The budding yeast PP2A^{Cdc55} protein phosphatase prevents the onset of anaphase in response to morphogenetic defects

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Faithful chromosome transmission requires establishment of sister chromatid cohesion during S phase, followed by its removal at anaphase onset. Sister chromatids are tethered together by cohesin, which is displaced from chromosomes through cleavage of its Mcd1 subunit by the separase protease. Separase is in turn inhibited, up to this moment, by securin. Budding yeast cells respond to morphogenetic defects by a transient arrest in G2 with high securin levels and unseparated chromatids. We show that neither securin elimination nor forced cohesin cleavage is sufficient for anaphase in these conditions, suggesting that other factors contribute to cohesion maintainance in G2. We find that the protein phosphatase PP2A bound to its regulatory subunit Cdc55 plays a key role in this process, uncovering a new function for PP2A^{Cdc55} in controlling a noncanonical pathway of chromatid cohesion removal.

Introduction

Balanced chromosome partitioning during anaphase relies on the prior establishment of sister chromatid cohesion, which takes place concomitantly to DNA replication. Sister chromatid cohesion is essential for bipolar attachment of chromosomes to the mitotic spindle and depends on a cohesin complex formed by the Smc1, Smc3, Mcd1/Scc1, and Scc3 proteins. The Pds5 protein binds less tightly to this core complex but also contributes to sister chromatid cohesion (Nasmyth and Haering, 2005).

To undergo chromosome segregation in anaphase, cohesin must be removed from chromosomes. This occurs through two distinct routes in higher eukaryotes, where a "prophase pathway" involving Polo and Aurora B kinases promotes the dissociation of most cohesin from chromosome arms as they condense. The remaining cohesin is removed at the metaphase-to-anaphase transition by separase that operates the proteolytic cleavage of Mcd1, in turn stimulated by Polo kinase–dependent Mcd1 phosphorylation (Uhlmann, 2003; Nasmyth and Haering, 2005). Such a prophase pathway does not seem to exist in budding and fission yeasts, where separase appears fully responsible for cohesin dissociation along the entire chromosome (Uhlmann, 2003).

Because of its irreversible nature, sister chromatid separation is tightly regulated and inhibited by several checkpoint mechanisms. Separase activation, for example, is finely tuned by its association with securin, which acts both as a molecular chaperone contributing to separase activation and as an inhibitor of its protease activity (Agarwal and Cohen-Fix, 2002; Uhlmann, 2003). Anaphase-promoting complex (APC)–dependent ubiquitylation of securin triggers its destruction, which is essential for anaphase onset (Peters, 2006), and both DNA and spindle damage inhibit anaphase by stabilizing securin (Uhlmann, 2001).

In budding yeast, the morphogenesis checkpoint prevents the onset of anaphase in case of budding defects or alterations of the actin cytoskeleton. This depends on the Swe1 kinase that triggers the inhibitory phosphorylation of Cdk1 (Lew, 2003). By investigating how the morphogenesis checkpoint controls sister chromatid separation, we found that neither securin inactivation nor forced Mcd1 cleavage are sufficient to allow anaphase when the morphogenesis checkpoint is activated. Rather, the protein phosphatase PP2A associated with its regulatory subunit Cdc55 is necessary to inhibit sister chromatid separation under these circumstances. Altogether, our data highlight a novel mechanism for controlling sister chromatid severing and segregation that involves the PP2A^{Cdc55}-regulated release of cohesion.

Results

The morphogenesis checkpoint prevents sister chromatid separation independently of Pds1

High levels of a truncated version of the budding yeast p21-activated kinase Cla4 (Cla4t) activate the morphogenesis checkpoint by

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Abbreviations used in this paper: APC, anaphase-promoting complex; Lat-A, latrunculin-A; TEV, tobacco etch virus.

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inhibiting endogenous Cla4 and its paralogue Ste20 (Chiroli et al., 2003), which share essential functions in bud neck formation, septin ring assembly, and cytokinesis (Johnson, 1999). Upon *CLA4t* overexpression from the *GAL1* promoter, haploid yeast cells arrest with wide bud necks, replicated chromosomes, undivided nuclei, short metaphase spindles, and high levels of the securin Pds1 (Chiroli et al., 2003). In addition, they markedly delay activation of the Polo kinase Cdc5 (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200609088/DC1), suggesting that they arrest in G2.

As *PDS1* deletion is sufficient to allow anaphase in most mutants arresting in mitosis, we asked whether it could bypass the G2 arrest caused by high Cla4t levels. Elutriated G1 cells of a *pds1Δ* strain with four copies of the *GAL1-CLA4t* construct integrated in the genome (4X *GAL1-CLA4t pds1Δ*) were released into the cell cycle in the presence of galactose. As expected, DNA replication (Fig. 1 A) and bipolar spindle formation (Fig. 1 B) took place normally in these conditions, whereas bud neck formation was abnormal because of *CLA4t* overexpression (not depicted). Surprisingly, pericentromeric chromosomal sequences marked by a tet operator array that binds TetR-GFP (Michaelis et al., 1997) could not separate in these cells (Fig. 1 B), indicating that sister chromatid separation did not occur. Nuclear division and spindle elongation did not take place throughout the course of the experiment (Fig. 1, B and C), similar to 4X *GAL1-CLA4t* cells under the same conditions (Fig. 1, A, B, and C). Thus, deletion of *PDS1* is not sufficient to bypass the G2 arrest caused by high levels of Cla4t.

As shown in Fig. 1 (D–F), latrunculin-A (Lat-A), which activates the morphogenesis checkpoint by depolymerizing the actin cytoskeleton, induced, like Cla4t, a securin-independent G2 arrest. In fact, cells released from a G1 arrest in the presence of Lat-A did not bud (Fig. 1 E) but replicated DNA (Fig. 1 D) and formed bipolar spindles (Fig. 1, E and F). However, neither wild-type nor *pds1Δ* cells underwent sister chromatid separation, nuclear division, or spindle elongation (Fig. 1, E and F). In contrast, the same events took place promptly in the morphogenesis checkpoint–defective *swe1Δ* cells, which also exited mitosis and entered a new round of DNA replication, as indicated by the appearance of 4C DNA contents (Fig. 1 D). Altogether, these data indicate that the morphogenesis checkpoint appears to prevent the onset of anaphase independently of securin.

CLA4t overexpression does not impair securin-mediated nuclear import of separase

Besides its inhibitory function, securin also has a positive role in separase activation in several eukaryotic systems, prompting us to test whether Cla4t overproduction might impair Pds1 interaction with the Esp1 separase and/or Esp1 nuclear import. Wild-type, 4X *GAL1-CLA4t*, and 4X *GAL1-CLA4t swe1Δ* cells expressing HA-tagged Pds1 (Pds1-HA) and myc-tagged Esp1 (Esp1-myc18) were grown in raffinose, arrested in G1 by α-factor, and released in the presence of galactose, followed by the analysis of Pds1 and Esp1 nuclear localization and physical interaction. As shown in Fig. 2 A, budding was delayed in 4X *GAL1-CLA4t* cells compared with wild type, but kinetics of Pds1-HA and Esp1-myc18 nuclear accumulation were similar in the two strains.

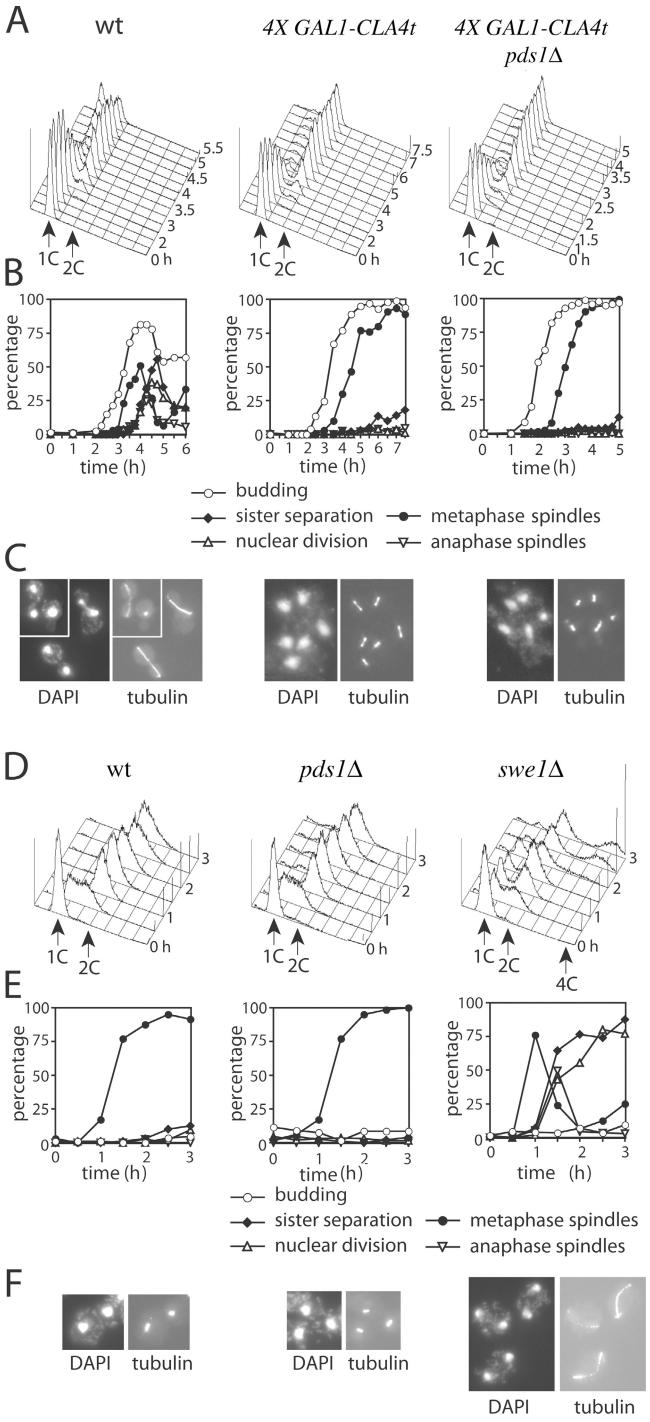

Figure 1. **G2 arrest by the morphogenesis checkpoint does not depend on securin.** (A–C) Strains with the indicated genotypes (ySP3575, ySP3435, and ySP3436) were grown at 25°C in YEPR. Elutriated G1 unbudded cells were released at 25°C in the presence of galactose (time 0), followed by FACS analysis of DNA contents (A) and determination of kinetics of budding, sister chromatid separation, spindle formation/elongation, and nuclear division (B). Micrographs (C) were taken at 240 min (wt), 420 min (4X *GAL1-CLA4t*), and 300 min (4X *GAL1-CLA4t pds1Δ*). (D–F) wt (ySP601), *pds1Δ* (ySP2894), and *swe1Δ* (ySP3887) cells were grown in YEPD at 23°C, arrested in G1 by α-factor, and released in the presence of Lat-A, followed by FACS analysis of DNA contents (D) and determination of kinetics of budding, sister chromatid separation, spindle formation/elongation, and nuclear division (E). (F) Micrographs represent cells at 90 min.

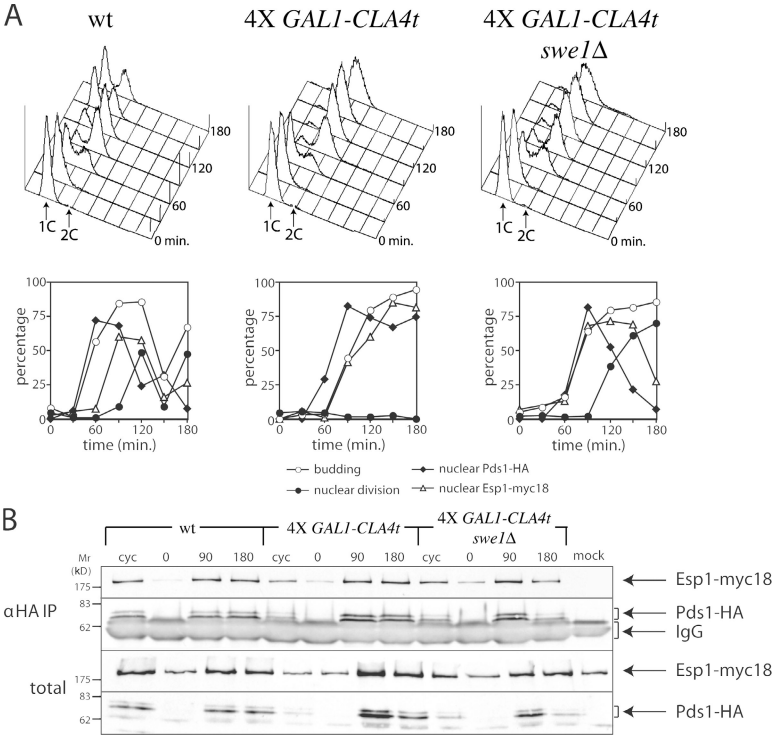

Figure 2. **Binding to securin and nuclear accumulation of separase are not affected by the morphogenesis checkpoint.** Strains with the indicated genotypes (ySP1735, ySP4690, and ySP4692) were grown in YEPR at 25°C, arrested by α-factor, and released in YEPRG (time 0). (A) At the indicated times, cells were analyzed for DNA contents (histograms), budding, nuclear division, and Pds1 and Esp1 nuclear accumulation (graphs). Pds1 and Esp1 localization was scored on fixed cells after in situ immunofluorescence. (B) Protein extracts from cells harvested at the indicated times were analyzed by immunoblotting with anti-HA (Pds1) or anti-myc (Esp1) antibodies either directly (total) or after Pds1 immunoprecipitation with anti-HA antibodies (αHA IP).

Thus, Pds1 can still act as an Esp1 molecular chaperone in the presence of high levels of Cla4t. Accordingly, similar levels of Esp1-myc18 were immunoprecipitated with Pds1-HA from both wild-type and 4X *GAL1-CLA4t* cell extracts (Fig. 2 B).

Cohesin cleavage is not sufficient for execution of anaphase in the presence of high Cla4t levels

Although lack of securin did not allow chromatid separation upon morphogenesis checkpoint activation, ectopic cohesin cleavage could be expected to trigger nuclear division in the same conditions. We engineered 4X *GAL1-CLA4t* cells to express a Mcd1–tobacco etch virus (TEV) variant, where the Esp1 cleavage site at position 268 is replaced by the recognition sequence for the TEV protease (Uhlmann et al., 2000). We then introduced in the same cells the TEV protease coding sequence under the control of the *GAL1* promoter. These cells grow normally under uninduced conditions because the Mcd1-TEV variant can be cleaved by separase at position 180, whereas it is cleaved and fully removed from chromosomes upon TEV induction even if separase is inactive. Small G1 cells of this strain were elutriated and released in the presence of galactose to trigger expression of both Cla4t and TEV. Remarkably, nuclear division did not take place (Fig. 3 A), suggesting that cohesin cleavage might be insufficient to allow chromosome segregation in 4X *GAL1-CLA4t* cells. Conversely, as previously reported (Uhlmann et al., 2000), cohesin cleavage by the TEV protease was sufficient to trigger anaphase in cells depleted for Cdc20 (Fig. 3 B), the APC regulatory subunit essential for Pds1 proteolysis and anaphase onset (Peters, 2006). Thus, cohesin cleavage seems to be sufficient to trigger anaphase in metaphase-arrested cells but not in cells arrested in G2 by the morphogenesis checkpoint.

Because it was formally possible that the lack of nuclear division in 4X *GAL1-CLA4t MCD1-TEV* cells was due to inefficient cohesin cleavage, we analyzed the kinetics of cohesin cleavage by the TEV protease in 4X *GAL1-CLA4t* versus wild-type cells after release from G1 in the presence of galactose. Full length of Mcd1-TEV tagged with 3 HA epitopes at the C terminus (Mcd1-HA3) and its cleavage product by separase (at position 180) were detectable in both strains in cycling cells and at time 0 (Fig. 3 C). Upon galactose addition, kinetics of TEV production, as well as appearance of the TEV-induced Mcd1-HA3 cleavage product (at position 268), were similar in the two strains. However, disappearance of full-length Mcd1 and its separase-induced cleavage product, which can both be cleaved by TEV, was slower in 4X *GAL1-CLA4t* than wild-type cells (Fig. 3 C). This might be due to delayed activation of the Polo/Cdc5 kinase, which stimulates Mcd1 cleavage (Alexandru et al., 2001), in 4X *GAL1-CLA4t* versus wild-type cells. In spite of that, most, if not all, Mcd1-HA3 was cleaved by 3 h in 4X *GAL1-CLA4t* cells, but nuclear division occurred only in a small fraction of them (Fig. 3 C). In contrast, >75% of wild-type cells had accomplished nuclear division under the same conditions. Therefore, other mechanisms besides cohesin-mediated sister chromatid cohesion likely contribute to prevent chromosome segregation when the morphogenesis checkpoint is active.

The spindle is functional under morphogenesis checkpoint activation

Because mitotic Cdks regulate spindle assembly and microtubule dynamics, the morphogenesis checkpoint might delay nuclear division through spindle misfunction. Upon bipolar attachment of sister kinetochores to microtubules, spindle forces overwhelm centromeric cohesion, leading to precocious separation of sister

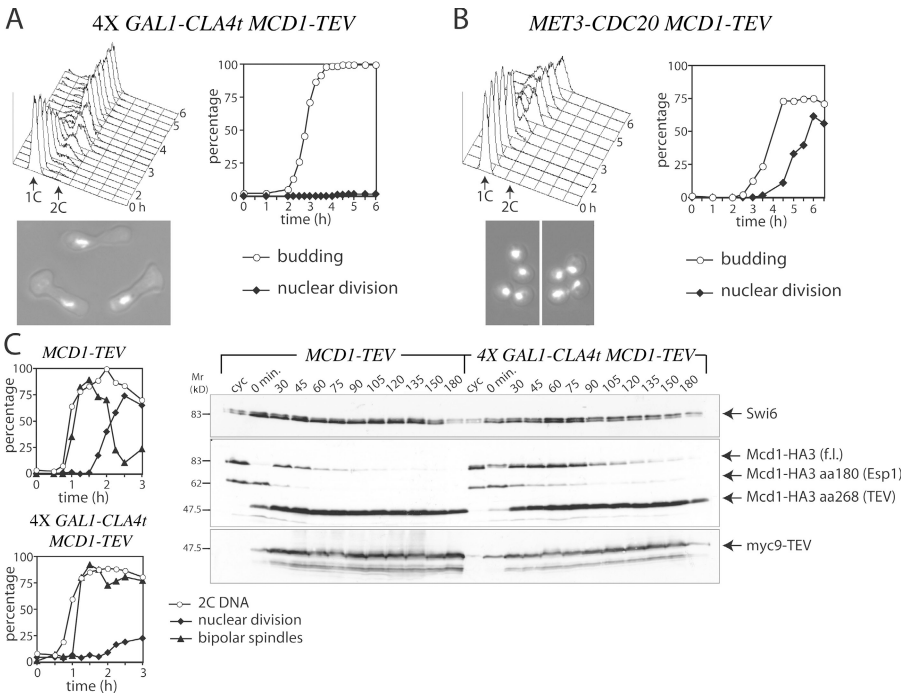

Figure 3. **Mcd1 cleavage is not sufficient for nuclear division upon *CLA4t* overexpression.** (A) *GAL1-CLA4t* cells expressing Mcd1-TEV and *GAL1-TEV* (ySP5871) were grown in YEPR at 25°C. Elutriated small G1 cells were released in YEPRG at 25°C (time 0). At the indicated time points, cell samples were analyzed for DNA contents (top left), budding, and nuclear division (top right). Micrographs represent cells at the end of the experiment. (B) *MET3-CDC20 MCD1-TEV GAL1-TEV* cells (ySP5870) were grown in raffinose medium lacking methionine. Elutriated G1 cells were released in YEPRG containing 2 mM methionine (time 0). Cell samples were analyzed as in A. (C) *MCD1-TEV* (ySP3448) and *GAL1-CLA4t MCD1-TEV* (ySP5871) cells were grown in YEPR at 25°C, arrested in G1 by α-factor, and released in YEPRG at 25°C at time 0. Cells were collected at the indicated times for Western blot analysis with anti-HA (Mcd1) and anti-myc (TEV) antibodies (left), FACS analysis of DNA contents (not depicted), and kinetics of nuclear division and bipolar spindle formation (right). Swi6 was used as loading control.

centromeres before anaphase (Goshima and Yanagida, 2000), thus providing a readout for spindle function. We found that sister centromeres of chromosome 15 could separate concomitantly with spindle formation in the presence of Lat-B (Fig. 4 B), suggesting that spindle forces are normal.

Because kinetochore inactivation by the *ndc10-1* mutation prevents kinetochore–microtubule attachment without affecting spindle formation and elongation (Goh and Kilmartin, 1993), we also asked whether spindle elongation could take place in *ndc10-1* cells under morphogenesis checkpoint activation. We induced morphogenetic defects by using a temperature-sensitive *cdc24* mutation, which alters a guanine-nucleotide exchange factor for the GTPase Cdc42 that is required for budding (Johnson, 1999). Upon release of synchronized G1 cells at 37°C, *cdc24* cells arrested in G2 as unbudded with undivided nuclei and short metaphase spindles. Lack of kinetochore attachment in *cdc24 ndc10-1* cells was sufficient to allow spindle elongation (Fig. 4 A), suggesting that spindle dynamics is not affected by morphogenetic defects. Therefore, residual sister chromatid cohesion, rather than a misfunctional spindle, is likely responsible for preventing chromosome segregation in the absence of Mcd1 upon morphogenesis checkpoint activation.

The phosphatase PP2A^{Cdc55} prevents sister chromatid separation upon morphogenesis checkpoint activation

Cdc55 is one of the two regulatory subunits of yeast protein phosphatase PP2A and was previously implicated in maintaining sister chromatid cohesion in response to spindle defects (Minshull et al., 1996). This prompted us to test whether *CDC55* deletion could allow sister chromatid separation in Cla4t-overexpressing cells. Elutriated G1 cells of a 4X *GAL1-CLA4t cdc55Δ* strain carrying the tetO/tetR-GFP constructs for monitoring sister chromatid separation were released into the cell cycle in the presence of galactose. As shown in Fig. 5 A, deletion of *CDC55* partially rescued the cytokinetic defects caused by high Cla4t levels, indicated by re-accumulation of a small fraction of cells with 1C DNA contents at the end of the first cell cycle. Most cells, however, displayed abnormal bud necks characteristic of 4X *GAL1-CLA4t* cells. In spite of that, they underwent efficient sister chromatid separation

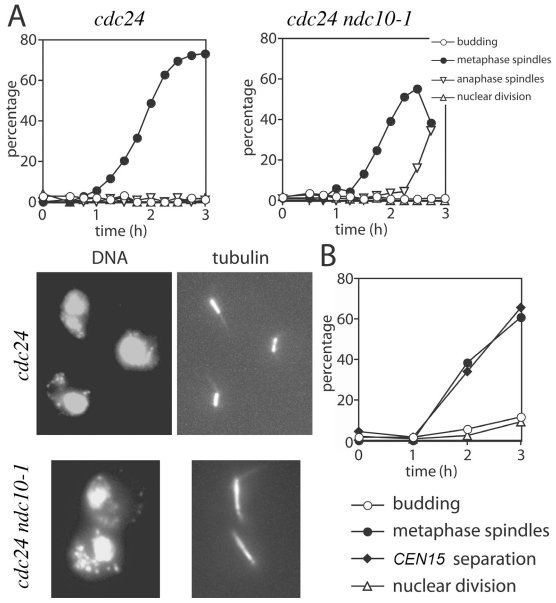

Figure 4. **Spindle dynamics is not affected by the morphogenesis checkpoint.** (A) *cdc24* (ySP305) and *cdc24 ndc10-1* (ySP6207) cell cultures were arrested in G1 by α-factor and released at 37°C (time 0). Cells were analyzed at the indicated times for DNA contents (not depicted), budding, spindle formation/elongation, and nuclear division (graphs). Micrographs represent cells at 150 min after release. (B) Wild-type cells with tagged *CEN15* (ySP1717) were arrested in G1 by α-factor and released in the presence of Lat-B (time 0). Cells were analyzed at 1-h intervals for DNA contents (not depicted), *CEN15* separation, tubulin immunostaining, and nuclear division.

and nuclear division (Fig. 5, B and C), suggesting that Cdc55 prevents anaphase onset when p21-activated kinases are inactive.

Nuclear division could also be induced in 4X *GAL1-CLA4t* cells by expressing a mutant form of the Pph21 catalytic subunit (Pph21-L369Δ; Fig. S2, available at http://www.jcb.org/cgi/content/full/jcb.200609088/DC1) that was shown to preferentially fail to interact with Cdc55 (Jiang, 2006). Therefore, chromatid cohesion upon morphogenesis checkpoint activation requires the protein phosphatase PP2A bound to Cdc55.

The catalytic and structural PP2A subunits can form mutually exclusive complexes with either one of the regulatory subunits Cdc55 and Rts1 (Evans and Hemmings, 2000). $PP2A^{Rts1}$ and its human counterpart have recently been shown to prevent precocious dissociation of centromeres both in mitosis and in meiosis I (Kitajima et al., 2006; Riedel et al., 2006). In an experiment similar to the one described for *cdc55Δ*, we found that pericentromeric sequences could not separate in the majority of 4X *GAL1-CLA4t rts1Δ* cells (Fig. 5 B). When pericentromeric regions did split (∼25% of the cells), GFP dots were always found very close to each other (Fig. 5 C) and nuclear division was negligible (Fig. 5 B), suggesting that $PP2A^{Rts1}$ plays a minor role, compared with $PP2A^{Cdc55}$, in controlling chromatid cohesion under these circumstances.

Because Cdc55 and Rts1 compete for binding to the other PP2A subunits, sister chromatid separation in the absence of Cdc55 could be ascribed to increased levels of the $PP2A^{Rts1}$ complex. To investigate this possibility, we asked whether 4X *GAL1-CLA4t* cells lacking both Cdc55 and Rts1 could undergo anaphase. Elutriated G1 cells of the 4X *GAL1-CLA4t cdc55Δ rts1Δ* strain released in the presence of galactose progressed into the cell cycle very slowly, as a result of budding and replication defects (Fig. 5, A and B). In spite of that, those that could finish chromosome replication underwent efficient dissociation of sister chromatids and nuclear division (Fig. 5 B), suggesting that anaphase onset in 4X *GAL1-CLA4t* cells lacking Cdc55 is not due to increased levels of $PP2A^{Rts1}$ activity.

We then asked whether $PP2A^{Cdc55}$ also controls sister chromatid cohesion in other conditions that activate the morphogenesis checkpoint. Wild-type and *cdc55Δ* cells were arrested in G1 by α-factor and then released in the presence of Lat-A. In these conditions, neither wild-type nor *cdc55Δ* cells budded throughout the course of the experiment (Fig. 5 D).

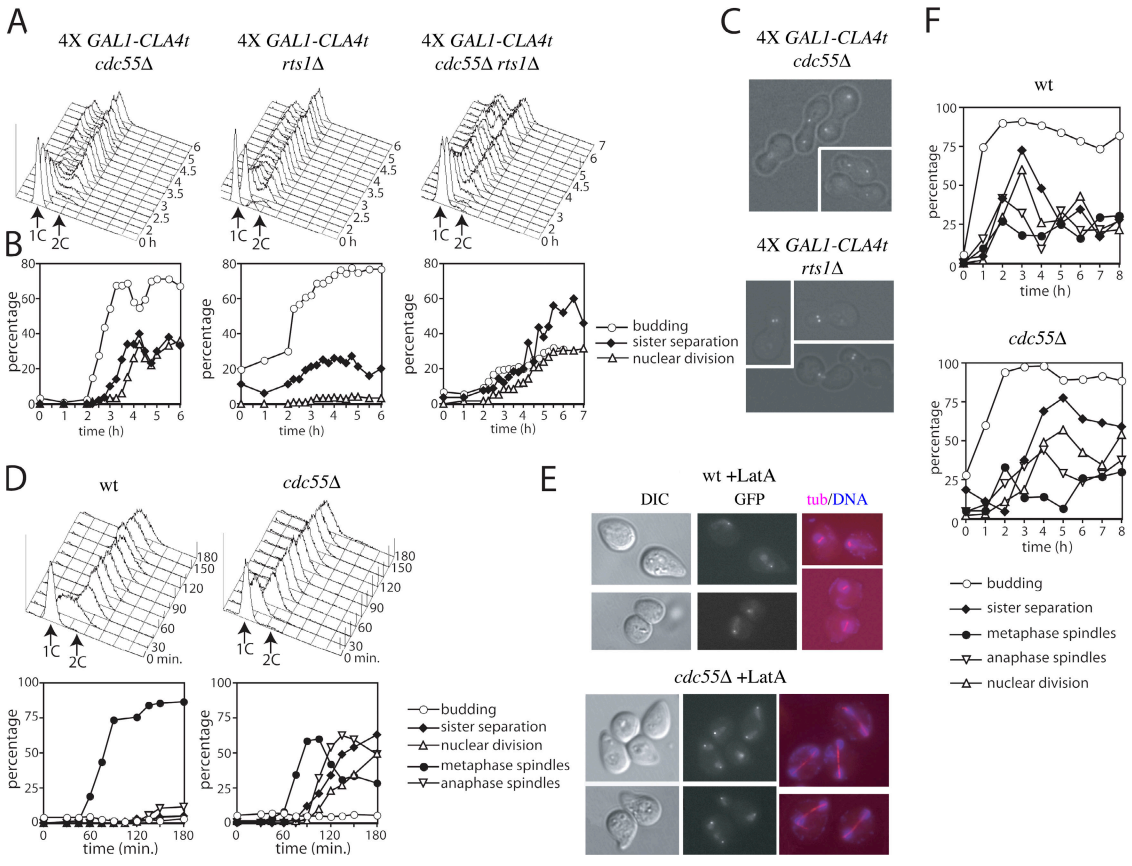

Figure 5. **$PP2A^{Cdc55}$ prevents sister chromatid separation upon activation of the morphogenesis checkpoint.** (A–C) Strains with the indicated genotypes (ySP5115, ySP5112, and ySP5165) were grown at 30°C in YEPR. Elutriated G1 cells were released in YEPRG at 25°C at time 0. Cell samples were analyzed at the indicated times for DNA contents (A), budding, sister chromatid separation, and nuclear division (B). (C) Micrographs represent sister chromatid separation at 285 min (4X *GAL1-CLA4t cdc55Δ*) and 300 min (4X *GAL1-CLA4t rts1Δ*). (D) Wild-type (wt; ySP3575) and *cdc55Δ* (ySP5068) cells were grown in YEPD at 30°C, arrested in G1 with α-factor, and released in the presence of Lat-A (time 0). Cells were analyzed at the indicated times for DNA contents (histograms), budding, sister separation, nuclear division, and spindle formation/elongation (graphs). (E) Micrographs represent pericentromeric chromosomal sequences (GFP) and merged pictures of tubulin and DNA staining (tub/DNA) at 150 min. DIC, differential interference contrast. (F) Wild-type (ySP3575) and *cdc55Δ* (ySP5068) cells were arrested in G1 by α-factor and released at 16°C. Cells were analyzed at the indicated times for budding, sister chromatid separation, spindle formation/elongation, and nuclear division.

As expected, wild-type cells accumulated with 2C DNA contents, unsevered sister chromatids, undivided nuclei, and short metaphase spindles (Fig. 5, D and E). Strikingly, sister chromatids separated efficiently in *cdc55Δ* cells under the same conditions, thus allowing spindles to elongate and nuclei to divide (Fig. 5, D and E). Finally, because *CDC55* deletion causes by itself morphogenetic defects and Swe1 stabilization at low temperatures (Healy et al., 1991; Yang et al., 2000), we asked whether the *cdc55Δ* mutant could separate sister chromatids at 16°C. At this temperature, *cdc55Δ* cells showed prominent morphogenetic defects (not depicted), but nevertheless could split chromatids and divide nuclei, albeit with a delay compared with wild-type cells (Fig. 5 F).

To directly compare the effects of cohesin inactivation and lack of $PP2A^{Cdc55}$ on sister chromatid separation of cells with morphogenetic defects, we used the temperature-sensitive *scc1-73* allele, which inactivates Mcd1 and advances sister chromatid separation relative to wild type at the restrictive temperature (Michaelis et al., 1997). G1-arrested *cdc24* cells either lacking *CDC55* or carrying the *scc1-73* allele were released at 37°C. *cdc24 scc1-73* cells could efficiently separate chromosome V arm sequences, although with a delay compared with *scc1-73* cells, but did not elongate spindles or divide nuclei (Fig. 6 A). In contrast, *cdc24 cdc55Δ* cells underwent complete chromosome segregation under the same conditions (Fig. 6 A). Accordingly, the distance between separating chromatids at 150 min after release was significantly higher in *cdc24 cdc55Δ* cells than in *cdc24 scc1-73* cells (Fig. 6 B). Therefore, some residual chromatid cohesion likely persists even when cohesin is inactivated and $PP2A^{Cdc55}$ plays a crucial role in controlling sister chromatid separation when the morphogenesis checkpoint is activated.

Mcd1 cleavage does not occur in *cdc55Δ* cells undergoing anaphase under morphogenesis checkpoint activation

Although ectopic cohesin cleavage did not allow nuclear division during morphogenesis checkpoint activation, *CDC55* deletion might still allow anaphase onset in these conditions through cohesin cleavage. To test this possibility, *cdc24*, *cdc24 swe1Δ*, and *cdc24 cdc55Δ* cells were arrested in G1 by α-factor and then released at 37°C, followed by analysis of cell cycle parameters (Fig. 7, A and B) and Mcd1 cleavage by separase (Fig. 7 C). As expected, *cdc24* cells arrested with 2C DNA contents, unseparated sister chromatids, and metaphase spindles, whereas most *cdc24 swe1Δ* cells underwent anaphase and spindle elongation and eventually exited mitosis and rereplicated their chromosomes, accumulating DNA contents higher than 2C (Fig. 7, A and B), suggesting that lack of Swe1 overrides cells' ability to sense morphogenetic defects. Interestingly, *cdc24 cdc55Δ* cells could also undergo anaphase in the same conditions, albeit with a delay compared with *cdc24 swe1Δ* cells, but remained mostly arrested with 2C DNA contents. The Mcd1 cleavage product, which was readily apparent in *cdc24 swe1Δ* cells and preceded sister chromatid separation, was mostly negligible in *cdc24 cdc55Δ* cells (Fig. 7 C). Nevertheless, chromatin staining of Mcd1 after chromosome spreading revealed that cohesin remained bound to chromatin in wild-type cells (not depicted) but had dissociated from the chromosomes in nuclei of *cdc55Δ* cells that underwent anaphase (Fig. 7 D). Thus, sister chromatid separation and Mcd1 dissociation from chromosomes in *cdc55Δ* cells under morphogenesis checkpoint activation do not seem to correlate with separase-dependent cleavage of cohesin. Accordingly, the Mcd1 cleavage product was not detectable in 4X *GAL1-CLA4t cdc55Δ* cells undergoing anaphase in the presence of galactose, similar to 4X *GAL1-CLA4t* cells (Fig. S3, available at http://www.jcb.org/cgi/content/full/jcb.200609088/DC1), and Mcd1 disappeared from the nuclei of 4X *GAL1-CLA4t cdc55Δ* cells in anaphase (Fig. S3 E). Mcd1 displacement from chromatin did not correlate with increased Mcd1 phosphorylation, which could instead be detected as electrophoretic mobility shift in nocodazole-arrested cells (Fig. S3 D). It is interesting to note that *SWE1* deletion in Cla4t-overexpressing cells caused rapid Pds1 and Clb2 proteolysis, as well as appearance of the Mcd1 cleavage product, whereas Pds1 and Clb2 remained mostly stable upon deletion of *CDC55* (unpublished data).

Unlike in *cdc55Δ* cells under morphogenesis checkpoint activation, sister chromatid separation in nocodazole-treated *cdc55Δ* cells was accompanied by Pds1 degradation Mcd1 cleavage, although with a delay compared with the spindle checkpoint–defective *mad2Δ* cells (Fig. S4, available at http://www.jcb.org/cgi/content/full/jcb.200609088/DC1).

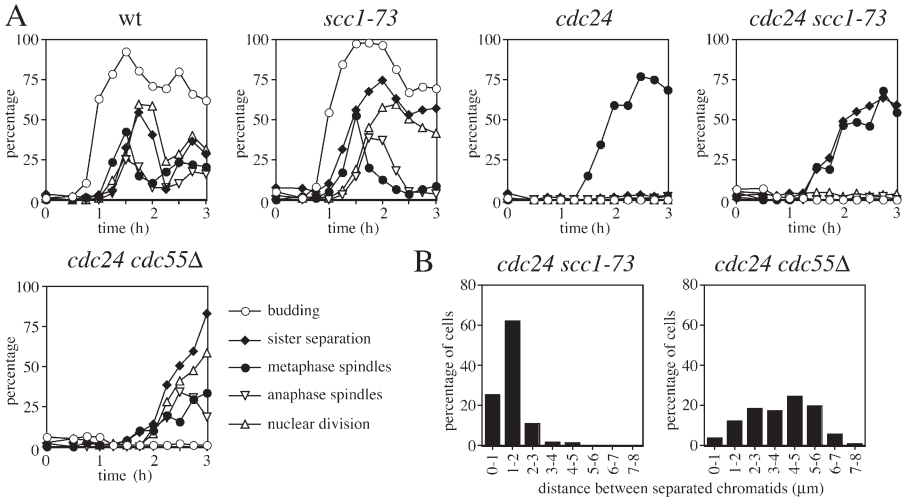

Figure 6. **Inactivation of $PP2A^{Cdc55}$, but not of cohesin, allows nuclear division in the presence of morphogenetic defects.** Strains with the indicated genotypes (ySP601, ySP818, ySP6236, ySP6241, and ySP6214) were arrested in G1 by α-factor at 25°C and released at 37°C (time 0). Cells were analyzed at the indicated times for DNA contents (not depicted), budding, sister chromatid separation, spindle formation/elongation, and nuclear division (A). Distances between separated chromatids were measured at time point 150 min (n = 180) with MetaMorph software.

Therefore, PP2A^{Cdc55} contributes to maintaining sister chromatid cohesion in nocodazole by impinging on the same targets of the spindle assembly checkpoint, as recently suggested by others (Yellman and Burke, 2006). In contrast, PP2A^{Cdc55} likely prevents sister chromatid separation in G2 through a different mechanism.

Sister chromatid dissociation induced by lack of PP2A^{Cdc55} does not require the Cdc14 phosphatase, Polo kinase, and condensin but can be reversed by topoisomerase II inhibition

PP2A^{Cdc55} has recently been shown to prevent Cdc14 early anaphase release from the nucleolus through Net1 dephosphorylation (Queralt et al., 2006). Cdc14 can in turn trigger Pds1 proteolysis in nocodazole-arrested cells (Visintin et al., 1998), and this mechanism has been proposed to be responsible for the precocious dissociation of sister chromatids in nocodazole-treated *cdc55Δ* cells (Yellman and Burke, 2006). We therefore asked whether Cdc14 was released from the nucleolus in *cdc55Δ* cells with morphogenetic defects and necessary for their onset of anaphase. Wild-type and *cdc55Δ* cells were arrested in G1 by α-factor and released in the presence of Lat-B. In situ immunostaining of Cdc14 showed that anaphase took place in *cdc55Δ* cells before Cdc14 release from the nucleolus (Fig. 8 A). In addition, analysis of *cdc55Δ* anaphase cells 150 min after release revealed that a high fraction of them (68.3%; $n = 120$) had undergone anaphase with Cdc14 in the nucleolus (Fig. 8 B), suggesting that premature Cdc14 release is not responsible for sister chromatid separation in these cells.

To test whether Cdc14 was required for the onset of anaphase in *cdc55Δ* mutants with morphogenetic defects, we inactivated Cdc14 in *cdc24 cdc55Δ* cells with the temperature-sensitive

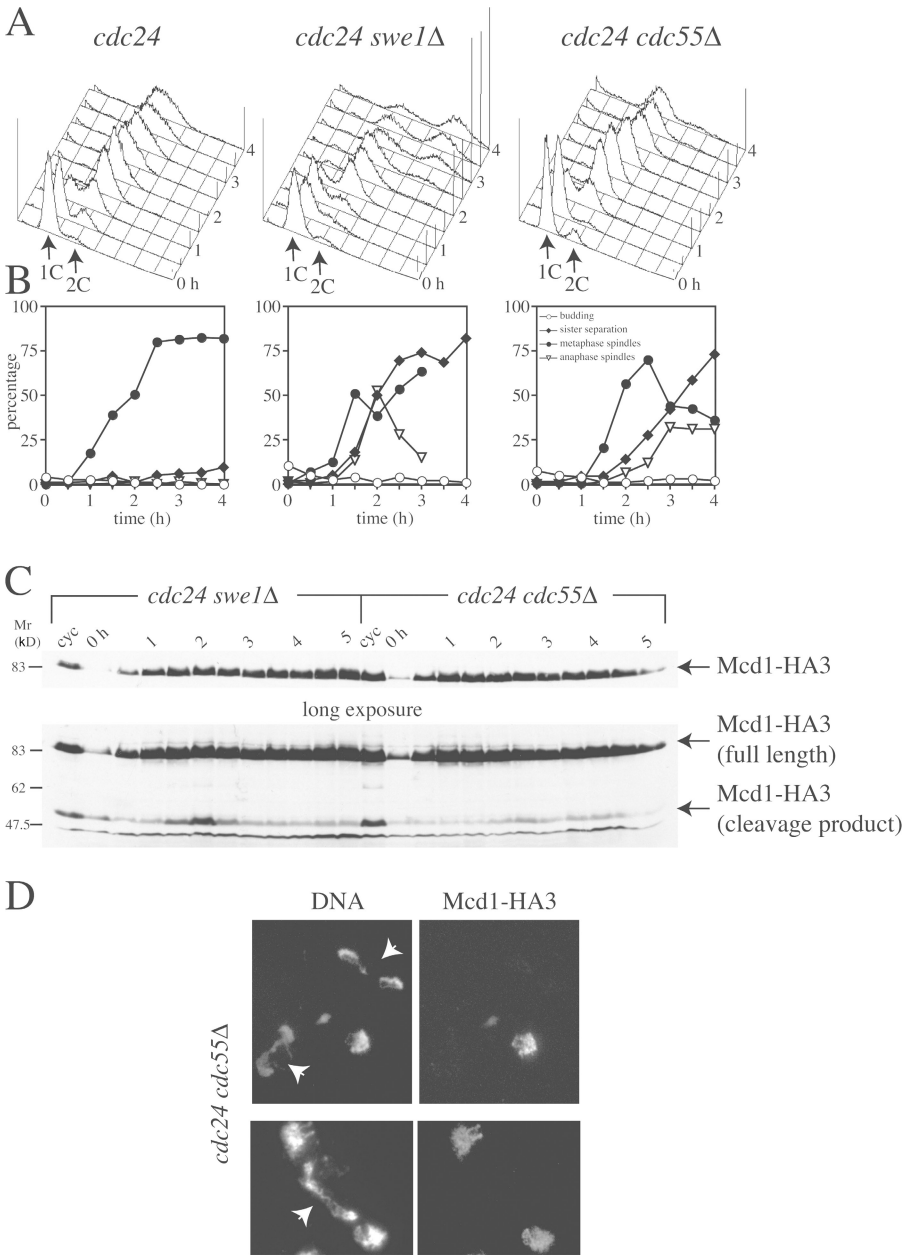

Figure 7. **Lack of Cdc55 upon Cla4t overproduction allows anaphase in the absence of Mcd1 cleavage.** Strains with the indicated genotypes (ySP6249, ySP6250, and ySP6463) growing at 25°C were arrested in G1 by α-factor and released at 37°C (time 0). At the indicated times, cell samples were collected for FACS analysis (A); kinetics of budding, sister chromatid separation, and spindle formation/elongation (B); Western blot analysis of TCA protein extracts (C); and chromosome spreads (D). Total extracts were immunoblotted with anti-HA antibodies to detect Mcd1-HA3 (C). Chromosome spreads (D) were immunostained with anti-HA antibodies (Mcd1-HA3) and mounted with DAPI to stain DNA. Arrows indicate anaphase nuclei.

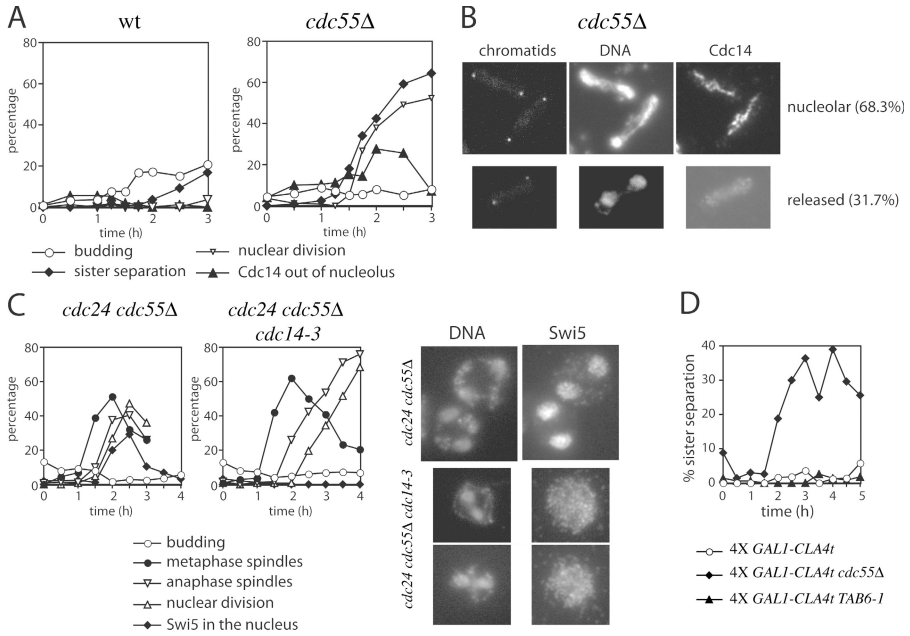

Figure 8. **Cdc14 is not required for nuclear division of *cdc55Δ* cells with morphogenetic defects.** (A) Wild-type (wt; ySP3575) and *cdc55Δ* (ySP5068) cells were arrested in G1 by α-factor at 30°C and released at 25°C in the presence of Lat-B (time 0). At the indicated times, cells were collected to determine the kinetics of budding, sister chromatid separation, nuclear division, and Cdc14 nucleolar export after in situ immunofluorescence. Micrographs (B) show examples of *cdc55Δ* cells at 150 min after release. (C) *cdc24 cdc55Δ* (ySP6503) and *cdc24 cdc55Δ cdc14-3* (ySP6499) cells expressing myc-tagged Swi5 were arrested in G1 by α-factor at 25°C and released at 37°C (time 0). Cells were analyzed at the indicated times for budding, spindle formation/elongation, nuclear division, and Swi5 nuclear import. Pictures were taken at 150 min after release. (D) Strains with the indicated genotypes (ySP5704, ySP5710, and ySP5893) were grown in YEPR at 30°C, arrested in G1 by α-factor, and released in YEPRG at 25°C (time 0), followed by scoring GFP dots to determine the kinetics of sister chromatid separation.

cdc14-3 allele. As a control for Cdc14 inactivation, we analyzed the subcellular localization of the Swi5 transcription factor, whose nuclear import in telophase is strictly dependent on its dephosphorylation by Cdc14 (Visintin et al., 1998). Cell cultures of *cdc24 cdc55Δ* and *cdc24 cdc55Δ cdc14-3* strains expressing a myc-tagged Swi5 protein were synchronized in G1 by α-factor and released at 37°C to analyze, over time, budding kinetics, Swi5 localization, and nuclear division. Swi5 was cytoplasmic in both strains throughout most of the cell cycle. However, although it was imported into the nucleus of *cdc24 cdc55Δ* telophase cells, it always remained in the cytoplasm of *cdc24 cdc55Δ cdc14-3* cells, indicating that Cdc14 had been inactivated (Fig. 8 C). Lack of Cdc55 allowed a fraction of *cdc24* cells to divide nuclei irrespective of Cdc14 function (Fig. 8 C), indicating that Cdc14 is dispensable for the onset of anaphase in these conditions. Accordingly, Cdc14 was also insufficient to promote sister chromatid separation in *CLA4t*-overexpressing cells carrying the dominant *TAB6-1* allele, which encodes a hyperactive Cdc14 variant with reduced affinity to its inhibitor Net1 (Shou et al., 2001; Fig. 8 D).

Although we did not detect any increase in Mcd1 phosphorylation in *cdc55Δ* versus wild-type cells overproducing Cla4t (Fig. S3), it was still possible that $PP2A^{Cdc55}$ could prevent sister chromatid separation by counteracting the Cdc5-mediated phosphorylation of a small fraction of Mcd1 or other cohesin subunits. However, inactivation of Cdc5 with the *cdc5-2* temperature-sensitive allele did not prevent anaphase in *cdc24 cdc55Δ* cells (Fig. 9 A), suggesting that Cdc5 is not required for this process.

Timely sister chromatid segregation, especially of ribosomal DNA and chromosome sequences far from centromeres, depends on condensin and DNA topoisomerase II (DiNardo et al., 1984; Holm et al., 1985; Bhalla et al., 2002; D'Amours et al., 2004; Sullivan et al., 2004). We therefore tested the effects of the temperature-sensitive *ycg1-10* and *top2-4* mutations, affecting condensin and DNA topoisomerase II, respectively, on the unscheduled anaphase of *cdc24 cdc55Δ* cells. Although inactivation of Ycg1 had no significant effect, inactivation of topoisomerase II in *cdc24 cdc55Δ top2-4* cells mostly prevented anaphase (Fig. 9 A), suggesting that the presence of topological linkages prevents sister chromatid separation under these conditions. Consistently, the presence of the *top 2-4* allele could partially rescue the cold sensitivity of *cdc55Δ* cells (Fig. 9 B), which is presumably due to unscheduled sister chromatid separation in the presence of morphogenetic defects.

We then asked whether morphogenetic defects could arrest the cell cycle in a stage where topological linkages are not resolved, using an assay that allows detection of accumulation of catenated forms of a circular minichromosome (Koshland and Hartwell, 1987). Unlike *top2-4* mutants, however, neither *cdc24* (Fig. 9 C) nor *GAL1-CLA4t* cells (not depicted) accumulated minichromosome topoisomers. Although we cannot exclude the possibility that the behavior of natural chromosomes is different from that of minichromosomes, the delay of nuclear division caused by the morphogenetic checkpoint does not seem to be accompanied by lack of decatenation.

CDC55 overexpression delays sister chromatid separation independently of Pds1

If $PP2A^{Cdc55}$ acts as an inhibitor of sister chromatid separation, increasing its dosage might delay the onset of anaphase. We therefore introduced into the genome of otherwise wild-type cells multiple copies of a galactose-inducible *GAL1-CDC55* construct. Parental and transformed strains growing in raffinose were arrested in G1 with α-factor and released in the presence of galactose. We then monitored separation of the tetO array located 13 kb away from *CEN5*, as well as spindle formation and elongation (Fig. 10). *CDC55* overexpression did not affect bipolar spindle formation but delayed sister chromatid separation, nuclear division, and spindle elongation,

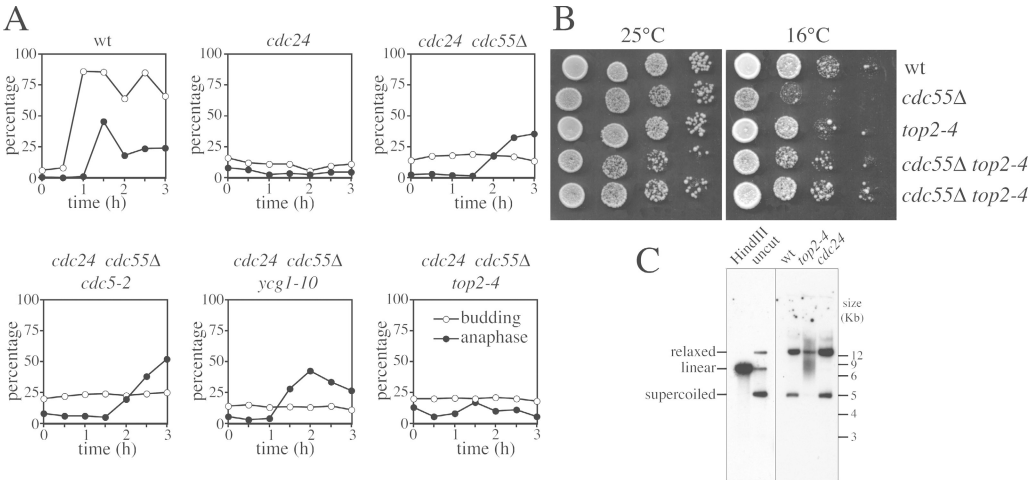

Figure 9. **Effects of Cdc5, condensin, and topoisomerase II inactivation on anaphase onset in the absence of Cdc55.** (A) Strains with the indicated genotypes (W303, ySP305, ySP6130, ySP6146, ySP6121, and ySP6105) were grown in YEPD at 26°C, arrested in G1 with α-factor, and released at 37°C (time 0). At different times, cells were collected to determine the kinetics of budding and anaphase after nuclear staining with propidium iodide. (B) Serial dilutions of strains with the indicated genotypes (W303, ySP5737, ySP5929, and ySP6066) were spotted on YEPD plates and incubated at 25°C for 2 d and at 16°C for 6 d. (C) Wild-type (wt), *top2-4*, and *cdc24* cells carrying a minichromosome (YCp50) were arrested in G1 with α-factor and released for 3 h at 37°C in the presence of nocodazole. Topological forms of plasmids isolated from these cells were examined by Southern blot after electrophoresis of total DNA in the presence of ethidium bromide using the 1.7-kb Amp^R PvuI–BglII fragment of YCp50 as a probe. Purified YCp50 DNA linearized with HindIII and uncut were run in parallel to define the mobility of the various conformational forms.

causing cells to accumulate in G2. This delay did not depend on functional securin, as *PDS1* deletion did not accelerate the onset of anaphase in *CDC55*-overexpressing cells. High levels of Cdc55 delayed sister chromatid separation at both pericentromeric and telomeric regions (unpublished data), suggesting that $PP2A^{Cdc55}$ prevents dissociation of sister chromatids along their length.

If $PP2A^{Cdc55}$ acted as anaphase inhibitor independently of securin, we could expect that simultaneous loss of Pds1 and Cdc55 might have additive effects, allowing precocious separation of sister chromatids during the unperturbed cell cycle. Indeed, concomitant deletion of *CDC55* and *PDS1* turned out to be lethal (unpublished data).

Discussion

The control of sister chromatid separation by the morphogenesis checkpoint

It has been well established that morphogenetic defects, such as lack of actin polarization or budding, cause a G2 arrest in budding yeast because of the inhibitory phosphorylation of Cdk1 on tyrosine 19 by the Swe1 kinase (Lew, 2003). This inhibitory phosphorylation likely involves only a small pool of mitotic Cdks. In fact, the morphogenesis checkpoint arrests the cell cycle after spindle formation, whereas complete inactivation of all mitotic Cdks by mutations or *SWE1* overexpression prevents spindle pole body separation and bipolar spindle assembly (Crasta and Surana, 2006). We show here that the morphogenesis checkpoint prevents sister chromatid separation independently of securin because *pds1Δ* cells treated with Lat-A or overexpressing the dominant-negative *CLA4t* allele do not attempt anaphase. Our data also indicate that morphogenesis checkpoint activation does not delay separase association to securin and its nuclear import, which depends on Pds1 phosphorylation by Cdks (Agarwal and Cohen-Fix, 2002; Uhlmann, 2003), consistent with only a minor pool of mitotic Cdks being inactivated under these conditions.

Inactivation of Mcd1 through the temperature-sensitive *scc1-73* allele or its ectopic cleavage also turned out to be insufficient for anaphase and chromosome segregation under morphogenesis checkpoint activation, raising the possibility that either spindle function is compromised or residual cohesion persists on chromosomes after Mcd1 inactivation. Because in our assays spindle forces seem normal, we favor the second interpretation. Whether residual cohesion depends on other cohesin subunits or on other proteins remains to be established. Cohesin-independent chromatid linkages have been reported for repetitive sequences (D'Amours et al., 2004; Dynek and Smith, 2004; Sullivan et al., 2004), and a role for condensin in chromatid cohesion has been recently described (Lam et al., 2006). Swe1-mediated phosphorylation of mitotic Cdks could prevent the release of these linkages in addition to inhibiting securin degradation. Although a direct role for mitotic Cdks in dismantling sister chromatid cohesion has not been reported so far, Cdks are required at different levels for Polo kinase activation, which in turn contributes to dissociation of sister chromatids by phosphorylating the cohesin Mcd1 and enhancing its susceptibility to cleavage by separase (Alexandru et al., 2001). In addition, in higher eukaryotic cells Polo and Aurora B kinases promote the prophase pathway of cohesin dissociation from chromosome arms that is independent of securin degradation and relies on phosphorylation of the SA2 cohesin subunit (Sumara et al., 2002; Hauf et al., 2005). In budding yeast, mitotic Cdks activate the Polo kinase through several mechanisms, including transcription (Spellman et al., 1998), phosphorylation (Mortensen et al., 2005), and inhibition of proteolysis (Zachariae et al., 1998). It is therefore not surprising that Cdc5 activation is dramatically delayed in response to the morphogenesis checkpoint.

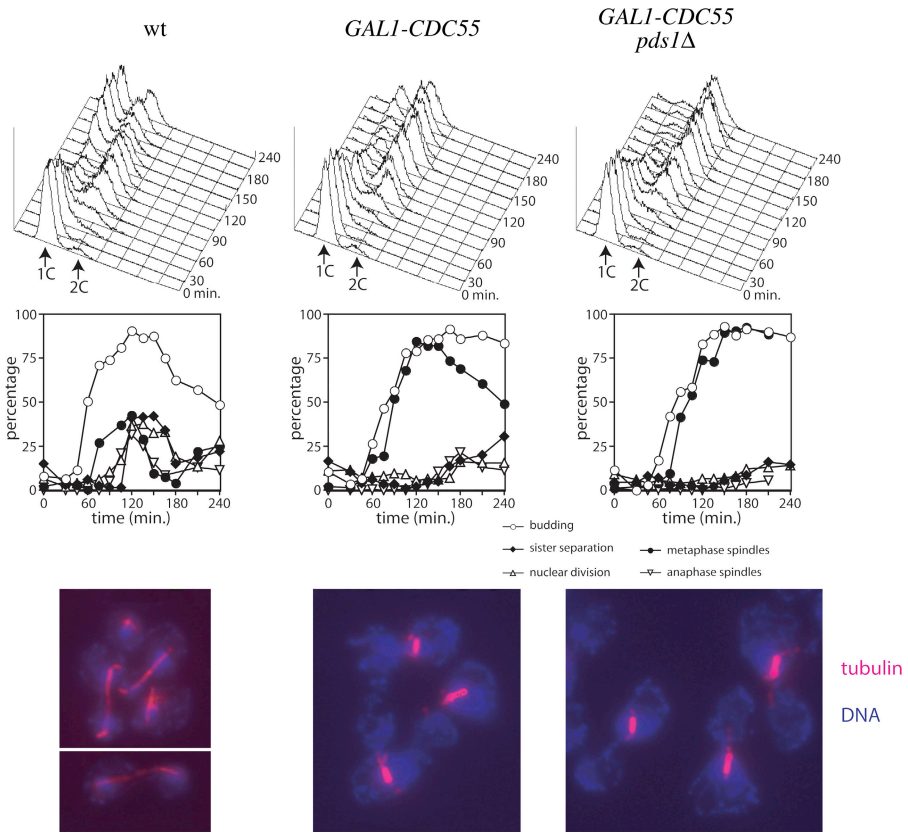

Figure 10. ***CDC55* overexpression delays anaphase independently of securin.** Wild-type (wt; ySP3575), *GAL1-CDC55* (ySP5690), and *GAL1-CDC55 pds1Δ* (ySP5752) strains carrying the tetO/tetR-GFP construct to detect pericentromeric sequences at chromosome V were grown in YEPR at 25°C, arrested in G1 by α-factor, and released in YEPRG at 25°C (time 0). Cells were analyzed at different times for DNA contents (histograms), budding, sister chromatid separation, spindle formation/elongation, and nuclear division (graphs). Pictures show merged micrographs of tubulin (red) and DNA (blue) at 150 min.

The failure to timely activate Cdc5 could contribute to the lack of sister separation in these conditions but cannot be the only culprit. In fact, Cdc5 inactivation leads to inefficient separation of telomeric regions but has no or little effect on that of centromeric and arm sequences (Alexandru et al., 2001). In addition, Cdc5 is not required for the onset of anaphase of *cdc24 cdc55Δ* cells. If the failure to separate sister chromatids when the morphogenetic checkpoint is active were merely due to delayed Cdc5 activation, anaphase should be resumed by ectopic Mcd1 cleavage, which we show not to be the case. Therefore, sister chromatid cohesion seems to be maintained by the morphogenesis checkpoint through a previously unanticipated mechanism that does not depend only on securin stabilization and Polo kinase inactivation.

PP2A^{Cdc55} and the control of sister chromatid separation

We find that inactivation of the protein phosphatase PP2A^{Cdc55} is sufficient to allow sister chromatid separation when the morphogenesis checkpoint is activated. Unlike upon deletion of *SWE1*, which completely abolishes the cell's ability to respond to morphogenetic defects, this is not achieved through switch off of checkpoint signaling, because lack of PP2A^{Cdc55} activates by itself the checkpoint and induces Swe1 stabilization by causing morphogenetic defects (Jiang, 2006). In agreement with a critical function for PP2A^{Cdc55} in controlling sister separation when the morphogenesis checkpoint is active, deletion of *CDC55* turned out to be lethal for *cla4* and *cdc12* mutants (unpublished data), whose morphogenesis defects are known to activate the checkpoint (Lew, 2003). Recently, PP2A bound to Rts1/B56, the other regulatory subunit, has been found to protect centromeric cohesion during mitosis and meiosis I, in both yeast and human cells (Kitajima et al., 2006; Riedel et al., 2006). In our experimental conditions, PP2A^{Rts1} seems to have only a minor role, perhaps restricted to centromeric regions, in preventing chromatid dissociation.

Cdc55 was previously implicated in maintaining sister chromatid cohesion in response to activation of the spindle assembly checkpoint (Minshull et al., 1996), suggesting that PP2A^{Cdc55} acts as anaphase inhibitor in several conditions. However, in nocodazole-treated *cdc55Δ* cells, sister chromatid separation is accompanied by Mcd1 proteolytic cleavage (Yellman and Burke, 2006; this study), whereas we could not find evidence for such event in *cdc55Δ* cells undergoing anaphase in the presence of morphogenetic defects. In agreement with our data, Cdc55 has recently been shown to prevent chromatid separation independently of securin degradation and Mcd1 cleavage in cells with telomeric DNA lesions (Tang and Wang, 2006).

How could PP2A^{Cdc55} prevent sister chromatid separation in G2? For instance, it could regulate a pathway of cohesin removal similar to the prophase pathway of higher eukaryotic cells, although so far Mcd1 cleavage by separase seems to be the only necessary and sufficient event for cohesin removal from yeast chromosomes (Uhlmann, 2003). If PP2A^{Cdc55} were to inhibit cohesin dissociation independently of Mcd1 cleavage, its inactivation could allow anaphase in the absence of separase. In contrast to recently published data (Tang and Wang, 2006), we find that both the *esp1-1* mutation (Ciosk et al., 1998) and

overexpression of nondegradable Pds1 (Cohen-Fix et al., 1996) prevent *cdc55Δ* cells from undergoing anaphase (unpublished data), suggesting that separase is still required for sister chromatid separation in the absence of $PP2A^{Cdc55}$. It should be noted, however, that separase has additional functions that are unrelated to its role in Mcd1 cleavage (Sullivan et al., 2001; Stegmeier et al., 2002; Sullivan and Uhlmann, 2003; Papi et al., 2005). Interestingly, Cdc55 has recently been shown to interact physically with Esp1 and to prevent the early anaphase release of Cdc14 by causing dephosphorylation of its inhibitor Net1 (Queralt et al., 2006). This raises the possibility that lack of $PP2A^{Cdc55}$ causes the unscheduled activation of Cdh1/APC, and thereby Pds1 degradation, by promoting Cdc14 release. Although this could partly explain the separation of sister chromatids in nocodazole-treated *cdc55Δ* cells, we show here that nuclear division of *cdc55Δ* cells when the morphogenesis checkpoint is active is independent of Cdc14 function, suggesting that $PP2A^{Cdc55}$ must have other roles, besides inhibiting Cdc14 dissociation from Net1, before the onset of anaphase. Therefore, a more direct role of $PP2A^{Cdc55}$ in controlling sister chromatid separation in G2 must be invoked.

Putative targets of $PP2A^{Cdc55}$ in the control of anaphase

The importance of $PP2A^{Cdc55}$ as anaphase inhibitor is underscored by the synthetic lethality of *pds1Δ cdc55Δ* double mutants, where sister chromatid separation could be so premature as to cause lethal chromosome missegregation. In addition, *CDC55* overexpression delays chromatid dissociation independently of securin. In agreement with a crucial function as anaphase inhibitor, $PP2A^{Cdc55}$ phosphatase activity decreases at the onset of anaphase (Queralt et al., 2006).

An obvious candidate for being dephosphorylated by $PP2A^{Cdc55}$ to prevent sister chromatid dissociation was Mcd1, especially in light of recent data indicating that the other PP2A complex, $PP2A^{Rts1/B56}$, prevents precocious loss of centromeric cohesion by counteracting Mcd1 phosphorylation by Polo kinase (Kitajima et al., 2006; Riedel et al., 2006). However, as discussed above, $PP2A^{Cdc55}$ might target other proteins beside Mcd1. For instance, it could dephosphorylate other cohesin subunits and prevent cohesin unloading through a pathway analogous to the vertebrate prophase pathway. Despite the efforts, we could not detect any difference in the electrophoretic mobility of other cohesin subunits, such as Scc3 and Pds5, in *cdc55Δ* versus wild-type cells (unpublished data). Alternatively, $PP2A^{Cdc55}$ could regulate other chromatin-bound proteins, such as the condensin complex. It is worth mentioning that the human condensin HCP-6 interacts with and is dephosphorylated by PP2A bound to the B subunit (Yeong et al., 2003). Finally, another putative target of $PP2A^{Cdc55}$ might be Esp1, which interacts physically with Cdc55 (Queralt et al., 2006). Although separase has been proposed to down-regulate $PP2A^{Cdc55}$ activity, separase regulation of by $PP2A^{Cdc55}$ can also be envisaged.

In summary, a crucial role for $PP2A^{Cdc55}$ in maintaining sister chromatid cohesion in response to several stress conditions is emerging, making it a key factor for preserving genome stability. Mutations in *Drosophila melanogaster* PP2A B subunit, the Cdc55 counterpart, cause chromosome segregation defects (Mayer-Jaekel et al., 1993), and mammalian PP2A is considered to be a principal guardian against malignant transformation (Janssens et al., 2005). Understanding the molecular mechanisms by which $PP2A^{Cdc55}$ controls the onset of anaphase under different conditions might shed light on processes that prevent chromosome missegregation, which is intimately linked to tumorigenesis.

Materials and methods

Strains, media, and reagents

All yeast strains (Table S1, available at http://www.jcb.org/cgi/content/full/jcb.200609088/DC1) were derivatives of or were backcrossed at least three times to W303 (*ade2-1, trp1-1, leu2-3,112, his3-11,15, ura3, ssd1*). Cells were grown in YEP medium (1% yeast extract, 2% bactopeptone, and 50 mg/l adenine) supplemented with 2% glucose (YEPD), 2% raffinose (YEPR), or 2% raffinose and 1% galactose (YEPRG). Unless otherwise stated, α-factor, nocodazole, Lat-A, and Lat-B were used at 3 μg/ml, 15 μg/ml, 0.1 mM, and 0.2 mM, respectively. For galactose induction of α-factor–synchronized cells, galactose was added half an hour before release. *MET3-CDC20* cells were grown in synthetic medium lacking methionine, whereas the *MET3* promoter was shut off by resuspending cells in YEPD medium supplemented with 2 mM methionine.

Plasmid constructions and genetic manipulations

To clone *CDC55* under the *GAL1-10* promoter (plasmid pSP376), a BglII–PstI PCR product containing the *CDC55* coding region and 140 bp of downstream sequence was cloned in the BamHI–PstI site of a *GAL1-10*–bearing YIplac211 vector. pSP376 integration was directed to the *URA3* locus by BglII digestion. Copy number of the integrated plasmid was verified by Southern analysis. *CDC55*, *SWE1*, *PPH21*, and *PPH22* chromosomal deletion were generated by one-step gene replacement (Wach et al., 1994).

Immunoprecipitations, kinase assays, and Western blot analysis

Immunoprecipitations were performed as described by Fraschini et al. (2001); lysis buffer was supplemented with 0.1% Triton X-100 (Fluka). Cdc5 kinase assays were performed according to Charles et al. (1998). For Western blot analysis, TCA protein extracts were prepared according to Fraschini et al. (1999). Nondenaturing protein extracts were prepared according to Chiroli et al. (2003). Proteins transferred to Protran membranes (Schleicher and Schuell) were probed with 9E10 mAb for myc-tagged proteins, with 12CA5 or 16B12 mAb (Babco) for HA-tagged proteins, and with polyclonal antibodies against Clb2 and Swi6. Secondary antibodies were obtained from GE Healthcare, and proteins were detected by an enhanced chemiluminescence system according to the manufacturer.

Other techniques

Flow cytometric DNA quantitation, in situ immunofluorescence, and chromosome spreads were performed according to Fraschini et al. (1999). Nuclear division was scored with a fluorescent microscope on cells stained with propidium iodide. Visualization of Tet operators using GFP was performed as described in Michaelis et al. (1997). Catenation assays were performed according to Bachant et al. (2002). To detect spindle formation and elongation, α-tubulin immunostaining was performed with the YOL34 monoclonal antibody (Serotec) followed by indirect immunofluorescence using rhodamine-conjugated anti-rat antibody (1:100; Pierce Chemical Co.). Cdc14 immunostaining was performed with sc-12045 polyclonal antibodies (Santa Cruz Biotechnology, Inc.) followed by indirect immunofluorescence using CY3-conjugated anti-goat antibody (GE Healthcare). Immunostaining of myc- and HA-tagged proteins was done by incubation with the 9E10 mAb and 16B12 mAb (Babco), respectively, followed by indirect immunofluorescence using CY3-conjugated goat anti–mouse antibody (GE Healthcare). Digital images were acquired on a fluorescent microscope (Eclipse E600; Nikon) equipped with a charge-coupled device camera (DC350F; Leica) at 20°C with an oil 100× 1.3 NA Plan Fluor objective (Nikon), using FW4000 software (Leica).

Online supplemental material

Fig. S1 shows that Cdc5 protein levels and associated kinase are delayed in response to morphogenetic defects. Fig. S2 shows that mutations in the catalytic subunit of PP2A that impair its interaction with Cdc55 promote

nuclear division when the morphogenesis checkpoint is activated. Fig. S3 shows that Mcd1 falls off chromatin but its proteolytic cleavage is undetectable in *cdc55Δ* cells overexpressing Cla4t. Fig. S4 shows that sister separation in the absence of Cdc55 upon nocodazole treatment correlates with Mcd1 cleavage. Table S1 describes the genotypes of strains used in this work. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200609088/DC1.

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