

Mutation of the polo-box disrupts localization and mitotic functions of the mammalian polo kinase Plk

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ABSTRACT Members of the polo subfamily of protein kinases play pivotal roles in cell proliferation. In addition to the kinase domain, polo kinases have a strikingly conserved sequence in the noncatalytic domain, termed the polo-box. The function of the polo-box is currently undefined. The mammalian polo-like kinase Plk is a functional homologue of *Saccharomyces cerevisiae* Cdc5. Here, we show that Plk localizes at the spindle poles and cytokinetic neck filaments. Without impairing kinase activity, a conservative mutation in the polo-box disrupts the capacity of Plk to complement the defect associated with a *cdc5-1* temperature-sensitive mutation and to localize to these subcellular structures. Our data provide evidence that the polo-box plays a critical role in Plk function, likely by directing its subcellular localization.

The polo subfamily of protein kinases has been identified in various eukaryotic organisms and plays pivotal roles in cell division and proliferation. A feature of the polo subfamily members is the presence of a distinct region of homology in the C-terminal noncatalytic domain, termed the polo-box (1) (Fig. 1*A* and *B*). Members of this subfamily include mammalian Plk (1–5), Snk (6), Fnk/Prk (7, 8), *Xenopus laevis* Plx1 (9), *Drosophila melanogaster* polo (10), *Schizosaccharomyces pombe* Plo1 (11), and *Saccharomyces cerevisiae* Cdc5 (12).

The M phase of the cell cycle is a highly regulated process that requires a series of coordinated biochemical and cellular events to ensure the faithful partitioning of genetic and cytoplasmic components. It is apparent from genetic and biochemical analyses that many polo kinases regulate diverse cellular events at various stages of M phase (for reviews, see refs. 13 and 14), such as centrosome maturation (15) and bipolar spindle formation (10, 11, 15). Polo kinases also appear to regulate important biochemical steps in G₂/M phase, such as activation of Cdc2 through Cdc25C phosphatase (9), DNA damage checkpoint adaptation (16), and regulation of the anaphase-promoting complex (17, 18).

One additional function attributed to polo kinases is the induction of cytokinesis-associated septal structures (11, 19). In an otherwise wild-type yeast genetic background, ectopic expression of a mutationally activated form of Plk induces a class of cells with unusually elongated buds that develop multiple septal structures (19). In addition, cells depleted of Cdc5 protein fail to complete cytokinesis and have a dumbbell-shaped terminal morphology (12). In fission yeast, loss of *plo1*⁺ function leads to mitotic arrest, with failure of both formation of the F-actin ring and deposition of septal components, whereas overexpression of Plo1 induces multiple septa at any phase of the cell cycle (11), suggesting that Plo1 is both essential and sufficient for septum and actin ring formation. In animal cells, current models of cytokinesis emphasize the potential of either the centrosomes or the midzone to provide

a signal for cleavage furrow induction at the equatorial cortex (20–24). Studies (25, 26) have shown that Plk localizes at both the centrosomes and the midzone/midbody during the late stage of the mammalian cell cycle. Thus, the subcellular localization of Plk supports its proposed role in cytokinesis (27).

Although it is apparent that polo kinases play multiple roles during M-phase progression and cytokinesis, the role of the polo-box, a strikingly conserved amino acid sequence present in all the members of polo kinase subfamily, is not yet known. We reported that murine Plk is a functional homolog of *S. cerevisiae* Cdc5 (19). In this communication, we used Plk expression in yeast to provide evidence that the polo-box is required for the capacity of Plk to complement functionally the *cdc5-1* defect. The data reported here suggest that the polo-box serves to target the catalytic activity of Plk to the spindle poles and cytokinetic neck filaments.

MATERIALS AND METHODS

Strains, Growth Conditions, and Transformations. Yeast strains used in this study are 1788 [isogenic diploid of EG123, *MATa leu2-3, 112 ura3-52 trp1-1 his4 can1^r* (28) and KKY921–2B (*MATa cdc5-1 leu2 trp1 ura1*) (12)]. Yeast cells were cultured in yeast extract/peptone (1% yeast extract/2% Bacto-peptone) supplemented with 2% glucose, 2% raffinose (Sigma), or 2% galactose (J.T. Baker) as required. Synthetic minimal medium (29) supplemented with the appropriate nutrients was employed to select for plasmid maintenance. Yeast transformation was carried out by the lithium acetate method (30).

Generation of Plk Mutants and Enhanced Green Fluorescent Protein (EGFP)-Plk Fusion Constructs. Site-directed mutagenesis in murine *PLK* cDNA was carried out by using the Sculptor *in vitro* mutagenesis system (Amersham). A 1.4-kb *SacI* fragment isolated from each partially digested mutant clone was ligated into YCplac111-*GAL1*-HA-*PLK* digested with *SacI* (19). All of the mutant clones were sequenced to confirm the mutations introduced into the *PLK* coding sequence. YCplac111-*GAL1*-HA-*PLK* and its C-terminal deletion mutant were constructed as described (19). The C-terminal deletion mutant lacks amino acid residues 356 to 604. YCplac111-*GAL1*-EGFP-*PLK* fusion constructs were generated by inserting a 700-bp *XhoI* fragment of the EGFP coding sequence into the *XhoI* site present at the N-terminal *PLK* coding sequence. The EGFP coding sequence was amplified by PCR by using the pEGFP-N1 plasmid (CLONTECH) as a template.

Expression of Plk in *S. cerevisiae*. To express wild-type and mutant forms of Plk in *S. cerevisiae* under the control of the *GAL1* promoter, yeast transformants harboring each construct were grown in yeast extract/peptone/raffinose to an OD₆₀₀ of

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Abbreviation: EGFP, enhanced green fluorescent protein.

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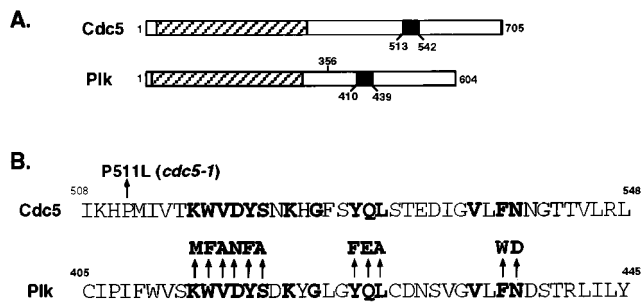


FIG. 1. (A) Structures of Cdc5 and Plk. A hatched box denotes the kinase domain, and a closed box denotes the polo-box. Plk lacking the C-terminal domain (Plk Δ C) has lost amino acid residues 356 to 604. (B) Identification of the *cdc5-1* mutation site and diagram showing the polo-box mutants generated in Plk. Yeast genomic DNAs prepared from the *cdc5-1* mutant (H5C1A1) and its parental wild-type strain (H4939-1b) (a gift of L. Hartwell, University of Washington) were used as templates to amplify full length *cdc5-1* and *CDC5* genes, respectively, by using the PCR. Restriction and complementation analyses confirmed the cloned genes (data not shown). DNA sequence analysis revealed a point mutation (conversion of Pro511 to Leu) in the *cdc5-1* allele. Introduction of the P511L mutation into the wild-type Cdc5 was sufficient to abolish its capacity to complement the *cdc5-1* defect (data not shown). Conserved amino acids among all of the polo family members known to date are in bold letters; arrows point to amino acids changed in the point mutations.

0.8 at 30°C. Cultures then were washed twice with water and were resuspended in yeast extract/peptone/galactose at an OD₆₀₀ of 0.05 and were cultured continuously. All the *cdc5-1* transformants were cultured at 23°C. To examine the complementation of the *cdc5-1* defect by Plk expression, cells were cultured at 37°C, however.

Flow Cytometry Analyses. Flow cytometry analyses were carried out as described (19). In brief, cells were harvested and were fixed with 70% ethanol, then were treated with RNase A (1 mg/ml) in PBS for 30 min at 37°C. After disrupting the cells for 1 min with a sonicator (model W-375, Heat Systems/Ultrasonics), cells were stained with propidium iodide (50 μ g/ml) in PBS. Flow cytometry analyses were performed with a CELLQUEST program (Becton Dickinson).

Immunoprecipitation, Kinase Assays, and Western Analyses. Yeast cells were lysed with an equal volume of glass beads (Sigma) as described (19). The obtained lysates were spun at 2,000 \times g for 2 min to remove unbroken cells and beads. The resulting supernatants were considered as total cellular lysates. For immunoprecipitation, total cellular lysates were subjected to further centrifugation at 15,000 \times g for 30 min to clarify heavy cellular materials. Before incubation with affinity-purified anti-Plk antibody, the resulting supernatants (S15) were diluted to 1 ml with TBSN buffer (20 mM Tris-Cl, pH 8.0/150 mM NaCl/0.5% Nonidet P-40/5 mM EGTA/1.5 mM EDTA/0.5 mM Na₃VO₄/20 mM *p*-nitrophenyl phosphate) supplemented with protease inhibitors. Protein A-Sepharose 4B (Zymed) was added and incubated for an additional 1 hr to precipitate the antibody.

Kinase assays and Western analyses were carried out as described (19). In brief, the kinase activity of Plk was measured in a kinase mixture (50 mM Tris-Cl, pH 7.5/10 mM MgCl₂/5 mM DTT/2 mM EGTA/0.5 mM Na₃VO₄/20 mM *p*-nitrophenyl phosphate) supplemented with 3 μ g of dephosphorylated casein (Sigma) and 25 μ M ATP (5 μ Ci of [γ -³²P]ATP; 1 Ci = 37 Gbq). Western analyses were carried out with affinity-purified Plk antibody or HA antibody at a concentration of 0.5 μ g/ml. Proteins that interact with antibodies were detected by the enhanced chemiluminescence (ECL) Western detection system (Amersham).

Cell Staining and Immunofluorescence Microscopy. Indirect immunofluorescence was performed as described (19).

Cdc10 was localized by using affinity-purified rabbit polyclonal anti-Cdc10 antibody (a gift of J. Chant, Harvard University, Cambridge, MA) and rhodamine-conjugated goat anti-rabbit IgG (Zymed). Microtubules were visualized by using YOL1/34 rat anti-tubulin antibody (Accurate Chemical and Scientific Corp., Westbury, NY) and goat anti-rat CY3 antibody (The Jackson Laboratory). DNA was visualized by using propidium iodide staining at 40 μ g/ml. Stained cells were viewed under a Zeiss LSM410 confocal microscope equipped with a Krypton/Argon laser.

RESULTS

Requirement of both the Polo-Box and the Kinase Activity of Plk for Functional Complementation of the *cdc5-1* Defect. It has been reported (19) that Plk is a functional homolog of *S. cerevisiae* Cdc5. At the restrictive temperature, cells with a *cdc5-1* mutation arrest late in mitosis as large budded cells (12, 16), with an accumulation of cells with 2N (G2/M) DNA content and a greatly diminished 1N (G1) population, as reflected in flow cytometry (Fig. 2A). We confirmed our previous observation that expression of wild-type Plk, but not the kinase-inactive PlkK82M, complements the cell division cycle defect of the *cdc5-1* mutants, regenerating the 1N DNA-containing population to a similar extent as CDC5 expression (19) (Fig. 2B–D).

To extend our previous analyses and investigate whether the polo-box is required for the ability of Plk to complement the *cdc5-1* defect, we created a Plk mutant lacking the C-terminal domain (Plk Δ C), which includes the polo-box (amino acids 410–439 in Plk) (Fig. 1A). Plk Δ C does not complement the *cdc5-1* defect (Fig. 2E), despite a 3- to 4-fold increase in specific kinase activity (19). These results are consistent with the abilities of these Plk constructs to restore the *cdc5-1* cell growth defect at the restrictive temperature when cultured on galactose-containing medium (data not shown) (19).

To examine specifically whether Plk requires its polo-box to complement the *cdc5-1* defect, we generated 11 single point mutations in the polo-box (Fig. 1B). The ability of these mutants to complement the *cdc5-1* defect was analyzed by flow cytometry. The most dramatic effect was seen with a W414F mutation, which essentially eliminated the capacity of Plk to complement the *cdc5-1* defect (Fig. 2F). Mutations at V415A, L427A, and N437D also significantly reduced the capacity of Plk to complement this defect (data not shown). These results indicate that the polo-box is critical for the ability of Plk to complement the *cdc5-1* defect. Consistent with the importance of the polo-box domain was our finding that the *cdc5-1* allele possesses a single point mutation at the upstream boundary of the polo-box (Fig. 1B) that may alter the structural integrity of this region.

Microscopic observation revealed that the majority of *cdc5-1* mutant cells at the restrictive temperature were enlarged greatly, with heterogeneous cell sizes and shapes (data not shown); in forward scatter, this phenotype results in a broad, spread out pattern (Fig. 2G). As with expression of Cdc5 (Fig. 2H), wild-type Plk expression, but not that of the kinase-inactive K82M mutant, restored the heterogeneously enlarged cell population to a uniform wild-type morphology (Fig. 2I and J). Neither W414F nor Plk Δ C expression was able to restore wild-type cell morphology (Fig. 2K and L), consistent with its inability either to rescue growth at the restrictive temperature (data not shown) or to relieve the *cdc5-1* cell cycle arrest. Taken together, the above data demonstrate the necessity of both the kinase activity and the polo-box for the ability of Plk to complement the *cdc5-1* defect.

The W414F Mutation Does Not Influence the Expression Level or the Kinase Activity of Plk. To eliminate the possibility that the inability of PlkW414F to complement the *cdc5-1* defect is caused by a decrease in stability or kinase activity of

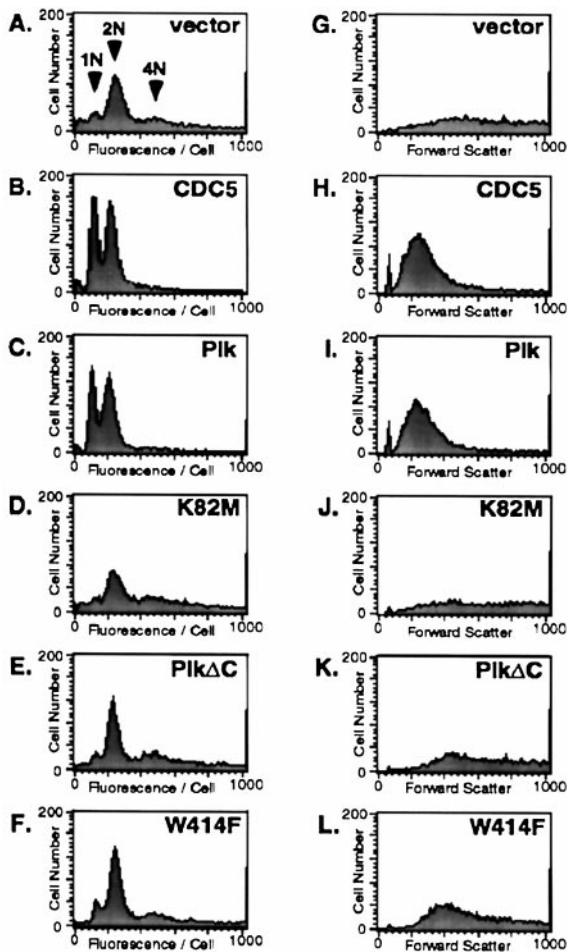


FIG. 2. Mutations in either the ATP-binding site or the polo-box abolish the capacity of Plk to complement the *cdc5-1* defect. A haploid *cdc5-1* mutant strain, KKY921-2B (*MATa cdc5-1 leu2 trp1 ura1*) (12) was transformed with various YCplac111-*GALI*-HA-PLK constructs or with YCplac111-*CDC5*. To examine the ability of Plk constructs to complement the *cdc5-1* defect, transformants were cultured at 37°C in yeast extract/peptone + 2% galactose medium for 10 hr as described (19) and were subjected to flow cytometry analyses. Vector, YCplac111-*GALI*; *CDC5*, YCplac111-*CDC5*; *Plk*, YCplac111-*GALI*-HA-*Plk*; *K82M*, YCplac111-*GALI*-HA-*PlkK82M*; *PlkΔC*, YCplac111-*GALI*-HA-*PlkΔC*; *W414F*, YCplac111-*GALI*-HA-*PlkW414F*. (A-F) A G1 population (1N arrow) and G2/M population (2N arrow) are indicated in the vector panel. Cells with a DNA content >2N are indicated by the 4N arrow (a broad cell population with the third 4N arrow). (G-L) The increase in forward scatter at the x axis reflects an increase in cell size. The broad, spread out pattern observed in the *cdc5-1* mutant transformed with vector (G) results from a heterogeneous population of enlarged cells whereas wild-type cells (data not shown) and the *cdc5-1* mutant complemented with *Cdc5* (H) or *Plk* (I) expression produce a distinct bell-shaped pattern of forward scatter.

W414F, we examined the expression level and the enzymatic activity of *PlkW414F*. Immune complex kinase assays revealed that both wild-type *Plk* and the *PlkW414F* mutant exhibit similar levels of specific activities, whereas *PlkK82M* does not possess a significant amount of activity (Fig. 3). In total cellular lysates, the expression levels of the wild-type and mutant forms of *Plk* were similar (data not shown). These results indicate that the inability of *PlkW414F* to complement the *cdc5-1* defect is likely caused by the disrupted function of the polo-box by the introduction of the *W414F* mutation.

The *W414F* Mutation Disrupts EGFP-*Plk* Localization at Spindle Poles and Septin Ring Structures. The inability of the *W414F* mutant to complement the *cdc5-1* defect led us to

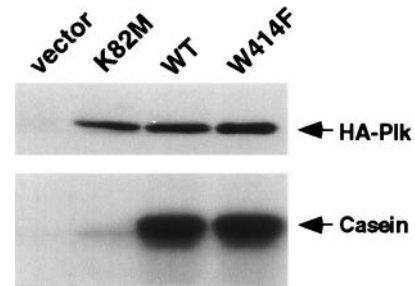


FIG. 3. Mutations in the polo-box do not impair *Plk* kinase activity *in vitro*. The *cdc5-1* (KKY921-2B) cells bearing various YCplac111-*GALI*-HA-*PLK* constructs were cultured under inducing conditions for 10 hr and were harvested. The lysates were centrifuged at $15,000 \times g$ for 30 min to clarify heavy cellular materials. From 500 μ g of cellular proteins present in the S15 fraction, the wild-type and mutant forms of HA-*Plk* were immunoprecipitated and subjected to *in vitro* kinase assays. (Upper) HA-tagged *Plk* was immunoprecipitated with affinity-purified anti-*Plk* antibody, was electrophoresed, and was detected on membranes by Western analysis with anti-HA antibody. (Lower) HA-tagged *Plk* was immunoprecipitated with anti-*Plk* antibody, and *in vitro* kinase assays were performed as described by using casein as a substrate (19). Vector, YCplac111-*GALI*; *K82M*, YCplac111-*GALI*-HA-*PlkK82M*; *WT*, YCplac111-*GALI*-HA-*Plk*; *W414F*, YCplac111-*GALI*-HA-*PlkW414F*.

speculate that the polo-box may serve either to target *Plk* to particular subcellular locations or to mediate the interaction of *Plk* with essential substrates or activators. We examined the cellular localization of *Plk* that is fused to an EGFP and expressed under the *GALI* promoter. At the restrictive temperature, EGFP-fused wild-type *Plk* (EGFP-*Plk*) and *PlkT210D* (EGFP-*PlkT210D*) complemented the *cdc5-1* defect to a similar degree as their parental unfused forms (data not shown). As expected, the introduction of the *W414F* mutation essentially eliminated the capacity of EGFP-*Plk* to complement the *cdc5-1* defect, and the C-terminal deletion abolished complementation completely (data not shown). These results show that the EGFP-fused *Plk* proteins behave in the same manner as their nonfusion counterparts.

Ectopic expression of both wild-type EGFP-*Plk* and EGFP-*PlkT210D* in yeast yielded two distinct fluorescent dots in the cytoplasm as well as either one or two bright bands at the bud neck in budded cells (Fig. 4). To investigate whether the signals correspond to spindle poles and septin ring structures, respectively, we carried out subsequent immunostaining with antibodies against either tubulin or *Cdc10*, one of four septin components (*Cdc3*, *Cdc10*, *Cdc11*, and *Cdc12*) of the cytokinetic neck filaments in budding yeast (for review, see ref. 31). Our data revealed that the two dots of staining represented the spindle poles whereas the bands corresponded to the cytokinesis-associated septin ring structures (see Fig. 5 *A* and *B*). In contrast, the localization of *Plk* to these subcellular structures was either greatly diminished or absent in cells expressing EGFP-*PlkW414F* or the EGFP-*PlkT210D*/*W414F* double mutant. A *Plk* construct lacking the C-terminal domain (EGFP-*PlkΔC*) was diffusely localized to the cell periphery and chromatin whereas a construct lacking both the kinase and the C-terminal domains (EGFP-*Plk-N*¹⁻⁴⁹) yielded a diffuse signal (Fig. 4). These data show that the polo-box directs *Plk* localization to the spindle poles and the bud-neck filaments and that tryptophan 414 appears to play a critical role in targeting *Plk* to these structures. These observations indicate that the inability of the *W414F* mutant to complement the *cdc5-1* defect is attributable to the loss of its specific cellular localization.

DISCUSSION

The role of the polo-box, a strikingly conserved amino acid sequence present in the C-terminal noncatalytic domain of

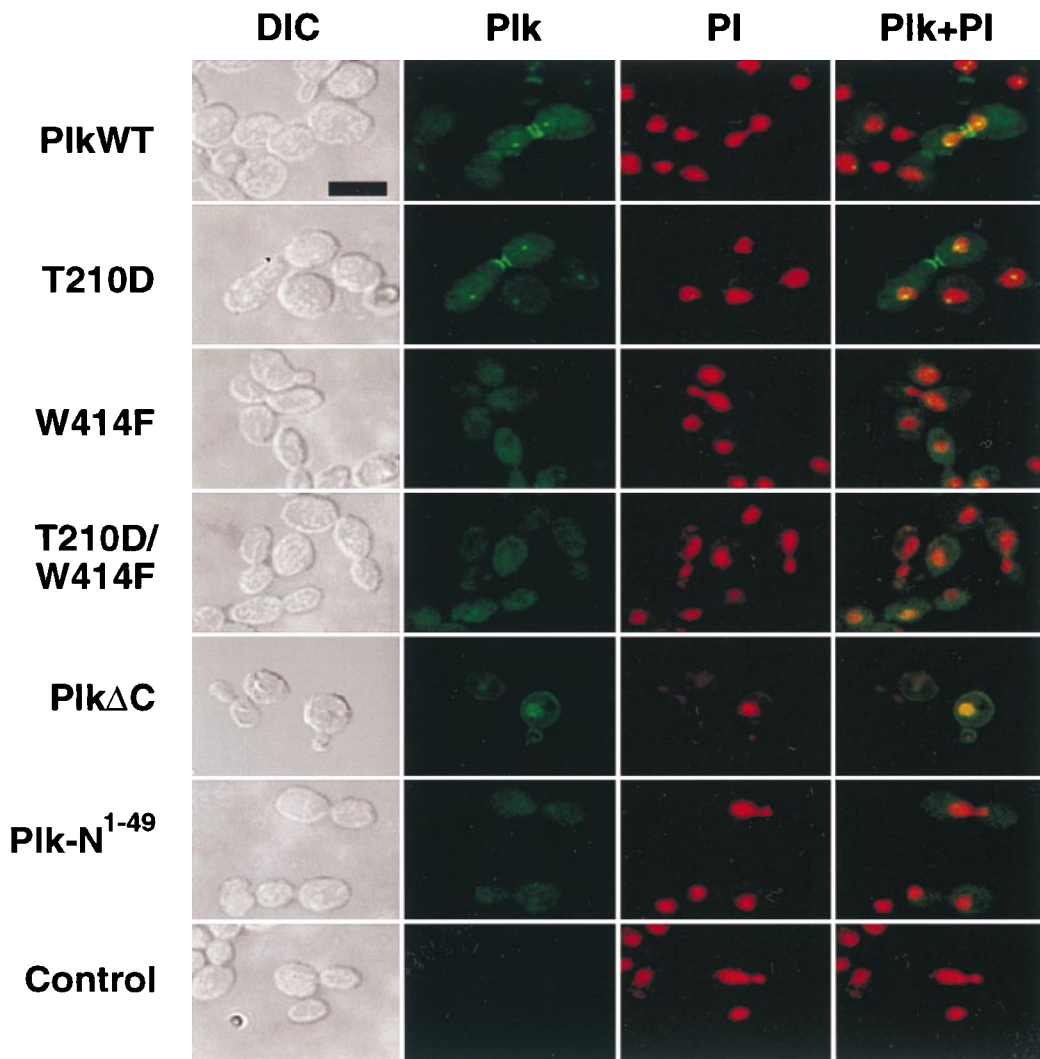


FIG. 4. The requirement of the polo-box for Plk localization. To localize wild-type and mutant forms of Plk in a diploid wild-type strain, 1788, EGFP-Plk fusion constructs were generated and expressed under the control of the *GALI* promoter. Transformants expressing EGFP fusion constructs were stained with propidium iodide to visualize chromosomal DNA and were examined by confocal microscopy. PlkWT, YCplac111-*GALI*-HA-EGFP-Plk; T210D, YCplac111-*GALI*-HA-EGFP-PlkT210D; W414F, YCplac111-*GALI*-HA-EGFP-PlkW414F; T210D/W414F, YCplac111-*GALI*-HA-EGFP-PlkT210D/W414F; Plk Δ C, YCplac111-*GALI*-HA-EGFP-Plk Δ C; Plk-N¹⁻⁴⁹, YCplac111-*GALI*-HA-EGFP-Plk-N¹⁻⁴⁹; control, an irrelevant plasmid without EGFP. Plk-N¹⁻⁴⁹ contains only the N-terminal 49-aa residues of Plk fused to EGFP and serves as a background EGFP signal. DIC, differential interference contrast; Plk, EGFP-Plk expression; PI, propidium iodide staining of nuclei; Plk + PI, EGFP-Plk and propidium iodide images superimposed. (Bar = 5 μ m.)

polo kinases, is not known. To shed light on the function of the polo-box, we used Plk expression in budding yeast. Either a conserved mutation in the polo-box, W414F, or a mutation inactivating the kinase activity of Plk, K82M, abolishes the capacity of Plk to functionally complement the *cdc5-1* defect (Fig. 2). These data indicate that both the polo-box and the kinase activity are required for the mitotic functions of Plk. The importance of the polo-box is further supported by our recent observation that the introduction of the W414F mutation also completely abolished the capacity of Plk to induce abnormally elongated buds with ectopic septal structures (K.S.L., T.Z.G., and R.L.E., unpublished data). We have demonstrated that the polo-box is required for the localization of Plk to spindle poles and cytokinetic neck filaments (Figs. 4 and 5). Thus, the polo-box appears to function as an interaction domain to target the catalytic activity of the enzyme to specific subcellular locations, thereby allowing efficient interaction with its physiological substrates or activators.

We show that a single conserved amino acid change of tryptophan to phenylalanine at position 414 in the polo-box disrupts Plk localization at both the spindle poles and cytoki-

netic structures to a similar degree (Fig. 4). This observation suggests that the polo-box domain of Plk specifically recognizes a single binding protein or perhaps different binding partners with similar binding motifs at these distinct subcellular locations. Functional complementation assays for the *cdc5-1* defect suggest that, among the examined amino acid residues conserved in the polo-box, W414 is the most critical amino acid residue for the function of Plk. However, additional amino acid residues (V415, L427, and N437) that are present in other conserved segments of the polo-box also appear to be important for Plk function (Fig. 2 and data not shown). Indeed, the critical interactions may extend well beyond the short module commonly referred to as the polo-box. In this regard, it should be noted that these data demonstrate that the polo-box is necessary for specific localization but do not demonstrate that it is sufficient. It is now of importance to determine the sequences that are sufficient to direct localization to the spindle poles and the bud neck.

Recent studies suggest that polo kinases have multiple roles during G2/M phase, such as activation of Cdc2 through Cdc25C phosphatase (9), maturation of mitotic centrosomes

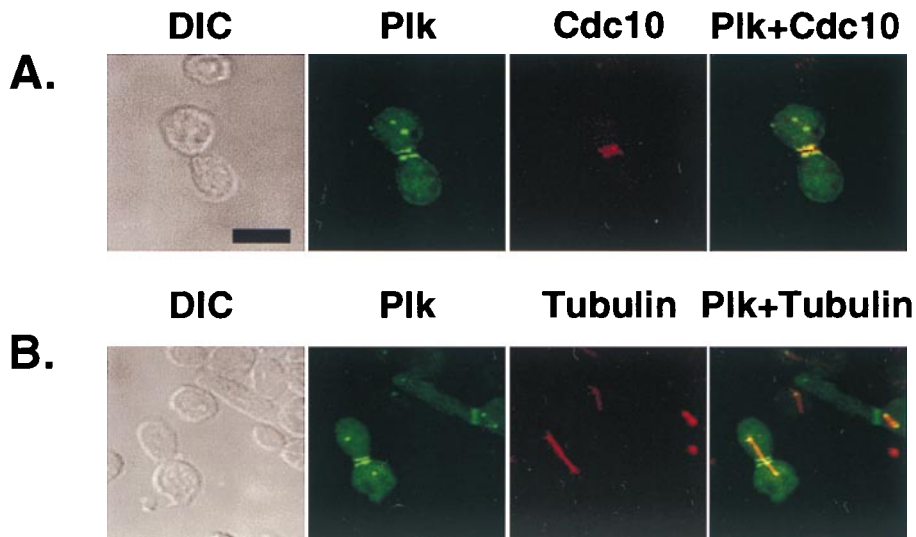


FIG. 5. Ectopically expressed Plk localizes at the spindle poles and bud neck filaments. EGFP-Plk fusion constructs were expressed under the control of the *GAL1* promoter in a diploid wild-type strain, 1788. To enhance the signals present at the spindle poles and the cytokinetic septal structures, two tandem EGFPs were inserted into the N terminus of the Plk coding sequence. Transformants were cultured for subsequent immunostainings to examine Cdc10 and tubulin localizations. (A) Plk (green) and Cdc10 (red) localize at the neck filaments. Septin rings (red) are viewed edge on and therefore appear as lines. (B) Plk (green) localizes at the spindle poles. Spindles are visualized by microtubule staining (red). The spindles appear to emanate from the structures with which Plk associates. DIC, differential interference contrast; Plk, EGFP-Plk expression; Cdc10, Cdc10 staining; Tubulin, tubulin staining. Superimposed images are shown as Plk + Cdc10 and Plk + Tubulin. (Bar = 5 μm .)

(15), DNA damage checkpoint adaptation (16), and regulation of the anaphase-promoting complex (17, 18, 32). It is not known whether all of the mitotic functions of Plk occur at the spindle poles and cytokinetic structures, therefore requiring Plk localization to these sites. Alternatively, it is possible that the polo-box interacts with other subcellular structures as well, and the strong EGFP-Plk signals at the spindle poles and cytokinetic neck filaments may simply reflect the enriched concentration of certain binding partners at these sites. Therefore, it will be interesting to investigate whether the polo-box is required for all of the proposed mitotic functions of polo kinases or for a subset of these events.

We have reported here that, in budding yeast, ectopically expressed EGFP-Plk localizes at the spindle poles and at the cytokinetic neck filaments. Consistent with our data, recent indirect immunofluorescence studies revealed that endogenous Cdc5 localizes at spindle poles (17). However, these studies did not detect Cdc5 at the bud neck filaments. Detection of Plk localization at the neck filaments could be attributable to the two different detection methods employed or to an additional property that Plk may have. Further experiments will be required to resolve this issue.

In mammalian cells, Plk localizes at the centrosomes and the midzone/midbody (2, 25), both of which have been implicated in directing the site of cytokinesis (20–24). Many eukaryotic cells rely on a mechanism in which the mitotic apparatus dictates the position of the cleavage plane during anaphase/telophase whereas in budding yeast, a future cleavage plane of septin ring structures is already present in late G1 in the absence of identifiable spindle structures. Although the morphological features and timing of certain events of cytokinesis in budding yeast and mammalian cells are strikingly different, it is likely that the molecular processes used for inducing cytokinesis have remained largely unchanged throughout evolution. Thus, the differences observed in Plk localization at the midzone/midbody as opposed to the cytokinetic neck filaments may reflect the difference in the temporal and spatial arrangement of mitotic events by which cytokinetic sites are specified in these evolutionarily distinct eukaryotic cells. In both systems, however, Plk localizes to the structures that are important for cytokinesis.

In this communication, we have demonstrated that complementation of the *cdc5-1* defect in *S. cerevisiae* by mammalian Plk requires both the protein kinase activity and the polo-box domain and that the polo-box plays a crucial role to target the catalytic activity of Plk to the spindle poles and cytokinetic neck filaments. Further experiments are necessary to identify polo-box-interacting proteins and additional Plk substrates and to determine their roles in regulating cell division and cytokinesis.

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