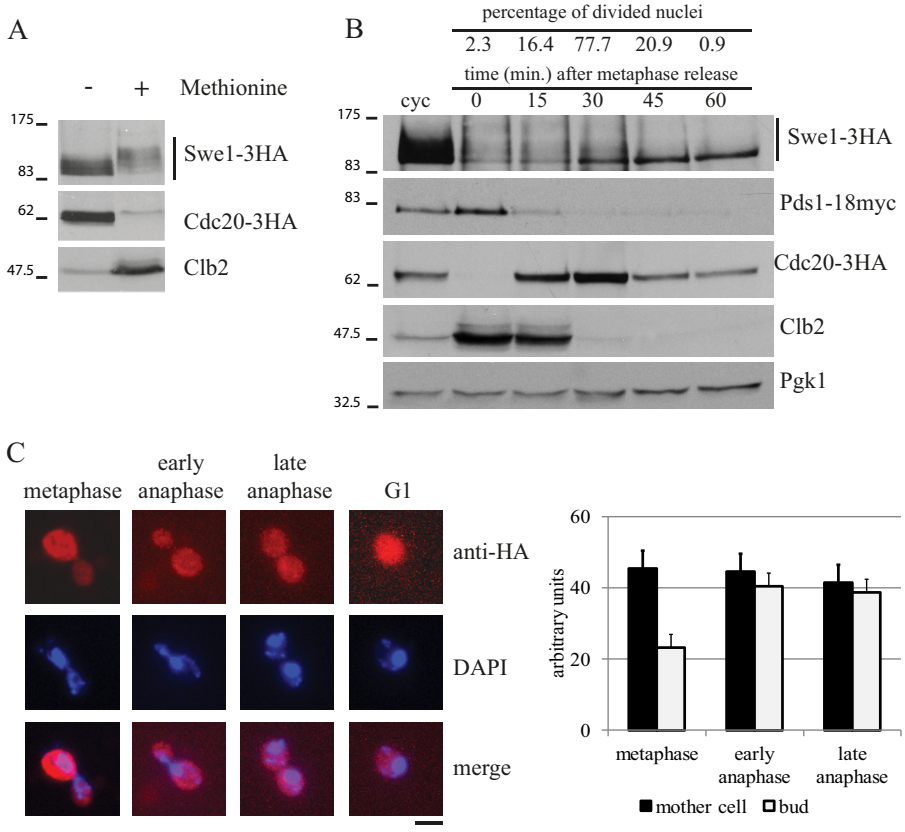

VOLUME 290 (2015) PAGES 1–12
DOI 10.1074/jbc.A114.590984

## Budding yeast Swe1 is involved in the control of mitotic spindle elongation and is regulated by Cdc14 phosphatase during mitosis.

**Erica Raspelli, Corinne Cassani, Elena Chiroli, and Roberta Fraschini**

**PAGE 4:**

The Clb2 data in Fig. 1*B* are not correct. The correct Clb2 data, shown here, were included in the initial version of Fig. 1*B* in the manuscript that was submitted for review, but a duplicate of the Cdc20–3HA data was mistakenly substituted for the Clb2 data in subsequent versions. This correction does not affect the interpretation of the results or the conclusions of this work.




Authors are urged to introduce these corrections into any reprints they distribute. Secondary (abstract) services are urged to carry notice of these corrections as prominently as they carried the original abstracts.