Independent modulation of the kinase and polo-box activities of Cdc5 protein unravels unique roles in the maintenance of genome stability

Hery Ratsima, Anne-Marie Ladouceur, Mirela Pascariu, Véronique Sauvé, Zeina Salloum, Paul S. Maddox, and Damien D'Amours[1]

Institute for Research in Immunology and Cancer and Département de Pathologie et Biologie Cellulaire, Université de Montréal, Montréal, QC, Canada H3C 3J7

Edited* by Angelika Amon, Massachusetts Institute of Technology, Cambridge, MA, and approved September 14, 2011 (received for review April 25, 2011)

Polo-like kinases (PLKs) are evolutionarily conserved kinases essential for cell cycle regulation. These kinases are characterized by the presence of a C-terminal phosphopeptide-interaction domain, the polo-box domain (PBD). How the functional domains of PLKs work together to promote cell division is not understood. To address this, we performed a genetic screen to identify mutations that independently modulate the kinase and PBD activities of yeast PLK/Cdc5. This screen identified a mutagenic hotspot in the F-helix region of Cdc5 kinase domain that allows one to control kinase activity in vivo. These mutations can be systematically engineered into other major eukaryotic cell cycle kinases to similarly regulate their activity in live cells. Here, using this approach, we show that the kinase activity of Cdc5 can promote the execution of several stages of mitosis independently of PBD activity. In particular, we observe that the activation of Cdc14 and execution of mitotic exit are uniquely sensitive to the modulation of Cdc5 kinase activity. In contrast, PBD-defective mutants are capable of completing mitosis but are unable to maintain spindle pole body integrity. Consistent with this defect, PBD-deficient cells progressively double the size of their genome and ultimately lose genome integrity. Collectively, these results highlight the specific contributions of Cdc5 functional domains to cell division and reveal unexpected mechanisms controlling spindle pole body behavior and genome stability.

Reversible protein phosphorylation plays critical roles in the regulation of most physiological processes in eukaryotes (reviewed in 1). This modification is catalyzed by protein kinases, a large family of enzymes regulating nearly every aspect of cell biology. The main structural features of protein kinases are highly conserved among various members of this family of proteins, and yet individual kinases display very high specificity in their modes of activation and substrate selection in vivo (1). Precise spatiotemporal regulation of protein kinase activity has been achieved, at least in part, through association of kinase domains with ancillary targeting domains and/or regulatory subunits (2, 3). Understanding the molecular mechanisms governing enzymatic activation and substrate specificity in multidomain kinases depends largely on the ability to dissociate the activity of regulatory and catalytic domains in these enzymes.

Polo-like kinases (PLKs) are multidomain kinases playing essential roles in cell division, proliferation, and development (reviewed in 4). A defining feature of this family of kinases is the presence at the protein C terminus of a phosphopeptide interaction module, the polo-box domain (PBD). The PBD is believed to stimulate phosphorylation of PLK substrates by mediating phospho-dependent interactions between a PLK and its substrates (5). The PBD can also target PLKs to specific subcellular domains, such as centrosomes (6, 7), where the increased local concentration of the kinase promotes the phosphorylation of specific substrates. Interestingly, a growing number of studies have shown that the PBD is capable of mediating interactions with unphosphorylated proteins as well (5). One such interaction involves the kinase domain of PLKs and is believed to suppress the activity of the kinase until a substrate is bound to the PBD (8). This intramolecular interaction does not require residues important for phosphopeptide binding, thereby suggesting the existence of distinct binding modes for the association of phosphorylated and unmodified proteins with the PBD (9). The PBD thus appears to be a key interaction hub for the regulation of PLK localization, substrate specificity, and enzymatic activity.

How the kinase domain and the PBD of PLKs work together to promote cell cycle progression in living cells is unclear. In particular, it is unknown whether the PBD plays kinase-independent functions in vivo (4). Discovering the specific roles of PLK functional domains in cells is a formidable challenge because kinase inactivation leads to lethality, and thus prevents detailed in vivo analyses. Indeed, it has not been possible so far to isolate PLK mutants constitutively defective in kinase activity. Furthermore, existing conditional mutations have undesirable side effects, such as impaired proliferation or changes in protein levels (Fig. S1 *A* and *C*). To address this, we developed an innovative genetic approach to isolate separation-of-function mutants in the biochemical activities of budding yeast PLK/Cdc5. We show here that the functions of the kinase domain and PBD of Cdc5 can be dissociated genetically with strikingly different consequences for cells. In particular, we unveil previously undescribed functions for Cdc5 PBD in cell division and in the maintenance of genome stability.

Results

Regulation of Cdc5 Kinase Activity by F-Helix Mutations. We first performed a genetic screen to identify mutations affecting the F-helix of Cdc5 kinase domain. This helix plays an integrative role in eukaryotic protein kinases (EPKs) by acting as an anchor for distinct parts of the kinase domain (10, 11). Although this helix is conserved in all EPKs, we noticed that it is absent in atypical kinases (10) and reasoned that it may not be absolutely required for catalysis. In light of this, we hypothesized that mutations in the F-helix may affect kinase activity in a moderate or conditional manner. To test this, we generated both random and targeted mutations in Cdc5 kinase domain. From this pool, we identified two specific mutations, D263N and V269D, that gen-

Author contributions: H.R. and D.D. designed research; H.R., A.-M.L., M.P., V.S., and Z.S. performed research; H.R., A.-M.L., P.S.M., and D.D. analyzed data; and H.R. and D.D. wrote the paper.

Conflict of interest statement: The authors plan to file a patent on the discovery that mutations in the catalytic domain of protein kinases can regulate their enzymatic activity.

*This Direct Submission article had a prearranged editor.

[1]To whom correspondence should be addressed. E-mail: damien.damours@umontreal.ca.

See Author Summary on page 17585.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1106448108/-/DCSupplemental.

Downloaded at Palestinian Territory, occupied on December 31, 2021

www.manaraa.com

erated temperature-sensitive (ts) alleles when introduced in the F-helix of Cdc5 (Fig. 1 *A* and *B*). One of the mutations affects the F-helix aspartate conserved in all EPKs (D263) and prevents the formation of an important network of hydrogen bonds with residues in the catalytic loop (10) (H196, H202, R203; Fig. 1*C*). The other mutation affects valine 269, a residue involved in the formation of several conserved hydrophobic interactions important for kinase-substrate association (10). Importantly, the severity of the phenotype of the original mutants could be modulated and optimized by changing the nature of the amino acid residue at the specific position affected in the original mutants (*cdc5-77*: D263Q and *cdc5-88*: V269E; Fig. 1 *A*–*D*). Together, these results indicate that it is possible to engineer kinase-specific conditional mutations in Cdc5.

Our ability to create Cdc5 mutants with conditional kinase activity prompted us to ask whether other essential kinases might be engineered in a similar manner. Introducing mutations corresponding to *cdc5-77* and *cdc5-88* in essential cell cycle kinases resulted in the creation of at least one novel conditional allele for each of these kinases (Fig. 1*E*). For example, engineering both positions in Ipl1 kinase resulted in two ts alleles, including a previously undescribed high-sensitivity ts allele, *ipl1-85*. Interestingly, the previously published *ipl1-321* and *mps1-6* alleles carry mutations that affect the same kinase positions as those modified in *cdc5-77* and *cdc5-88*, respectively (12, 13). It is important to note that F-helix mutations may not necessarily affect all EPKs in a similar manner. Nevertheless, our results indicate that the F-helix region is a mutagenic hotspot that can be exploited to create useful conditional mutants in eukaryotic protein kinases.

We next sought to determine whether Cdc5 kinase mutations could modulate the enzymatic activity of the engineered proteins. To this end, we purified wild-type (wt) Cdc5 and Cdc5-77 mutant and performed kinase activity assays at various temperatures. As expected, the kinase activity of wt Cdc5 increased with temperature from 22 °C to 34 °C and remained high at 38 °C (Fig. 2*A*). In contrast, the kinase activity of Cdc5-77 started to diminish at temperatures above 30 °C and was significantly reduced at 38 °C (Fig. 2*A*). We needed to use much larger amounts of mutant relative to wt Cdc5 to monitor phosphorylation accurately in these kinase assays. To better compare the absolute difference in activity of those kinases, we performed additional experiments in the presence of equal amounts of wt and mutant Cdc5. Strikingly, we observe that even at the optimal temperature of 30 °C, the kinase activity of Cdc5-77 is ≤1–2% of wt levels (Fig. 2*B*). Cell survival in the presence of very low levels of kinase activity has been reported with several essential kinases (14–16). This low level of activity is not attributable to reduced phosphorylation of the activation loop of Cdc5 because pre-

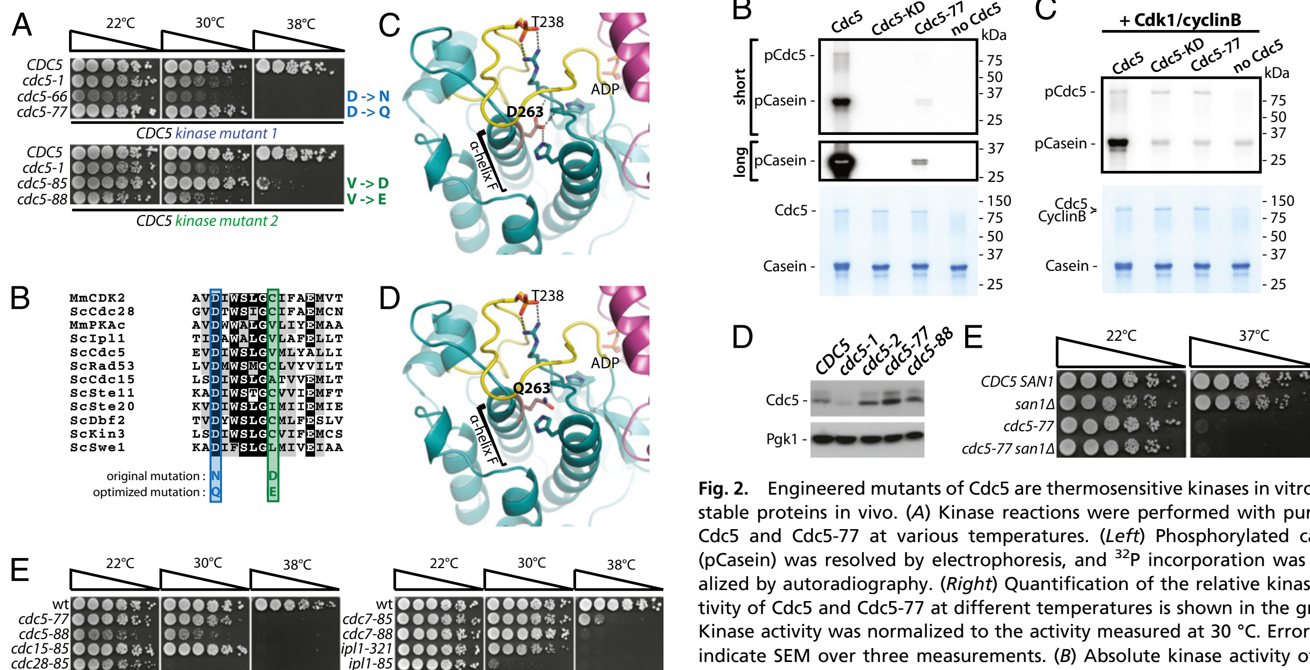

Fig. 1. Identification of *cdc5* mutants defective in kinase function. (*A*) Dilution series of wt cells and *cdc5* mutants were spotted on solid medium to evaluate growth at various temperatures. (*B*) Multiple local alignment of the sequence of the F-helix region in selected cell cycle kinases. (*C*) Model structure of the kinase domain of *Saccharomyces cerevisiae* Cdc5 showing the position of the conserved F-helix Asp263 in purple. Selected hydrogen bonds linking Asp263 in the F-helix with key conserved residues in the catalytic loop of the kinase domain are displayed by dashed lines (10). (*D*) Model structure of the Cdc5 mutant carrying Gln263 in its F-helix. (*E*) Fivefold dilution series of yeast cells carrying mutations at positions 1 and 2 of the F-helix of essential cell cycle kinases were spotted on solid medium to evaluate cell growth at various temperatures.

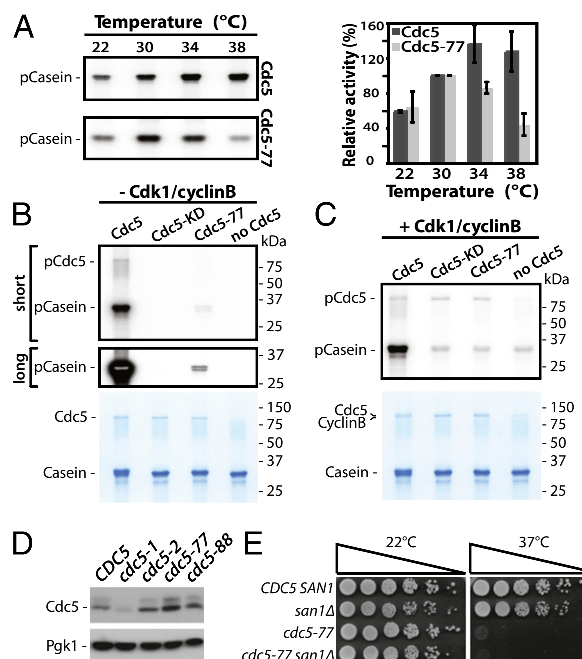

Fig. 2. Engineered mutants of Cdc5 are thermosensitive kinases in vitro and stable proteins in vivo. (*A*) Kinase reactions were performed with purified Cdc5 and Cdc5-77 at various temperatures. (*Left*) Phosphorylated casein (pCasein) was resolved by electrophoresis, and ^{32}P incorporation was visualized by autoradiography. (*Right*) Quantification of the relative kinase activity of Cdc5 and Cdc5-77 at different temperatures is shown in the graph. Kinase activity was normalized to the activity measured at 30 °C. Error bars indicate SEM over three measurements. (*B*) Absolute kinase activity of the Cdc5-77 mutant. All kinase reactions contained identical amounts of Cdc5, Cdc5-77, or Cdc5 kinase-dead [short-exposure autoradiogram (*Top*), long-exposure autoradiogram (*Middle*), and Coomassie-stained gel (*Bottom*)]. (*C*) Preactivation of Cdc5 by Cdk1 does not reactivate Cdc5-77 kinase. Assays were performed as above, except that Cdc5 was activated with Cdk1/cyclin B before conducting the kinase assays. The weak background signal in all lanes for casein is attributable to the inefficient phosphorylation of this substrate by Cdk1/cyclin B (14). (*D*) Protein levels of various *cdc5* mutants at restrictive temperature. Cells were synchronized in metaphase with nocodazole and incubated at 38 °C for 2 h before protein extraction and analysis of Cdc5 levels by Western blotting. (*E*) Inactivation of *SAN1* does not suppress the ts phenotype of *cdc5-77*. Fivefold dilution series of single and double mutants were spotted on solid medium to evaluate cell growth at 22 °C and 37 °C.

Downloaded at Palestinian Territory, occupied on December 31, 2021
www.manaraa.com
PNAS PLUS
GENETICS

phosphorylation of the mutant by Cdk1 (17) did not improve its kinase activity (Fig. 2*C*; note that Cdk1 can phosphorylate casein weakly, thereby covering up the weaker Cdc5 mutant signal). Although the kinase activity of Cdc5-77 is thermosensitive in vitro, it remains a possibility that the conditional phenotype of *cdc5-77* mutants is attributable, in part, to reduced protein levels in vivo. To address this, we directly monitored Cdc5 protein levels in mutant and wt cells. Fig. 2*D* shows that Cdc5-77 and Cdc5-88 protein levels were not reduced relative to wt Cdc5 at nonpermissive temperature. Likewise, the protein levels of another kinase mutant, Cdc15-85, were only weakly affected by incubation at nonpermissive temperature (Fig. S1*E*). Consistent with these observations, inactivation of the protein quality control pathway (18) did not suppress the ts phenotype of *cdc5-77* mutants (Fig. 2*E*) but did partially suppress the ts phenotype associated with the unstable Cdc5-1 protein (Fig. 2*D* and Fig. S1*D*). Together, these results indicate that the kinase activity of Cdc5-77 is thermosensitive in vitro and that Cdc5-77 protein levels are not reduced at nonpermissive temperature relative to wt Cdc5 in vivo.

Loss of Cdc5 Kinase Activity Leads to Mitotic Exit Defects. The creation of mutants that precisely modulate the kinase activity of Cdc5 allowed us to determine which of the known functions of Cdc5 require kinase activity in vivo. We first compared cell cycle progression of a synchronized culture of *cdc5-77* mutants (i.e., kinase-defective Cdc5) with that of a control *cdc15-2* strain. We used a *cdc15-2* mutant as a control in this experiment because this strain arrests during mitosis at a stage similar to *cdc5* mutants (19), allowing direct comparison of cell cycle progression between both strains. Fig. 3 *A–C* shows that *cdc5-77* mutants growing at nonpermissive temperature progressed normally from G1 up to early mitosis but arrested with elongated mitotic spindles and a 2n DNA content (135 min and later). The absence of preanaphase phenotypes in the *cdc5-77* mutant was also confirmed when comparing its cell cycle progression with that of wt cells (Fig. S2*A*). As expected for a kinase-defective strain, several proteins that normally become phosphorylated in mitosis, including Bfa1, Brn1, Mcd1/Scc1, and Nud1, showed little or no phosphorylation in *cdc5-77* mutants (Fig. 3*D*). In contrast, those substrates showed high levels of phosphorylation in *cdc15-2* cells arrested in late mitosis (Fig. 3*D*). Interestingly, attempts to test the impact of Cdc5 kinase activity on Slk19

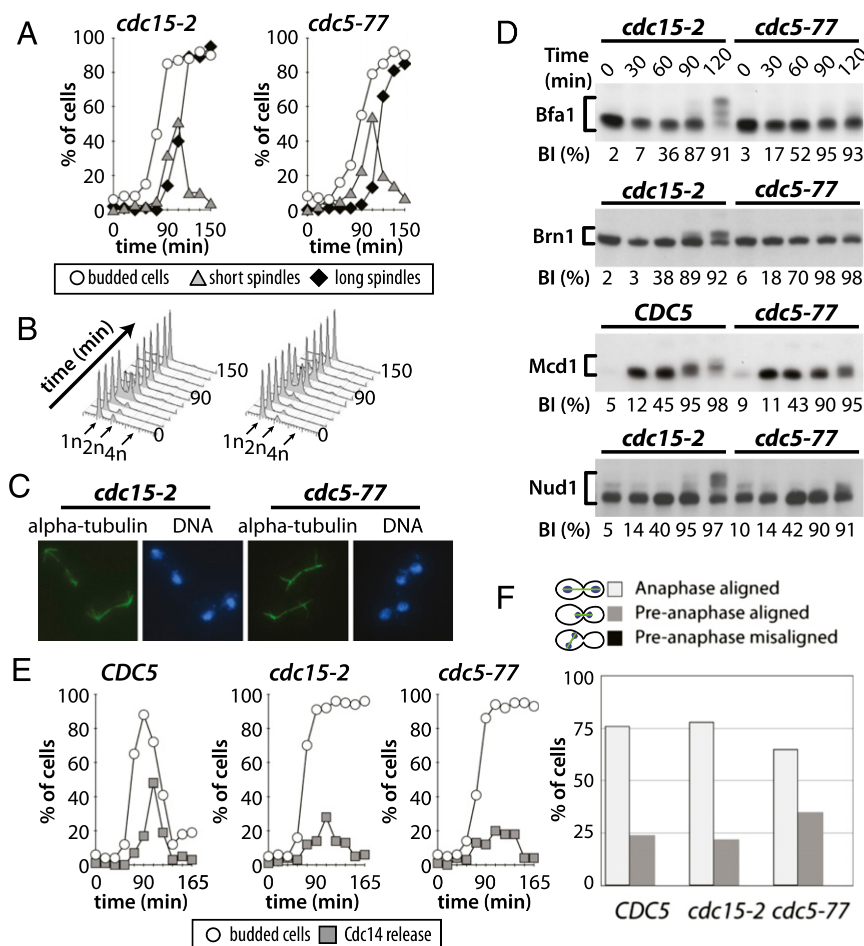

Fig. 3. Cell cycle phenotypes of *cdc5-77* mutants. The *cdc5-77* mutants, together with the control *cdc15-2* strain, were arrested in G1 with α-factor and released from their arrest into a synchronous cell cycle at 38 °C. Samples of cells were taken at the indicated time points to evaluate budding index and mitotic spindle length (*A*), cellular DNA content (*B*), and cell morphology at the end of the experiment (*C*). (*D*) *cdc15-2* and *cdc5-77* mutants were synchronized as above, and samples were taken at the indicated time points for analysis of phosphorylation-induced gel retardation of various Cdc5 substrates. For the analysis of Mcd1/Scc1 phosphorylation, cells were released from a G1 block into nocodazole-containing medium because this protein is degraded at the metaphase-anaphase transition (46, 47). The budding indexes (BI) are shown below the gels. (*E*) Analysis of Cdc14 release from the nucleolus in wt, *cdc15-2*, and *cdc5-77* mutants. Cells were synchronized and released as described above. (*F*) Mitotic spindle position relative to the mother-bud axis was determined at anaphase onset in the cells described in *E*.

Downloaded at Palestinian Territory, occupied on December 31, 2021
www.manaraa.com

phosphorylation were not successful because *cdc5-77* and *SLK19-3HA* are synthetic lethal when combined in yeast (Fig. S2*B*).

We next sought to better define the late mitotic defect of *cdc5-77* mutants. One of the key functions of Cdc5 in anaphase is to regulate the release and activation of Cdc14 from its nucleolar inhibitor, Cfi1/Net1 (20). To address the role of Cdc5 kinase activity in this process, we compared Cdc14 nucleolar release in wt, *cdc15-2*, and *cdc5-77* mutants released from α-factor arrest. It was necessary to include a wt strain in this experiment because *cdc15-2* mutants are defective in Cdc14 release by the mitotic exit network (MEN) (20). Immunofluorescence analysis of those strains revealed that the nucleolar release of Cdc14 was significantly diminished in *cdc5-77* mutants relative to wt (Fig. 3*E*). In fact, most *cdc5-77* cells contained little to no Cdc14 released from the nucleolus once they reached the end of the time course experiment (Fig. 3*E*). This phenotype indicates that *cdc5-77* mutants are completely defective in the activation of Cdc14 mediated by the MEN (Fig. 3*E*) (21). Mechanistically, this defect is consistent with the absence of phosphorylation of key MEN regulators, Bfa1 and Nud1, in *cdc5-77* cells (Fig. 3*D*). In contrast, the absence of defect in Cdc14 early anaphase release (FEAR) in *cdc5-77* mutants is consistent with FEAR network activity being at least partially competent in this mutant (21). This result indicates that the execution of FEAR network functions requires significantly less Cdc5 kinase activity than the execution of MEN functions. It is not possible to exclude, however, a requirement for very low levels of Cdc5 kinase activity in the execution of FEAR network functions.

Finally, we wanted to test the role of Cdc5 in spindle positioning in mid-mitosis because it has been shown recently that this function is defective in a specific *cdc5* mutant (22). The relative localization of the mitotic spindle was compared with the mother-bud axis at anaphase onset in *cdc5-77* mutants. We did not detect any increase in misaligned spindles in *cdc5-77* mutants relative to control strains (Fig. 3*F*). Taken together, these results show that the execution of late mitotic processes is acutely sensitive to the levels of Cdc5 kinase activity. Even though clear defects in Cdc5 substrate phosphorylation are visible before anaphase in *cdc5-77* mutants, their most striking cellular phenotype is a complete block in the execution of mitotic exit.

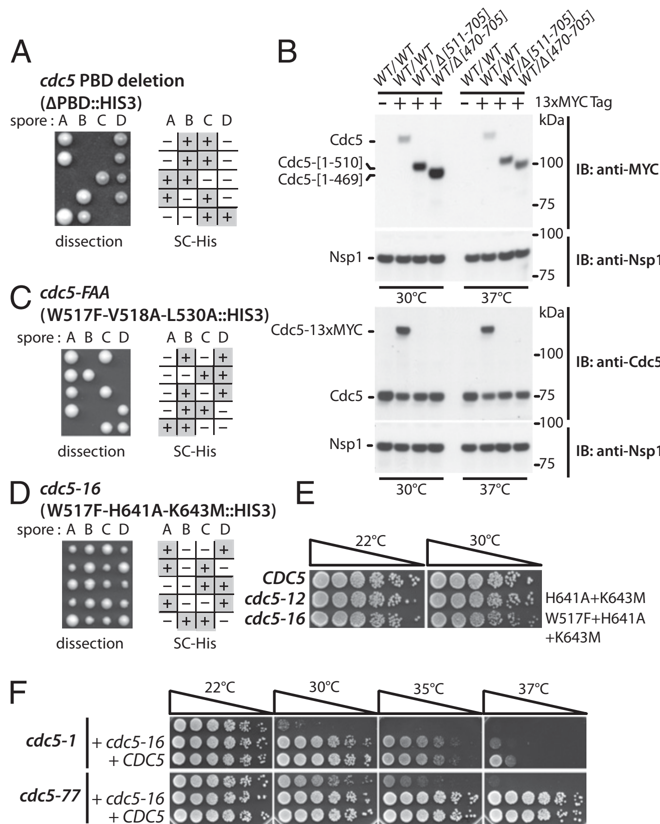

Fig. 4. Cdc5 phosphopeptide-binding activity is not essential for viability. (*A*) Genetic analysis of a sporulated heterozygous diploid strain carrying a complete deletion of Cdc5 PBD. The genotype of the resulting segregants was deduced using the *HIS3* marker associated with the *cdc5* mutation. (*B*) Effects of PBD deletion on Cdc5 levels at 30 °C and 37 °C. Cdc5 levels were determined by Western blot analysis in heterozygous diploid strains carrying epitope-tagged versions of Cdc5 lacking its PBD with [511–705] or without [470–705] the adjacent polo cap region. Cellular extracts were probed with anti-MYC (*Upper*), anti-Cdc5 (*Lower*), and anti-Nsp1 antibodies (loading control). Our Cdc5 antibody recognizes an epitope in the PBD of Cdc5, thus explaining the absence of signal with the PBD deletion mutant. (*C* and *D*) Genetic analysis of sporulated heterozygous diploid strains carrying *cdc5-FAA* or *cdc5-16* alleles. (*E*) Growth of *cdc5-12* and *cdc5-16* mutants at 22 °C and 30 °C. (*F*) Suppression of *cdc5-1* and *cdc5-77* ts phenotype by *cdc5-16*. A single copy of *CDC5* or *cdc5-16* was integrated at the *URA3* locus of yeast strains carrying *cdc5-1* or *cdc5-77*, and the growth phenotype of the resulting transformants was tested at 22 °C, 30 °C, 35 °C, and 37 °C.

The Phosphopeptide-Binding Activity of Cdc5 Is Not Essential for Viability. Having isolated kinase-specific mutants, we next wanted to identify mutations that would specifically inactivate Cdc5′s PBD. To this end, we created a diploid yeast strain carrying one copy of the *CDC5* gene with a deletion in its PBD (residues 511–705). Sporulation and dissection of this diploid strain revealed that spores carrying a deletion in Cdc5 PBD were inviable (Fig. 4*A*). Similar results were obtained with a strain carrying a previously described allele affecting Cdc5 PBD (i.e., *cdc5-FAA*) (6) (Fig. 4*C*). This result suggested that the PBD activity of Cdc5 is essential for yeast viability or, alternatively, that such mutations have deleterious secondary effects on the shorter version of the protein. To discriminate between these two possibilities, we tagged wt *CDC5* and PBD truncation mutants with MYC epitopes in heterozygous diploid strains and compared the levels of these proteins by Western blot analysis. Deletion of the PBD alone (residues 511–705) or together with the adjacent polo-cap region (residues 470–705) (9) resulted in a massive increase in the levels of the mutant proteins relative to wt Cdc5 (Fig. 4*B*). Previous studies have shown that supraphysiological levels of Cdc5 or excess PLK activity is deleterious to yeast proliferation (23, 24). This may explain why PBD truncation mutants were inviable in our experiments.

Since deletion of the entire PBD of PLKs/Cdc5 has secondary consequences on both protein levels and polo kinase activity (8), we hypothesized that a more targeted approach may be more successful at deciphering the precise role(s) of Cdc5 PBD. Specifically, creating point mutations in PBD residues known to be required for phosphopeptide-binding activity might be more amenable to genetic analysis because such mutations would leave other functions of the PBD intact. In particular, it is known that the inhibitory function of the PBD on the kinase activity of PLKs is maintained in phosphopeptide-binding mutants (9). These observations prompted us to create both moderate (*cdc5-12*: H641A, K643M) and severe (*cdc5-16*: W517F, H641A, K643M) alleles of Cdc5 PBD based on the observation that polo-like kinase 1 (Plk1) mutants corresponding to W517F or H641A-K643M have no detectable phosphopeptide-binding activity in vitro (9, 25). Consistent with this observation, introducing these mutations in Cdc5 PBD prevented binding to all its known phospho-substrates (9, 22, 26, 27). Heterozygous diploid yeast strains carrying one copy of *cdc5-12* or *cdc5-16* were sporulated, and the viability of the resulting spores was investigated. Haploid cells carrying PBD-defective alleles of *CDC5* at the endogenous locus were viable (Fig. 4*D*) and showed little to no growth defect

PNAS PLUS
GENETICS
Downloaded at Palestinian Territory, occupied on December 31, 2021
www.manaraa.com

(Fig. 4*E*). This result is consistent with a recent demonstration that expression of similar PBD mutants of *CDC5* from plasmids can complement *cdc5*Δ mutants (27). Together, these experiments revealed that the phosphopeptide-binding activity of the PBD of Cdc5 is largely dispensable for both cell proliferation and survival.

Previous studies have shown that a *cdc5* allele carrying point mutations in Cdc5 PBD (i.e., W517F, V518A, and L530A) was incapable of complementing the growth defect of *cdc5-1* mutants (6). We wanted to test whether *cdc5-16* behaved in a similar fashion. Thus, *cdc5-16* was integrated at the *URA3* locus in *cdc5-1* and *cdc5-77* mutants, and its ability to complement the ts phenotype of its host strains was tested. Remarkably, expression of *cdc5-16* or *CDC5* (both from their native promoter) complemented the ts phenotype of the *cdc5-77* mutant at all temperatures tested (Fig. 4*F*). Similar complementation was obtained in a *cdc5-1* mutant background, with the exception that this strain could not be fully complemented at 37 °C, even with wt *CDC5* (Fig. 4*F*). This may suggest that the *cdc5-1* mutant acts in a dominant-negative manner at high temperature, thus explaining why complementation of this allele has previously been reported at 35 °C (6). Importantly, the ability of *cdc5-16* PBD mutant to complement the growth defect of the kinase-specific mutant *cdc5-77* is consistent with both alleles being affected in distinct biochemical functions.

Cdc5 Kinase and PBD Mutants Have Distinct Phenotypes. We next turned our attention to the phenotypic characterization of the PBD mutants of Cdc5. In contrast to the strong mitotic phenotype of Cdc5 kinase mutants, no striking defect in cell cycle progression was detected in *cdc5-16* mutants progressing from G1 to mitosis (Fig. 5 *A*–*C*). Inactivation of the phosphopeptide-binding activity of the PBD did affect the duration of late mitotic events, as evidenced by an accumulation of cells with elongated spindles and a ∼15-min delay in spindle disassembly relative to wt cells (Fig. 5*A*). However, *cdc5-16* mutants exited mitosis rapidly after this delay and formed individual, albeit slightly enlarged and deformed cells (Fig. 5 *A*–*C*). Inspection of cell morphology by microscopy revealed no detectable "chain-like" phenotype indicative of a defect in cell separation in *cdc5-16* mutants (>93% single unbudded cells in 500 G1-arrested cells; Fig. 5 *A*–*C*).

The modest mitotic phenotype of *cdc5-16* cells prompted us to investigate how the loss of PBD phosphopeptide-binding activity affected the phosphorylation of Cdc5 substrates. Interestingly, loss of PBD activity affected Cdc5 substrate phosphorylation to very different degrees depending on the substrate analyzed. For instance, phosphorylation of Mcd1/Scc1 and Nud1 appeared to be mostly unaffected by the loss of PBD activity, whereas Slk19 was hyperphosphorylated in *cdc5-16* mutants (Fig. 5*D*). These results indicate that the intrinsic kinase activity of the Cdc5-16 mutant is not severely reduced by the PBD mutations. On the other hand, phosphorylation of Brn1 was significantly delayed in *cdc5-16* cells but reached normal levels later in mitosis (Fig. 5*D*). Finally, loss of PBD activity resulted in an almost complete abrogation of Bfa1 phosphorylation during mitosis (Fig. 5*D*). Together, these results reveal that inactivation of Cdc5 PBD can affect substrate phosphorylation both positively and negatively during the cell cycle. We cannot exclude at this stage the possibility that a yet-to-be discovered kinase is responsible for Scc1 and Slk19 phosphorylation in *cdc5-16* mutants. Indeed, in an attempt to test this possibility, we discovered that an allele of Cdc5 that is defective in both kinase and PBD activities (i.e., D263Q, W517F, H641A, K643M; *cdc5-20*) is inviable in yeast (Fig. S2*C*).

The mitotic delay experienced by *cdc5-16* cells could be the result of a defect in a number of anaphase events. To better define the anaphase phenotype of these cells, we first evaluated the kinetics of sister-chromatid (SC) segregation in *cdc5-16* mutants and control cells carrying tetO-GFP dots on chromosome 5 (Chr5). As expected, both strains segregated Chr5 with similar kinetics in a synchronous time-course experiment (Fig. 5*E*). Specifically, initiation of SC segregation occurred 60 min following α-factor release in both strains, and maximal segregation was reached at 75 min post-release (Fig. 5*E*). The absence of SC segregation defect in *cdc5-16* cells is consistent with the fact that phosphorylation of the cohesin subunit Mcd1/Scc1 is not affected in this mutant (Fig. 5*D*).

Next, we examined whether defects in spindle positioning might contribute to the anaphase delay of *cdc5-16* cells. Mutant and wt cells were processed as described above for evaluation of the relative position of their mitotic spindles at anaphase onset (22). Cytological examination of mitotic spindles in these cells did not reveal any evidence for defective spindle positioning in *cdc5-16* mutants (Fig. 5*F*). This prompted us to evaluate whether a later event in mitosis (i.e., Cdc14 nucleolar release) might be affected in *cdc5-16* cells. Immunofluorescence analysis of synchronous cell populations revealed that both mutant and wt strains initiated Cdc14 release at the same time in anaphase (∼60–75 min after α-factor release; Fig. 5*G*). However, *cdc5-16* mutants appeared to maintain this release significantly longer than wt cells (∼30 min longer; Fig. 5*G*). To test whether this apparent increase in Cdc14 release is physiologically relevant or is a consequence of slow anaphase progression, we monitored Clb2 levels in *cdc5-16* mutants. Our results show that Clb2 is degraded with identical timing in wt and *cdc5-16* mutants (Fig. 5*H*). Because Clb2 degradation depends on Cdc14 activity (20), we conclude from this experiment that there is no effective increase in Cdc14 activity in *cdc5-16* mutants. Collectively, these results indicate that the PBD activity of Cdc5 promotes timely progression through anaphase.

Inactivation of Cdc5 PBD Causes Defects in Spindle Pole Body Integrity. We next wanted to identify how the loss of PBD activity might affect the regulation and function of Cdc5 in vivo. To achieve this, we integrated 3xGFP-tagged versions of *CDC5* or *cdc5-16* at the *URA3* locus of wt cells and asked whether the subcellular localization of the mutant protein was altered relative to wt. Consistent with previous studies, we observed that Cdc5 accumulated in the nucleus as cells entered mitosis and was specifically enriched in two perinuclear foci corresponding to spindle pole bodies (SPBs) in mitotic cells (6, 24) (Fig. 6*A*). In contrast, we did not detect a specific enrichment of GFP–Cdc5-16 in any particular structure of the cell other than the nucleus during mitosis (Fig. 6*A*). The diffuse nuclear localization of the Cdc5 PBD mutant was not attributable to changes in expression levels of the mutant protein (Fig. S3*B*).

The fact that Cdc5-16 did not localize to the SPBs suggests that the functionality of microtubule-organizing centers (MTOCs) might be affected by the absence of Cdc5 PBD activity. To investigate this possibility, we fused the SPB component Spc42 to GFP in strains carrying wt *CDC5* or the PBD mutant allele and counted the number of SPB-like fluorescent structures/dots in these cells. Surprisingly, we observed that *cdc5-16* cells carried a highly abnormal number of Spc42-GFP structures relative to wt cells. Specifically, more than 30% of *cdc5-16* mutants showed two or more Spc42-GFP dots per cell, whereas normal cells arrested in nocodazole showed mostly a single Spc42-GFP dot per cell, as expected (Fig. 6 *B* and *C*). A noticeable fraction of *cdc5-16* mutants carried as many as three or four Spc42-GFP foci, whereas wt controls never showed this phenotype (Fig. 6B and Fig. S3*C*). Importantly, this phenotype was not caused by leaky checkpoint arrest in response to nocodazole treatment in the absence of Cdc5 PBD activity (27).

Although no other *cdc5* mutant shares the supernumerary Spc42 foci phenotype of *cdc5-16* mutants, it is intriguing that overexpression of wt Cdc5 has also been reported to increase

Downloaded at Palestinian Territory, occupied on December 31, 2021
www.manaraa.com

Downloaded at Palestinian Territory, occupied on December 31, 2021

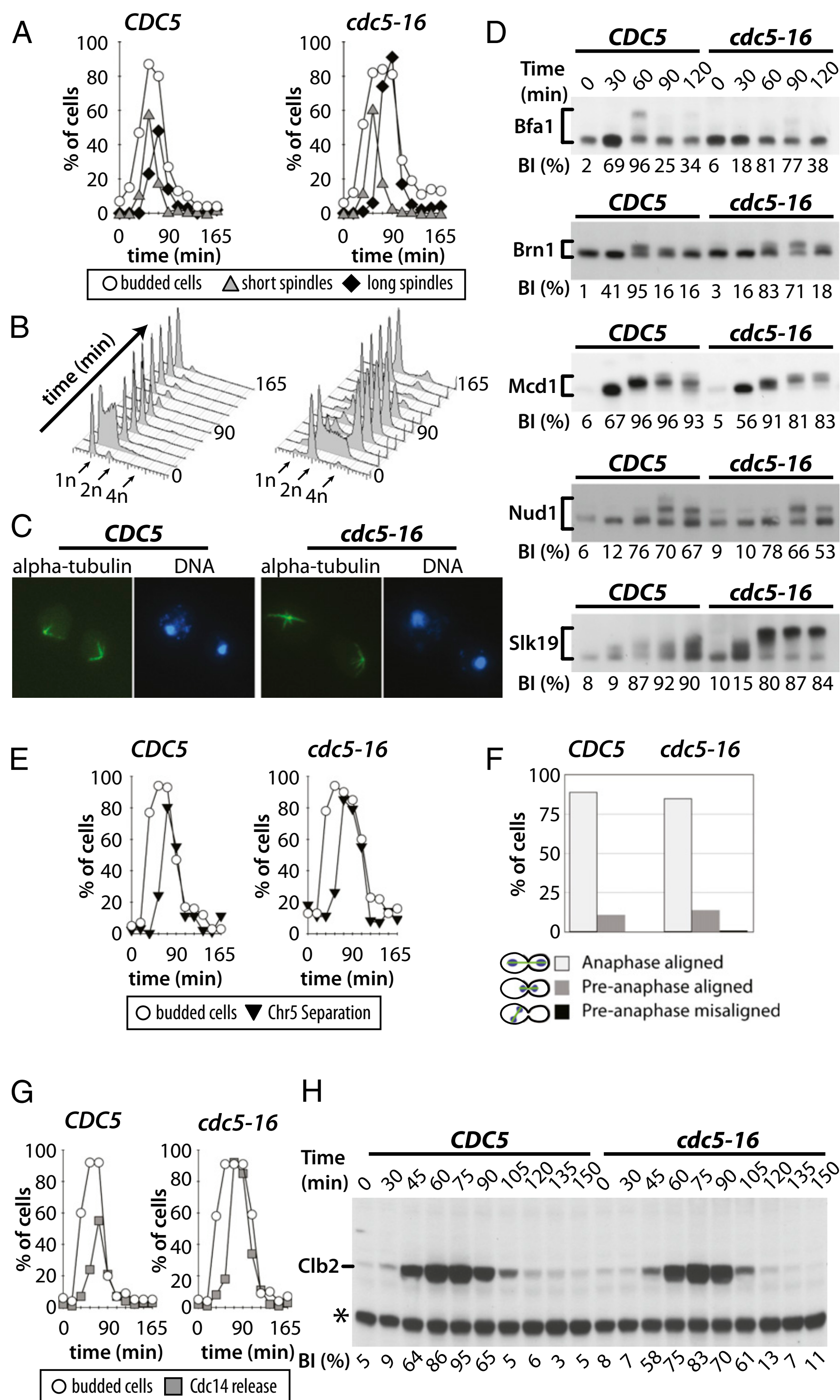

Fig. 5. Cell cycle phenotype of *cdc5* mutants defective in PBD activity. Cell cycle progression of wt and *cdc5-16* mutants was evaluated after G1 arrest with α-factor and release from the arrest at 30 °C. Samples of cells were taken at the indicated time points to evaluate BI and mitotic spindle length (*A*), cellular DNA content (*B*), cell morphology at the end of the experiment (*C*), and phosphorylation status of various Cdc5 substrates (*D*), as described in Fig. 3. Analysis of Mcd1/Scc1 and Slk19 phosphorylation was performed in cells that were released from a G1 block into nocodazole-containing medium because these proteins are degraded at the metaphase-anaphase transition (46, 47). (*E*) Analysis of SC segregation in control and *cdc5-16* cells released from a G1 block into a synchronous cell cycle. Note that the small proportion of *cdc5-16* cells carrying two GFP dots at the beginning of the segregation experiment is attributable to a change in the ploidy of this strain (i.e., a "generation 1" strain described in more detail below). (*F*) Analysis of mitotic spindle position relative to the mother-bud axis at anaphase onset in control and *cdc5-16* cells. (*G*) Analysis of Cdc14 release from the nucleolus in wt and *cdc5-16* mutants. Cells were synchronized and released as described above. (*H*) Analysis of Clb2 levels in wt and *cdc5-16* mutants progressing synchronously in the cell cycle. The asterisk marks the position of a cross-reacting band in the cell lysate. BIs are shown under the figure.

Spc42 foci numbers in yeast (6). However, the functional significance of this phenomenon, if any, has not been investigated until now. One possibility is that some of the supernumerary Spc42-GFP dots may represent a pool of cellular Spc42 that

www.manaraa.com

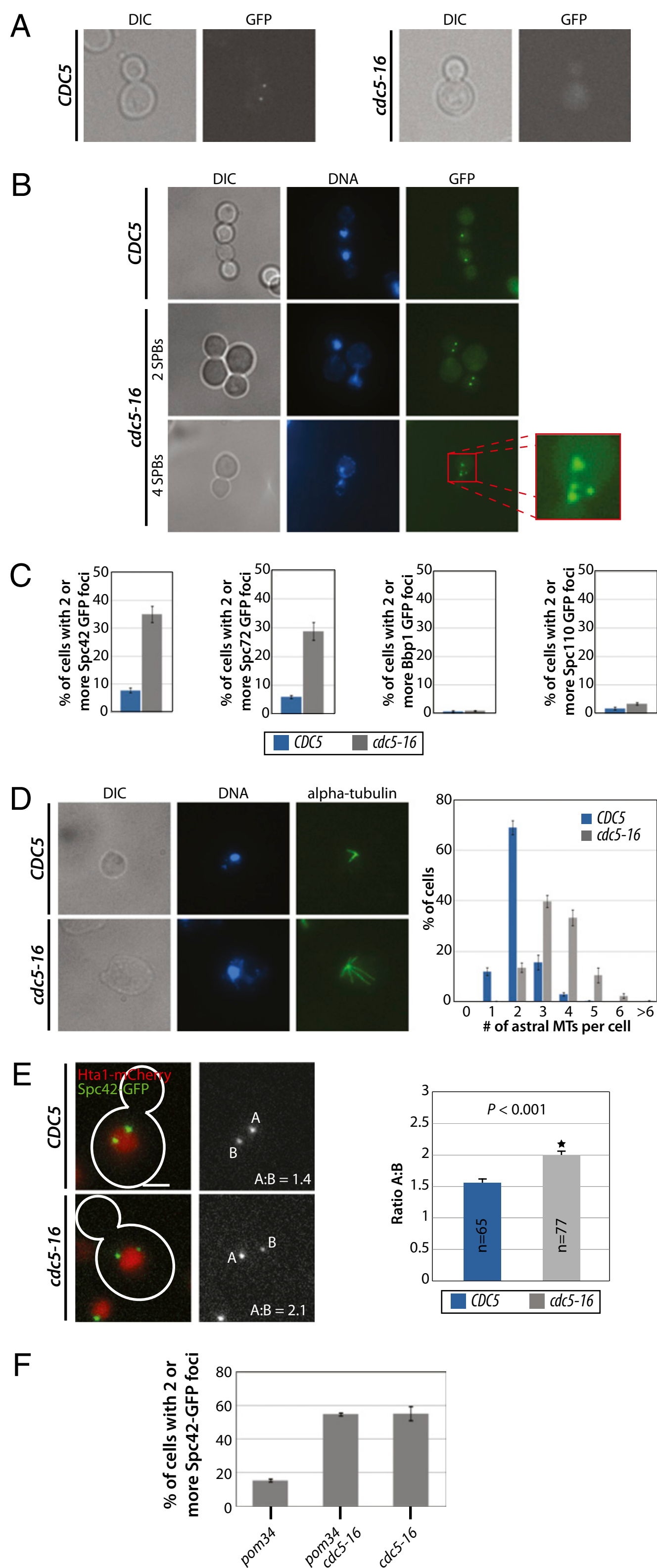

Fig. 6. Cdc5 PBD activity regulates SPB functionality. (*A*) Differential localization of Cdc5 and Cdc5-16 in mitotic cells. Wild-type and mutant Cdc5-GFP were imaged in exponential cultures of live cells. (*B*) Representative images of wt or *cdc5-16* cells carrying abnormal numbers of Spc42-GFP dots. (*Inset*) Magnification of a region of a *cdc5-16* cell containing four Spc42-GFP dots. Spc42-GFP was imaged in nocodazole-arrested cells. (*C*) Quantification of the number of SPB-like foci in *cdc5-16* cells. The number of GFP dots was determined in nocodazole-arrested cells carrying GFP-fused Spc42, Spc72, Bbp1, or Spc110. Error bars indicate SEM over four measurements. The small fraction of wt cells with two GFP dots represents cells that have escaped from the nocodazole arrest. (*D*) Aberrant astral microtubule (MT) numbers in *cdc5-16* cells. (*Left*) Representative images of wt or *cdc5-16* cells with abnormal astral microtubule numbers. (*Right*) Quantification of the astral microtubule number phenotype is shown in the graph. Error bars indicate SEM over three measurements. (*E*) (*Left*) Representative time-lapse images of wt and *cdc5-16* yeast cells expressing Spc42-GFP and Hta1-mCherry. The fluorescence intensity ratio of duplicated SPBs is reported in the lower corner. The ratio for each cell was obtained by dividing the high-intensity SPB "A" by the low-intensity SPB "B". (*Right*) Quantification of the ratios in wt and mutant cells is shown in the graph. The ratio of fluorescence intensity in *cdc5-16* cells was more than 30% higher ($P < 0.001$). Error bars indicate SEM. (Scale bar: 2.5 μm.) (*F*) Quantification of the number of Spc42-GFP foci in *cdc5-16* and *pom34Δ* mutants. Error bars indicate SEM over three measurements.

failed to insert into functional SPBs and self-assembled into aberrant SPB-like structures in mutant cells (12). In order to better understand the nature of these aberrant structures, we tagged multiple components of the SPB with GFP and monitored their localization in nocodazole-arrested *cdc5-16* cells. Analysis of Spc72-GFP localization revealed the existence of a large fraction of *cdc5-16* mutants carrying supernumerary Spc72-GFP dots relative to wt controls (Fig. 6*C*). In striking contrast, other components of the SPB, such as Bbp1-GFP and Spc110-GFP, did not form supernumerary foci in nocodazole-arrested *cdc5-16* cells (Fig. 6*C*). These observations indicate that some but not all components of the SPB are mislocalized in *cdc5-16* mutants. Importantly, the absence of the γ-tubulin receptor Spc110 (28) in the supernumerary Spc42/72 foci indicates that these structures do not represent bona fide MTOCs. This interpretation is supported by the fact that multipolar spindles were not detected at an appreciable frequency during our analysis of the cell cycle phenotypes of *cdc5-16* mutants (Fig. 5*A*).

The targeting of a subset of SPB components to aberrant structures in *cdc5-16* cells is expected to alter the balance and/or levels of components within active SPBs, which, in turn, may affect their functionality. Consistent with this view, we observed that SPBs in *cdc5-16* cells carried an abnormal number of astral microtubules relative to wt controls (Fig. 6*D*). Quantification of this defect revealed that *cdc5-16* cells have almost twice as many astral microtubules as wt cells (Fig. 6*D*). Furthermore, examination of live cells revealed that duplication of SPBs in *cdc5-16* mutants led to the formation of structures containing wide differences in their levels of Spc42 (Fig. 6*E*). Specifically, quantification of the Spc42-GFP fluorescence in the newly duplicated SPBs of *cdc5-16* cells indicated that Spc42 was distributed in a preferential manner to one SPB (labeled "A" in Fig. 6*E*), whereas the other SPB inherited much smaller amounts of this component (SPB "B" in Fig. 6*E*). Although detectable in wt cells as well, this asymmetry in the distribution of Spc42 was significantly weaker than in *cdc5-16* cells (Fig. 6*E*; ratio of SPB A:B). Importantly, the depletion of Spc42 from one SPB cannot be ascribed to differences in protein expression levels because wt and *cdc5-16* yeast contained similar total amounts of Spc42-GFP fluorescence on a cell-to-cell basis (Fig. S3*D*). This result indicates that, on average, the "weaker" SPB in *cdc5-16* mutants displayed a significant reduction (~25–50%) in Spc42 levels relative to normal SPBs. Collectively, these results indicate that formation of aberrant SPB structures in *cdc5-16* mutants affects the composition and functionality of active SPBs.

We next wanted to understand the molecular basis for this defect. In particular, the formation of extra Spc42/Spc72 foci in *cdc5-16* mutants could indicate that these proteins are not

Downloaded at Palestinian Territory, occupied on December 31, 2021
www.manaraa.com

properly incorporated into functional SPBs during their biogenesis. Deletion of *POM34* in SPB duplication mutants is known to suppress defects in the assembly of SPB components (29). However, loss of *POM34* in *cdc5-16* mutants did not result in an effective transfer of Spc42 from supernumerary foci to functional SPBs (compare Spc42 foci numbers in *cdc5-16* and in *pom34 cdc5-16* double mutants in Fig. 6*F*). This result shows that *POM34*-dependent SPB duplication defects are not the cause of the formation of incomplete SPBs in *cdc5-16* mutants. Taken together, these results indicate that Cdc5 PBD activity promotes effective assembly of SPB components into functional MTOCs and/or their maintenance in a single cohesive unit.

The PBD Activity of Cdc5 Is Required to Maintain Cellular Ploidy. Mutants defective in SPB/centrosome functionality are typically unable to maintain cellular ploidy (12). A prediction from this and from the SPB phenotypes described above is that *cdc5-16* mutants should show defects in the control of their ploidy. Consistent with this prediction, we noticed in our previous experiments that *cdc5-16* mutants arrested in G1 with α-factor have a 2n genomic content, whereas they reach a 4n state during mitosis (Fig. 5*B*). This situation corresponds to a doubling in genomic content for a haploid strain. Similar but less extensive changes were observed with the weaker PBD mutant, *cdc5-12* (Fig. S3*A*). To better understand the timing and mechanism underlying the loss of ploidy control in Cdc5 PBD mutants, we constructed diploid strains carrying wt *CDC5* and mutant *cdc5-16*. After sporulation and dissection of the diploid strain, haploid segregants carrying either *CDC5* or *cdc5-16* were subcultured once (generation 1) and submitted to flow cytometry analysis after synchronization in G1 or metaphase (Fig. 7*A*). *CDC5* segregants showed the predicted 1n and 2n peaks of DNA content under these conditions. In contrast, the *cdc5-16* mutant already contained at generation 1 a small fraction of its population with twice the normal amount of DNA when arrested in G1 and metaphase, respectively (i.e., 2n and 4n; Fig. 7*A*). Remarkably, further subculturing of *cdc5-16* cells (generations 2–4) resulted in a progressive doubling of their genomic content until most of the cells in the population became diploids (Fig. 7*A*). We did not observe *cdc5-16* cells with greater than 2n genomic content (in a G1 state; Fig. S3*E*), which is consistent with the fact that *cdc5* mutants are incapable of growing as tetraploid cells (30). This defect in ploidy control was confirmed by directly counting the number of copies of Chr5 in G1-synchronized *cdc5-16* cells carrying a fluorescent tetR-GFP signal at *URA3*. Wild-type cells always had a single Chr5 dot, whereas *cdc5-16* cells showed a progressive accumulation in the number of cells carrying two Chr5 dots, from ∼18% at generation 1 to ∼50% at generation 4 (Fig. 7*B*). Taken together, these results unravel a novel role for Cdc5 and its PBD in the control of cellular ploidy.

Next, we wanted to investigate whether the ploidy defect of *cdc5* mutants was attributable to the inability of Cdc5-16 protein to localize to SPBs (Fig. 6*A*). To test this notion, we fused the SPB-localization domain of Bbp1 (31) to *cdc5-16* sequence and integrated this allele at the *URA3* locus in a *CDC5*/*cdc5-16* diploid strain. Control cells carried an unmodified version of *cdc5-16* gene at the *URA3* locus. After sporulation and dissection of the diploid strains, haploid segregants were grown for four generations and the size of their genome was determined. As shown before, more than 95% of *cdc5-16* mutants (without an additional copy of *cdc5-16*) doubled the size of their genome under these conditions. The presence of an extra copy of *cdc5-16* in a *cdc5-16* mutant background caused only a modest suppression of the ploidy phenotype compared with control cells (Fig. 7*C*). In striking contrast, the ploidy defect of *cdc5-16* mutants was significantly improved by targeting Cdc5-16 to SPBs (Fig. 7*C*). Indeed, ∼60% of cells expressing the *cdc5-16–bbp1* fusion remained haploid during the time frame of this experiment (Fig. 7*C*). Collectively, these results reveal that the ploidy defect of *cdc5-16* cells is caused, at least in part, by the inability of mutant Cdc5 to localize to SPBs. In addition, these results show that Cdc5 promotes changes in SPBs that directly impact the maintenance of genome size and integrity.

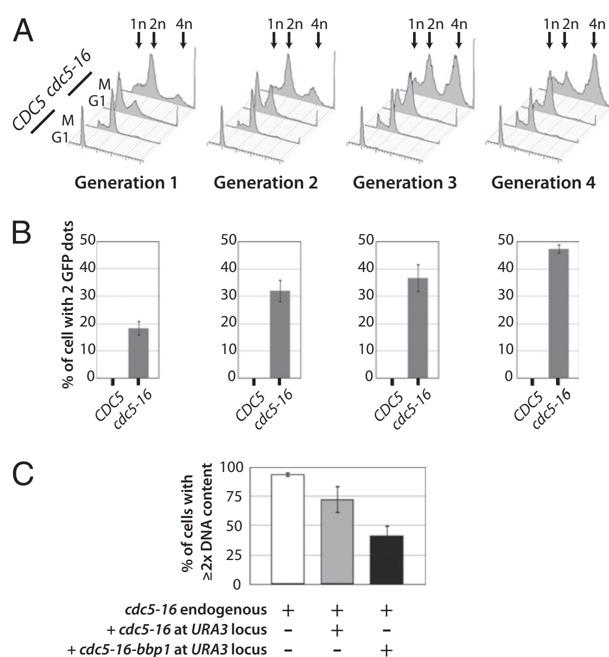

Fig. 7. Loss of ploidy control in *cdc5-16* mutants. Heterozygous diploid strains carrying *cdc5-16* were induced to sporulate, dissected, and haploid spores were allowed to grow until they formed visible colonies. The resulting *CDC5* and *cdc5-16* colonies were then transferred on fresh medium and subcultured a total of four times on plates ("generations 1–4"). (*A*) Cellular DNA content was evaluated by flow cytometry in cells arrested in G1 or metaphase. (*B*) Number of Chr5-derived tetO dots was evaluated in G1-arrested cells from generations 1 through 4, as described above. Error bars indicate SEM over three measurements. (*C*) Suppression of *cdc5-16* ploidy defect by localization of Cdc5-16 to SPBs. Haploid *cdc5-16* mutants carried no additional Cdc5 or an extra copy of either *cdc5-16* or *cdc5-16-bbp1* integrated at the *URA3* locus. The proportion of generation 4 cells having increased the size of their genome was determined after G1 arrest, as described above. Error bars indicate SEM over three measurements.

Discussion

In this study, we show that the functions of the kinase domain and PBD of Cdc5 can be dissociated genetically with strikingly different consequences for cells. This dichotomy is particularly evident in the effects of PBD inactivation on cellular ploidy. Indeed, this phenotype is only seen in PBD mutants and unravels a novel function for Cdc5 as an important regulator of cellular ploidy. Our ability to successfully separate the phenotypes of the kinase and PBD domains of Cdc5 highlights the benefit of examining mutants affected in specific biochemical activities in order to obtain an integrative view of the roles of multifunctional kinases in vivo. Consistent with this, PBD-specific mutants of Cdc5 have previously been very useful in the identification of novel functions for PLKs (27).

At a cellular level, one of the most striking defects in *cdc5-16* mutants is their inability to incorporate and/or maintain all SPB components in fully functional MTOCs. Multiple lines of evidence support a direct functional relationship between impaired regulation of SPB components and ploidy defects in *cdc5-16* mutants. First, loss of ploidy control is frequently observed in mutants defective in the regulation of SPB components (e.g., 12). Second, multiple components of the SPB are regulated by Cdc5-

PNAS PLUS
GENETICS
Downloaded at Palestinian Territory, occupied on December 31, 2021
www.manaraa.com

mediated phosphorylation (22, 32, 33). Third, Cdc5 localizes to SPBs (6, 24) and interacts physically with multiple SPB components (22, 33, 34). Fourth, mutants of SPB outer plaque components that are known to interact physically with Cdc5 [i.e., Spc72, Cnm67 (22, 34)] show an uncommon age-dependent increase in ploidy that is reminiscent of the progressive changes in ploidy experienced by *cdc5-16* cells (35). Finally, the specific constellation of cytological defects seen in *cdc5-16* mutants (i.e., supernumerary Spc42/Spc72 structures, reduced functionality of SPBs, aberrant numbers of astral microtubules) is also associated with loss of ploidy control in mutants affecting the SPB components Ndc1 and Cnm67 (36–38). Collectively, these observations indicate that Cdc5 plays an important role in the regulation of SPB function and in ploidy control.

Our results are consistent with previous studies implicating PLKs in the regulation of centrosome maturation in metazoans (4, 39). In particular, the mislocalization of a number of SPB components in *cdc5-16* mutants is reminiscent of the inability of human and *Drosophila* cells to effectively localize γ-tubulin–containing complexes to centrosomes in the absence of polo kinase activity (4, 39). This defect is also associated with loss of ploidy control in cells derived from polo mutant flies, similar to what we observe in *cdc5-16* mutants in yeast (40). The presence of aberrant assemblies of centrosome/SPB components in both Plk1- and Cdc5-defective cells further highlights the phenotypic similarity between these two mutant conditions (41). These observations strongly suggest that the critical role played by PLKs in MTOC maturation will be conserved from yeast to humans. From a mechanistic standpoint, the results obtained with *cdc5-16* mutants suggest that the PBDs of mammalian PLKs will likely play critical roles in the control of MTOC functionality and in the maintenance of genome ploidy. In light of this, it is perhaps not surprising that *PLK1* PBD mutations have been found in human tumor cell lines (16) and that many human cancers are characterized by misregulation of PLKs (4).

At the molecular level, the observation that not all Cdc5 substrates are affected to the same extent by the loss of PBD activity is highly interesting. Indeed, whereas some substrates are phosphorylated later or to a lesser extent in *cdc5-16* cells (i.e., Bfa1, Brn1), others appear to be unaffected (i.e., Mcd1) or even hyperphosphorylated (i.e., Slk19). One likely reason for this phenotype is that loss of PBD activity will effectively increase the availability of Cdc5 for those substrates whose phosphorylation does not normally depend on the PBD activity. This increased availability of Cdc5 will lead to enhanced phosphorylation of "PBD-insensitive" substrates, whereas those substrates that normally require the PBD to be phosphorylated will be less so in *cdc5* PBD mutants. This possibility is intriguing because it suggests that the phenotype associated with PBD inactivation may be the end result of two qualitatively different consequences: hyperphosphorylation of some substrates and lack of phosphorylation of others. Thus, in addition to its role in stimulating substrate phosphorylation, another key function of the PBD may be to prevent over-phosphorylation of a specific subset of PLK substrates. This substrate over-phosphorylation could be mediated directly by Cdc5 or indirectly by another kinase. In any case, it would be advantageous to limit the phosphorylation of specific substrates when such modification affects the substrate activity in a quantitative manner. This putative inhibitory role for Cdc5 PBD is based on the assumption that Cdc5 kinase activity is limiting in cells. Whether this assumption and the PBD inhibition model are taking place in vivo remains to be shown experimentally.

Another key observation from our work and from that of others is that the MTOC functions of PLKs likely require the localization of these kinases to SPBs and centrosomes (5). Paradoxically, this localization to SPBs is a potential reason why Cdc5′s role in the regulation of SPB functions may have been overlooked until now in budding yeast. Indeed, Cdc5 association with SPBs serves a functionally separate role in the regulation of mitotic exit (34), providing one reason other than SPB regulation for its enrichment at this location. In this context, the use of the *cdc5-16* mutant (W517F-H641A-K643M) has been instrumental to differentiate the SPB phenotypes from the mitotic exit phenotypes of Cdc5. A similar mutant has also been useful to identify a novel role for Cdc5 in mitotic spindle elongation (27). Importantly, defects in spindle elongation and SPB regulation are functionally distinct because it is possible to observe the former without the later, for example, in cells defective in microtubule motor activity (e.g., 42, 43). In the course of our study, we noticed that another allele partly defective in PBD activity, *cdc5-12* (H641A-K643M), had a much weaker phenotype than the *cdc5-16* mutant. In particular, the progression of *cdc5-12* mutants from 1n to 2n genomic content was slow, and only half of the population of mutant cells reached a 2n state after extended growth periods (Fig. S3*A*). Compared with *cdc5-16* mutants, the weak mitotic defects of the *cdc5-12* mutant [and of the similar *cdc5-HK* mutant (27)] indicate that this allele is not completely null in its PBD activity. It is also noteworthy that several ts alleles of *CDC5* carry point mutations affecting the PBD (Fig. S1*A*). From a functional standpoint, these alleles are not PBD-specific, however, because they severely reduce Cdc5 protein levels in vivo (Fig. S1*A*). As a consequence, these alleles should be considered defective in all biochemical activities of Cdc5, not exclusively in PBD activity.

Our ability to distinguish between the PBD and kinase-related functions of Cdc5 was made possible by the creation of kinase-specific mutations. It is remarkable that mutations in the F-helix of several essential kinases create conditional alleles of these enzymes. This observation indicates that the F-helix region of EPKs is a mutagenic hotspot that can be used to create unique mutants with conditional activity. The ability to quantitatively modulate the enzymatic activity of kinases using predictable mutations has multiple applications in biology. In particular, F-helix mutants could be useful to understand the specific roles of multifunctional kinases and, in addition, may allow the identification of specific thresholds of kinase activity required for particular functions in cells. These mutations may thus be regarded as temperature-controlled rheostats to modulate the enzymatic activity of specific kinase domains in vivo.

Materials and Methods

All yeast strains are derivatives of W303 and are described in Table S1. Standard procedures were used for yeast culture, genetics, molecular biology, NextGel (Amresco) electrophoresis, Western blotting, fluorescence microscopy, flow cytometry, Cdc5 overexpression, purification, and kinase assays (44). Preparation of yeast for time-lapse imaging was done as described previously (45). A comprehensive description of all experimental procedures is included in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank S. Michnick, F. Sicheri, J. St-Pierre, M. Therrien, and members of our laboratories for their comments on the manuscript and J. Gerton for yeast strains. This study was supported by Canadian Institutes of Health Research (CIHR) Grant MOP 82912 (to D.D.) and by Canadian Cancer Society Research Institute (CCSRI) Grant 20304 (to D.D.). P.S.M. and A.-M.L. are supported by Grant MOP 106548 (to P.S.M.) from the CIHR. This research was also made possible by a charitable donation from Monique and Guy Sauvageau Sr. D.D. is recipient of a Canada Research Chair in Cell Cycle Regulation and Genomic Integrity.

1. Ubersax JA, Ferrell JE, Jr. (2007) Mechanisms of specificity in protein phosphorylation. *Nat Rev Mol Cell Biol* 8:530–541.
2. Mahajan A, et al. (2008) Structure and function of the phosphothreonine-specific FHA domain. *Sci Signal* 1:re12.
3. Pawson T, Kofler M (2009) Kinome signaling through regulated protein-protein interactions in normal and cancer cells. *Curr Opin Cell Biol* 21:147–153.
4. Archambault V, Glover DM (2009) Polo-like kinases: Conservation and divergence in their functions and regulation. *Nat Rev Mol Cell Biol* 10:265–275.

Downloaded at Palestinian Territory, occupied on December 31, 2021

www.manaraa.com

5. Park JE, et al. (2010) Polo-box domain: A versatile mediator of polo-like kinase function. *Cell Mol Life Sci* 67:1957–1970.
6. Song S, Grenfell TZ, Garfield S, Erikson RL, Lee KS (2000) Essential function of the polo box of Cdc5 in subcellular localization and induction of cytokinetic structures. *Mol Cell Biol* 20:286–298.
7. Seong YS, et al. (2002) A spindle checkpoint arrest and a cytokinesis failure by the dominant-negative polo-box domain of Plk1 in U-2 OS cells. *J Biol Chem* 277: 32282–32293.
8. Jang YJ, Lin CY, Ma S, Erikson RL (2002) Functional studies on the role of the C-terminal domain of mammalian polo-like kinase. *Proc Natl Acad Sci USA* 99: 1984–1989.
9. Elia AE, et al. (2003) The molecular basis for phosphodependent substrate targeting and regulation of Plks by the Polo-box domain. *Cell* 115:83–95.
10. Kannan N, Neuwald AF (2005) Did protein kinase regulatory mechanisms evolve through elaboration of a simple structural component? *J Mol Biol* 351:956–972.
11. Kornev AP, Taylor SS, Ten Eyck LF (2008) A helix scaffold for the assembly of active protein kinases. *Proc Natl Acad Sci USA* 105:14377–14382.
12. Schutz AR, Winey M (1998) New alleles of the yeast MPS1 gene reveal multiple requirements in spindle pole body duplication. *Mol Biol Cell* 9:759–774.
13. Biggins S, et al. (1999) The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. *Genes Dev* 13:532–544.
14. Simanis V, Nurse P (1986) The cell cycle control gene cdc2+ of fission yeast encodes a protein kinase potentially regulated by phosphorylation. *Cell* 45:261–268.
15. Hermosilla GH, Tapia JC, Allende JE (2005) Minimal CK2 activity required for yeast growth. *Mol Cell Biochem* 274:39–46.
16. Simizu S, Osada H (2000) Mutations in the Plk gene lead to instability of Plk protein in human tumour cell lines. *Nat Cell Biol* 2:852–854.
17. Mortensen EM, Haas W, Gygi M, Gygi SP, Kellogg DR (2005) Cdc28-dependent regulation of the Cdc5/Polo kinase. *Curr Biol* 15:2033–2037.
18. Gardner RG, Nelson ZW, Gottschling DE (2005) Degradation-mediated protein quality control in the nucleus. *Cell* 120:803–815.
19. Surana U, et al. (1993) Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast. *EMBO J* 12:1969–1978.
20. D'Amours D, Amon A (2004) At the interface between signaling and executing anaphase—Cdc14 and the FEAR network. *Genes Dev* 18:2581–2595.
21. Stegmeier F, Visintin R, 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.
22. Snead JL, et al. (2007) A coupled chemical-genetic and bioinformatic approach to Polo-like kinase pathway exploration. *Chem Biol* 14:1261–1272.
23. Lee KS, Erikson RL (1997) Plk is a functional homolog of Saccharomyces cerevisiae Cdc5, and elevated Plk activity induces multiple septation structures. *Mol Cell Biol* 17: 3408–3417.
24. Shirayama M, Zachariae W, Ciosk R, Nasmyth K (1998) The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in Saccharomyces cerevisiae. *EMBO J* 17:1336–1349.
25. García-Alvarez B, de Cárcer G, Ibañez S, Bragado-Nilsson E, Montoya G (2007) Molecular and structural basis of polo-like kinase 1 substrate recognition: Implications in centrosomal localization. *Proc Natl Acad Sci USA* 104:3107–3112.
26. Yoshida S, et al. (2006) Polo-like kinase Cdc5 controls the local activation of Rho1 to promote cytokinesis. *Science* 313:108–111.
27. Chen YC, Weinreich M (2010) Dbf4 regulates the Cdc5 Polo-like kinase through a distinct non-canonical binding interaction. *J Biol Chem* 285:41244–41254.
28. Knop M, Schiebel E (1997) Spc98p and Spc97p of the yeast gamma-tubulin complex mediate binding to the spindle pole body via their interaction with Spc110p. *EMBO J* 16:6985–6995.
29. Sezen B, Seedorf M, Schiebel E (2009) The SESA network links duplication of the yeast centrosome with the protein translation machinery. *Genes Dev* 23:1559–1570.
30. Storchová Z, et al. (2006) Genome-wide genetic analysis of polyploidy in yeast. *Nature* 443:541–547.
31. Park CJ, et al. (2004) Requirement for Bbp1p in the proper mitotic functions of Cdc5p in Saccharomyces cerevisiae. *Mol Biol Cell* 15:1711–1723.
32. Hu F, et al. (2001) Regulation of the Bub2/Bfa1 GAP complex by Cdc5 and cell cycle checkpoints. *Cell* 107:655–665.
33. Park CJ, et al. (2008) Requirement for the budding yeast polo kinase Cdc5 in proper microtubule growth and dynamics. *Eukaryot Cell* 7:444–453.
34. Park JE, et al. (2004) Novel functional dissection of the localization-specific roles of budding yeast polo kinase Cdc5p. *Mol Cell Biol* 24:9873–9886.
35. Hoepfner D, Schaerer F, Brachat A, Wach A, Philippsen P (2002) Reorientation of mispositioned spindles in short astral microtubule mutant spc72Delta is dependent on spindle pole body outer plaque and Kar3 motor protein. *Mol Biol Cell* 13:1366–1380.
36. Thomas JH, Botstein D (1986) A gene required for the separation of chromosomes on the spindle apparatus in yeast. *Cell* 44:65–76.
37. Chial HJ, Giddings TH, Jr., Siewert EA, Hoyt MA, Winey M (1999) Altered dosage of the Saccharomyces cerevisiae spindle pole body duplication gene, NDC1, leads to aneuploidy and polyploidy. *Proc Natl Acad Sci USA* 96:10200–10205.
38. Hoepfner D, Brachat A, Philippsen P (2000) Time-lapse video microscopy analysis reveals astral microtubule detachment in the yeast spindle pole mutant cnm67. *Mol Biol Cell* 11:1197–1211.
39. Glover DM (2005) Polo kinase and progression through M phase in Drosophila: A perspective from the spindle poles. *Oncogene* 24:230–237.
40. Sunkel CE, Glover DM (1988) polo, a mitotic mutant of Drosophila displaying abnormal spindle poles. *J Cell Sci* 89:25–38.
41. Oshimori N, Ohsugi M, Yamamoto T (2006) The Plk1 target Kizuna stabilizes mitotic centrosomes to ensure spindle bipolarity. *Nat Cell Biol* 8:1095–1101.
42. Straight AF, Sedat JW, Murray AW (1998) Time-lapse microscopy reveals unique roles for kinesins during anaphase in budding yeast. *J Cell Biol* 143:687–694.
43. Tytell JD, Sorger PK (2006) Analysis of kinesin motor function at budding yeast kinetochores. *J Cell Biol* 172:861–874.
44. St-Pierre J, et al. (2009) Polo kinase regulates mitotic chromosome condensation by hyperactivation of condensin DNA supercoiling activity. *Mol Cell* 34:416–426.
45. Maddox PS, Bloom KS, Salmon ED (2000) The polarity and dynamics of microtubule assembly in the budding yeast Saccharomyces cerevisiae. *Nat Cell Biol* 2:36–41.
46. Sullivan M, Lehane C, Uhlmann F (2001) Orchestrating anaphase and mitotic exit: Separase cleavage and localization of Slk19. *Nat Cell Biol* 3:771–777.
47. Michaelis C, Ciosk R, Nasmyth K (1997) Cohesins: Chromosomal proteins that prevent premature separation of sister chromatids. *Cell* 91:35–45.

Downloaded at Palestinian Territory, occupied on December 31, 2021

www.manaraa.com