Polo-Like Kinase Is Expressed in S/G2/M Phase and Associated with the Flagellum Attachment Zone in both Procyclic and Bloodstream Forms of *Trypanosoma brucei*\(^7\)

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Received 30 April 2008/Accepted 4 July 2008

*Trypanosoma brucei*, the etiologic agent of African sleeping sickness, divides into insect (procyclic) and bloodstream forms. These two forms are subject to distinct cell cycle regulations, with cytokinesis controlled primarily by basal body/kinetoplast segregation in the procyclic form but by mitosis in the bloodstream form. Polo-like kinases (PLKs), known to play essential roles in regulating both mitosis and cytokinesis among eukaryotes, have a homologue in *T. brucei*, TbPLK, which regulates only cytokinesis. In our previous study, overexpressed triply hemagglutinin-tagged TbPLK (TbPLK-3HA) in the procyclic form localized to a mid-dorsal point and the anterior tip of the cell along the flagellum attachment zone (FAZ). In our current study, TbPLK-3HA expressed at the endogenous level was identified at the same dorsal location of both procyclic and bloodstream forms, albeit it was no longer detectable at the anterior tip of the cell. Endogenously expressed TbPLK fused with an enhanced yellow fluorescent protein (EYFP) localized to the same dorsal location along the FAZs in living procyclic and bloodstream cells. Fluorescence-activated cell sorter analysis of hydroxyurea-synchronized procyclic cells revealed that TbPLK-EYFP emerges during S phase, persists through G2/M phase, and vanishes in G1 phase. An indicated TbPLK-EYFP association with the FAZs of G2/M cells may thus represent a timely localization to a potential initiation site of cytokinesis, which agrees with the recognized role of TbPLK in cytokinetic initiation.

*Trypanosoma brucei* is a unicellular protozoan parasite that causes African sleeping sickness in humans and nagana in cattle. Its life cycle can be roughly divided into metacyclic and procyclic forms in tsetse flies and long slender and short stumpy bloodstream forms in mammalian hosts. Only the procyclic form and the long slender bloodstream form can be cultured in vitro, and their mechanisms of cell division during these two stages of development have thus been extensively examined (28). Trypanosomes divide by a binary fission from the anterior to the posterior end. Prior to this, the nucleus, the single mitochondrion, the mitochondrial DNA complex known as the kinetoplast, the basal body, and the flagellum are each duplicated and segregated into two daughter cells (42). The nuclear cycle has the usual G1, S, and G2/M phases (53), which must be coordinated with the kinetoplast cycle for a fruitful cytokinetic initiation (34). Distinctive cell cycle regulations have been observed for the two forms of *T. brucei* (29). While cytokinesis in the procyclic form is triggered primarily by the segregation of two kinetoplast/basal body pairs (37), it appears under the strict control of mitosis in the bloodstream form (15, 48). This change of command during two stages of development of the same species of organism may constitute one of the most intriguing aspects of trypanosome biology. It will be interesting to find out how proteins known to control both mitosis and cytokinesis in eukaryotes would function in trypanosomes.

\(^7\) Published ahead of print on 11 July 2008.
its localization by immunofluorescence microscopy, we observed that TbPLK was not localized to the nucleus at all, as in other eukaryotes (24). Instead, TbPLK was identified at a midpoint on the dorsal side of the cell and the anterior tip of the cell. In the cytoskeleton preparation, the subcellular localization of TbPLK coincided with the dorsal-side flagellum attachment zone (FAZ) (24). In a subsequent report, however, the immunofluorescence assay indicated the presence of overexpressed TbPLK in the cytoplasm (16). More recently, however, a third research group used affinity-purified antibodies raised against TbPLK and was able to confirm our results by finding TbPLK at the growing tip of the new FAZ (8). These conflicting claims prompted us to reexamine the localization of TbPLK and repeat our previous observation. We then expressed the HA-tagged TbPLK at endogenous levels in both the procyclic and bloodstream forms of trypanosome and found it localized to the same midpoint of the dorsal side of the cell. When TbPLK was fused with an enhanced yellow fluorescent protein (EYFP) and also expressed at the endogenous levels in both forms of trypanosome, it was also found to be localized at the midpoint of the dorsal side of the cells corresponding to the FAZ.

A difficulty in the localization study was that only about 20% of the cells appeared to express TbPLK, suggesting that the protein is expressed only during a certain phase of the cell cycle. In procyclic cells of T. brucei synchronized by hydroxyurea (6), TbPLK was found to appear in late S phase and persist in G2/M phase but vanish in G1 phase, which agrees with the postulated function of TbPLK in controlling cytokinesis in trypanosomes.

**MATERIALS AND METHODS**

**Cell culture.** Procyclic-form cells of strains 427 and 29-13 (52) were grown at 26°C in Cunningham’s medium supplemented with 10% fetal bovine serum (HyClone, UT). Bloodstream-form cells of strains 221 and 90-13 (52) were grown at 37°C with 5% CO2 supplied in HMI-9 medium containing 10% fetal bovine serum (HyClone, UT) and 10% serum plus (SAFC Bioscience Inc., KS). To maintain the T7 RNA polymerase and tetracycline repressor gene constructs within the cells, 15 μg/ml G418 and 50 μg/ml hygromycin B were added to the Cunningham’s medium for the 29-13 cell line, whereas 2.5 μg/ml G418 and 5 μg/ml hygromycin B were added to the HMI-9 medium for the 90-13 cell line. Cells were routinely diluted with fresh medium when their density reached 5 × 106 cells/ml.

**Plasmids and cell lines.** For the overexpression of triplet HA-tagged TbPLK (TbPLK-3HA) in the procyclic form, the 29-13 cell line harboring pLeu100-PLK-3HA was cultured in the presence of 2.5 μg/ml phenocyclin (24). For endogenous tagging with three tandem repeats of HA, a 600-bp fragment from the C-terminal portion of TbPLK was amplified by PCR and cloned into the KpnI-BamHI sites of plasmid pCMV-MCS (39; with three tandem repeats of HA). The resulting plasmid, pNeo-PLK-3HA, was linearized by EcoRI digestion and electroporated into procyclic-form cell line 29-13 and bloodstream-form cell line 90-13 cultured in the presence of 10 μg/ml blasticidin.

**Western blotting.** Cells were washed twice in phosphate-buffered saline (PBS), boiled for 3 min in sodium dodecyl sulfate-polyacrylamide gel electrophoresis sample buffer (Bio-Rad, CA), and centrifuged at 20,000 × g for 10 min. Total protein from the supernatant was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto immunoblot polyvinylidene difluoride membranes (Bio-Rad, CA). These membranes were blocked for 1 h in TBST (20 mM Tris-HCl [pH 7.4], 150 mM NaCl, 0.1% Tween 20) containing 5% skim milk and then incubated for 1 h with anti-HA mouse monoclonal antibody (MAb) conjugated with horseradish peroxidase (HRP; Sigma, MO) at a dilution ratio of 1:10,000 for detecting 3HA-tagged protein. EYFP-fused protein was detected using anti-green fluorescent protein (GFP) MAb (1:5,000) at a dilution ratio of 1:5,000 (Clontech Laboratory Inc.). The signal strength was quantified using ImageJ software (http://rsb.info.nih.gov/ij/).

**Immunofluorescence staining**. Cells were washed twice in PBS and fixed in 4% paraformaldehyde for 5 min. After being washed, the fixed cells were loaded on poly-l-lysine-coated slides for 20 min, treated with cold methanol for 5 min, and rehydrated in PBS for 5 min. The protocol for preparing cytoskeleton from procyclic-form T. brucei was followed for the cytoskeleton from the bloodstream-form (24), except that live bloodstream-form cells were fixed in poly-l-lysine-coated coverslips for 20 min and then washed three times in PBS containing 1% Triton X-100. Antibodies were added, the mixture was incubated for an hour, and cells were washed three times in PBS containing 0.1% Triton X-100. Corresponding secondary antibodies conjugated with fluorescent dyes were then added for another hour. Antibodies used in the study included rabbit anti-α-tubulin (Sigma, MO), mouse anti-PTP (39) with three tandem repeats of HA conjugated with AlexaFluor 488 (A21202; Invitrogen, CA) and anti-mouse IgG conjugated with Texas Red (D62; Invitrogen, CA). Following incubation with the secondary antibodies, cells were washed three times in PBS containing 0.1% Triton X-100. AlexaFluor 488- and Texas Red-labeled secondary antibodies were added, and cells were washed twice in PBS containing 0.1% Triton X-100. Corresponding secondary antibodies conjugated with fluorescent dyes were then added for another hour. Antibodies used in the study included rabbit anti-α-tubulin (Sigma, MO) and mouse anti-PTP (39) with three tandem repeats of HA conjugated with AlexaFluor 488 (A21202; Invitrogen, CA) and anti-mouse IgG conjugated with Texas Red (D62; Invitrogen, CA). Following incubation with the secondary antibodies, cells were washed three times in PBS containing 0.1% Triton X-100. AlexaFluor 488- and Texas Red-labeled secondary antibodies were added, and cells were washed twice in PBS containing 0.1% Triton X-100. AlexaFluor 488- and Texas Red-labeled secondary antibodies were added, and cells were washed twice in PBS containing 0.1% Triton X-100. AlexaFluor 488- and Texas Red-labeled secondary antibodies were added, and cells were washed twice in PBS containing 0.1% Triton X-100. AlexaFluor 488- and Texas Red-labeled secondary antibodies were added, and cells were washed twice in PBS containing 0.1% Triton X-100. AlexaFluor 488- and Texas Red-labeled secondary antibodies were added, and cells were washed twice in PBS containing 0.1% Triton X-100.

**Immunofluorescence microscopy**. Immunofluorescence was performed using a Zeiss Axioscop microscope equipped with a Photometrics Cool Snap HQ charge-coupled-device camera and processed by use of the software MetaVue version 5.0 and ImageJ.

**FACS analysis**. Cell samples for fluorescence-activated cell sorter (FACS) analysis were prepared as described previously (30) with minor modifications. Briefly, T. brucei cells were collected, centrifuged at 2,000 × g for 10 min, and washed twice in PBS. Cell pellets were gently suspended in 100 μl of PBS and mixed with 200 μl of 10% ethanol and 5% glycerol in PBS. They were then mixed with another 200 μl of 50% ethanol and 5% glycerol in PBS prior to incubation on ice for 5 min. One milliliter of 70% ethanol and 5% glycerol in PBS was added. The cells were then suspended in PBS. DNA-free RNase (Roche Diagnostics, IN) and propidium iodide (PI) were added to the suspension to final concentrations of 10 and 20 μg/ml, respectively, and incubated for 30 min at room temperature before the FACS analysis. The DNA content (FL2 channel) and EYFP signal strength (FL1 channel) of PI-stained cells were analyzed with a FACScan analytical flow cytometer using CellQuest software (BD Biosciences, CA).

**RESULTS**

Overexpressed TbPLK-3HA localizes to the midpoint of the dorsal side and anterior tip of procyclic cells. An anti-HA antibody staining of the paraformaldehyde-fixed procyclic cells overexpressing TbPLK-3HA indicated two areas of focal presence of the protein at the anterior tip of the cell and at the
midportion on the dorsal side of the cell (Fig. 1A). These results are in agreement with those published previously (24). This localization was also observed for the cells cultured for only 1 day after tetracycline induction instead of 5 days (data not shown). Similar results were obtained from TbPLK tagged with 3HA at either the C terminus (24) or the N terminus, indicating that the tagging position does not affect the localization of the protein (data not shown). The subcellular localization of overexpressed TbPLK-3HA in the procyclic cytoskeleton was also examined using the cells cultured for 5 days after the induction of overexpression, as done in the previous study (24). Mouse L3B2 MAb, which recognizes the FAZ (23) in the cytoskeleton, and anti-HA conjugated with TRITC were used to stain the cytoskeleton. The results (Fig. 1B) indicate that spots of TbPLK-3HA were associated with the FAZ. Although they are not present along the entire length of the FAZ as observed previously, they are always present at the posterior ends of both the old and the new FAZ, which is the originating point of the FAZ (42), and at the anterior end of the new FAZ. For some cells, localization at the anterior end of the old FAZ was also observed. Repeated examinations suggested that the positions of the anterior ends of the new and the old FAZ may correspond to the stained dorsal midpoint and the anterior tip, respectively, of the cells shown in Fig. 1A. Thus, a tentative conclusion one may derive from the data in Fig. 1 is that TbPLK could be associated with both ends of the FAZ when overexpressed in the procyclic form.

TbPLK-3HA expressed at the endogenous level also localizes to the dorsal midpoints of both procyclic and bloodstream cells. There is always the concern of whether an overexpressed protein can localize to the same place as that protein when it is expressed at the endogenous level. To cope with this uncertainty, the level of overexpressed TbPLK-3HA in the procyclic cells studied as shown in Fig. 1 was analyzed by Western blotting using the anti-HA MAb and given a relative quantity of 100% (Fig. 2A). Linearized DNA encoding TbPLK-3HA,
which was integrated into the chromosome through homologous recombination, was expressed in the procyclic and bloodstream forms of trypanosome in the in vitro cultures. The levels of these endogenously expressed proteins in the procyclic and bloodstream forms were also monitored by Western blotting. They turned out to correspond to 22% and 37% of the overexpressed protein, regardless of what tag was employed. In repeated trials, only about 20% of the procyclic form and 34% of the bloodstream form were found expressing TbPLK tagged with either 3HA or EYFP. These low percentages of cells expressing TbPLK at a given time led us to suspect that TbPLK could be specifically expressed only during a certain phase of the cell cycle in trypanosomes. Trypanosomes are known to have a very specific recombination, was expressed in the procyclic and bloodstream forms. To verify that the localization of TbPLK-3HA was not attributed to unknown artifacts from the procedure of immunostaining, TbPLK was tagged with EYFP at its C terminus and expressed at the endogenous levels in the two forms of the trypanosome. Among the transfected cells, PcF-PLK-EYFP could be seen in the form of bright spots numbering from one to three and distributed in the midportion along the dorsal side (Fig. 3A). When the cells were converted to cytoskeletal preparations and stained with L3B2 antibody against the FAZ, the few fluorescent spots of TbPLK-EYFP were clearly associated with the FAZ, with one of the spots invariably localized to the anterior end of the FAZ, whereas the other was always at the posterior end (Fig. 3B). These observations, equally applicable to both procyclic and bloodstream forms of trypanosome (Fig. 3), suggest that TbPLK is associated with the FAZ in both forms of trypanosome. The fact that more discrete spots of TbPLK-EYFP than of TbPLK-3HA could be observed along the FAZ could reflect the higher intensity of EYFP fluorescence.

**TbPLK emerges in S phase, reaches the highest level in G2/M phase, and vanishes in G1 phase.** One of the difficulties we encountered in trying to localize TbPLK in trypanosome cells was that not all the cells were found to express the protein, regardless of what tag was employed. In repeated trials, only about 20% of the procyclic form and 34% of the bloodstream form were found expressing TbPLK tagged with either 3HA or EYFP. These low percentages of cells expressing TbPLK at a given time led us to suspect that TbPLK could be specifically expressed only during a certain phase of the cell cycle in trypanosomes. Trypanosomes are known to have a very long G1 phase (34). We thus anticipated that TbPLK could be absent during that phase but emerge in the S or G2/M phase of the cell cycle.

**Procyclic and bloodstream form cells expressing TbPLK-**
EYFP at endogenous levels were each analyzed for DNA content by flow cytometry in the FL2 channel while using the FL1 channel to sort the cells according to the signal strength of EYFP fluorescence (Fig. 4A and C). A significant shift of the EYFP fluorescence peak was detected between the cells having no TbPLK-EYFP expression (peak a) and the cells having TbPLK-EYFP expression (peak b), and the shift was found in both forms of trypanosome. A small population of the cells

FIG. 3. Endogenous TbPLK-EYFP localization in both procyclic and bloodstream forms. (A) Procyclic-form (PCF) 29-13 cells or bloodstream-form (BSF) 90-13 cells harboring pcBla-PLK-EYFP were fixed with 4% paraformaldehyde and stained with DAPI for DNA (blue). TbPLK-EYFP was visualized in green. (B) The cytoskeleton from the same cell culture was stained with L3B2 antibody for the FAZ (red), and with DAPI for DNA (blue). TbPLK-EYFP was visualized in green. Open and closed arrowheads indicate kinetoplast and TbPLK-EYFP signals, respectively. Bars, 5 μm.
FACScan analysis as previously described. The cells were
samples were collected once every 2 hours and subjected to
culturing was resumed in the absence of hydroxyurea. Cell
night in the presence of 0.2 mM hydroxyurea and washed, and
TbPLK-EYFP at the endogenous level were cultured over-
cells with hydroxyurea (6). The procyclic cells expressing
developed by Chowdhury et al. to synchronize the procyclic
within the gate is indicated at the top right of each histogram.
analyzed, the number (along with the percentage) of events that fell
within the gate are indicated by gray color. Of 500,000 events that were
exclude most of the autofluorescent cells from the group of cells having
channel) from total cells are indicated by solid lines. To determine the
showing the EYFP signal was then sorted for DNA contents by
flow cytometry. The results indicate that for both forms of
trypanosome, the G1-phase cells showed no sign of fluores-
cence, whereas the S-phase cells and G2/M-phase cells showed
clear fluorescence signals (Fig. 4B and D).
To further clarify that TbPLK is expressed only in the S and
G2/M phases of the trypanosome cell cycle, we utilized a method recently
developed by Chowdhury et al. to synchronize the procyclic
cells with hydroxyurea (6). The procyclic cells expressing
TbPLK-EYFP at the endogenous level were cultured overnight
in the presence of 0.2 mM hydroxyurea and washed, and
the culturing was resumed in the absence of hydroxyurea. Cell
samples were collected once every 2 hours and subjected to
FACScan analysis as previously described. The cells were
found synchronized in the late S phase when hydroxyurea was
first removed at 0 h (Fig. 5A). After 2 h, most of the cells were
shifted to the G2/M phase in a neatly synchronized manner. From 4 to 6 h, there was a steady shift of cells from the G2/M
phase to the G1 phase, until most of the cells ended up in the
G1 phase (Fig. 5A, left). The EYFP fluorescence detected by
the FL1 channel had the highest intensity from 0 to 2 h after
the release from hydroxyurea but diminished steadily from 2 to
6 h (Fig. 5A, right). The cells were then gated and sorted
described for Fig. 4 (Fig. 5A, left). The EYFP fluorescence was detected in the S-phase cells at 0 h and in the G2/M-phase cells from 2 to 4 h but disappeared from the G1 cells that began to appear after 4 h (Fig. 5A, left). The data thus provided a clear indication that TbPLK
is present in abundance during the S and G2/M phases but
vanishes in the G1 phase.
To further confirm this conclusion, lysates of the cells released
from hydroxyurea treatment for 0, 2, 4, and 6 h were examined by Western blotting using the anti-GFP antibody for
quantification of TbPLK-EYFP. The expression level of the
protein was given a relative amount of 1 for the 0-h sample
(Fig. 5B). It increased to 1.22 after 2 h but decreased to 0.76
and 0.59 after 4 and 6 h, respectively (Fig. 5B). The Western
data thus agree well with those from the FACScan analysis.
Cells released from hydroxyurea treatment for different
lengths of time were also examined with a fluorescence micro-
scope for the presence of fluorescent spots in the dorsal mid-
portions of the cells. The results from examining ~120 cells in
each sample indicated the presence of fluorescent dorsal spots
among 45%, 52%, 15%, and 2% of the 0-h, 2-h, 4-h, and 6-h
cell samples, respectively (Fig. 5B). These data once again
agree with those from the FACScan and Western blot analyses.
Two microscopic photos from the 2-h and 6-h samples are
presented in Fig. 5C to demonstrate the difference in the
numbers of TbPLK-EYFP-expressing cells. There is little
doubt that TbPLK localizes along the FAZ and is expressed in
S and G2/M phases but vanishes in the G1 phase of the try-
panosome in both forms.

**DISCUSSION**

In the current investigation, we employed different experi-
mental approaches to demonstrate the discrete presence of
TbPLK at the midportion of the dorsal line in both procyclic
and bloodstream forms of trypanosomes. The close association
of TbPLK with the FAZ in the cytoskeleton preparations fur-
ther suggests that the protein could be a part of the FAZ. A
selective expression of TbPLK during only the S and G2/M
phases of the trypanosome cell cycle was also clearly presented
in the current study. All these unique features of TbPLK differ
significantly from what is known about PLKs in other eu-
karyotes.

The PLK of *S. cerevisiae*, Cdc5, is known to localize to the
nucleus, the spindle pole body, and the bud neck in accordance
with the progression of the cell cycle from S to G2/M phase and
eventually to cytokinesis (43, 45). Similarly, in mammalian
cells, the Plk1 level is known to peak in late G2 and early M
phase, accompanied by dramatic changes in subcellular local-
ization from the cytoplasm and the nucleus during G2 phase to
the centrosomes and kinetochores during early mitosis. A frac-
tion of Plk1 then translocalizes to the spindle midzone/midbody late in mitosis, where the contractile cleavage furrow will eventually be fused (11). Thus, in the yeast, which has a closed mitosis like that of trypanosomes, and in mammalian cells, with an open mitosis, extensive translocalizations of the PLKs are apparently necessary during the cell cycle (3, 9, 25, 49), a fact which could be attributed to the multiple essential functions of PLK in regulating the progression of both mitosis and cytokinesis. The single localization of TbPLK to the dorsal spots of cells thus distinguishes it from all the other known PLKs, which could be due to the sole function of TbPLK in regulating only the cytokinesis in trypanosomes (16, 24). This limited function of TbPLK could be due to the fact that none of the Cdc5 substrates that appear to play a role in controlling the mitosis of yeast (33, 44) or the proteins involving PLKs in mitosis in metazoans (12, 20, 35, 36, 41, 46) can find a structural homologue in the *T. brucei* genome database (4).

An Aurora B homologue (TbAUK1) in the trypanosome plays important roles in controlling both mitosis and cytokinesis (27, 47). The chromosomal passenger complex (CPC) in the trypanosome, composed of TbAUK1 and two other novel proteins, TbCPC1 and TbCPC2 (26), has a typical pattern of CPC translocalization during mitosis but moves all the way to a specific midpoint on the dorsal line of the cell, where the anterior tip of the daughter cell is tethered to the FAZ, where cytokinesis is apparently initiated (22, 26, 38). It has not yet been experimentally demonstrated whether the position of CPC ever overlaps with that of TbPLK during the cell cycle.

FIG. 5. Hydroxyurea (HU) synchronization of procyclic-form cells expressing endogenous TbPLK-EYFP. Procyclic-form 29-13 cells with pcBla-PLK-EYFP were treated with 0.2 mM HU overnight, washed twice with growth medium, and collected at the times indicated (0, 2, 4, and 6 h). The collected cells were fixed with ethanol, stained with propidium iodide, and analyzed by flow cytometry. (A) Histograms on the left indicate results from cell sorting for DNA contents (FL2 channel [solid line]). Cells showing fluorescence from TbPLK-EYFP (gray area) were processed in a way similar to that described for Fig. 4. Histograms on the right show results from cell sorting by TbPLK-EYFP fluorescence (FL1 channel). Dashed lines a and b indicate the peak locations in the histograms from the cells collected at 6 h and 2 h after release from HU treatment, respectively. (B) Western blot showing the changing TbPLK-EYFP levels after release from HU treatment. Total proteins from 10^6 cells stained with anti-GFP antibody (top) or Coomassie brilliant blue G-250 as a loading control (bottom) were applied to the Western analysis. The relative amount of TbPLK-EYFP, determined using ImageJ software, is indicated under each lane. The population of cells expressing PLK-EYFP on the dorsal side (n > 120) is also indicated under each lane. (C) Merged phase-contrast (gray) and PLK-EYFP fluorescence (white, arrowheads) images from the cells at 2 h (predominantly G2/M cells) and 6 h (predominantly G1 cells) after their release from HU treatment. Bars, 10 μm.
However, if the positioning of CPC is aimed at separating the two closely parallel FAZs, an interaction between CPC and TbPLK would be anticipated. Since TbPLK is expressed in the late S phase and located at the FAZ along the dorsal line of the cell, it is not unlikely that TbPLK may be expressed in this specific position at an earlier time to recruit the CPC, which moves to the mid-dorsal position late in telophase (26). An examination of the proteins complexed with TbPLK at the dorsal location would be thus a logical step in the next phase of this study.

Recently, a RNAi knockdown of FAZ1, a component of the FAZ structure, resulted in a compromised flagellar attachment and generation of 0N1K cells (zoids) and multinucleated cells, suggesting that destabilization of the FAZ structure causes defective cytokinesis (50). The tip of the daughter FAZ tethering to the existing FAZ could be the site where the initiation of cytokinesis occurs (22, 38). The TbPLK localization close to that tethering point suggests a role for TbPLK in initiating cytokinesis, as suggested by the outcome from its RNAi knockdown (16, 24). It is, however, unclear whether the presence or the timed absence of TbPLK from this location would trigger cytokinesis. In *S. cerevisiae*, the activation of a ubiquitin ligase known as the anaphase-promoting complex triggers the degradation of PLK Cdc5 (43) to repress the Cdc14 early anaphase network and to initiate the mitotic exit network (51).

**REFERENCES**


