Changing Roles of Aurora-B Kinase in Two Life Cycle Stages of *Trypanosoma brucei*†

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*Trypanosoma brucei* is an ancient unicellular eukaryote whose propagation involves a cyclic transmission between the mammalian host and the insect vector tsetse fly. A trypanosome cell contains a single flagellum, basal body, nucleus, mitochondrion, and the mitochondrial DNA complex known as the kinetoplast. Each of these organelles/structures is replicated and segregated into the two daughter cells in a well-synchronized manner during cell division, implying a delicate coordination among these events (25). Other than the usual sequential G1, S, G2, and M phases, there is also an S phase for the kinetoplast completed prior to the nuclear S phase (45). The kinetoplast is replicated prior to mitosis and closely associated with the flagellum basal body, with its segregation dependent on the separation of replicated basal bodies, leading the cell through cytokinesis and eventual cell division in good coordination with nuclear division (30).

Treatment of the procyclic form of *T. brucei* cells with okadaic acid prevented kinetoplast/basal body segregation and resulted in multinucleated cells, suggesting a normal progression of mitosis in cells defective in cytokinesis, which is controlled largely by the kinetoplast cycle (7). Treatment with aphidicolin or rhizoxin (29) or RNA interference (RNAi) silencing of the G1, S, or mitotic cyclins (15, 24) or the cdc2-related kinases (CRKs) (37) in the procyclic-form cells resulted in inhibition of nuclear division but not kinetoplast/basal body segregation and subsequent cytokinesis, resulting in anucleate daughter cells designated the zoids. These results suggest that the checkpoint linking mitosis with cytokinesis may be weak or absent in the procyclic form of *T. brucei* and that kinetoplast/basal body segregation alone is sufficient to drive some of the cells through cytokinesis and cell division.

In the bloodstream form of *T. brucei*, however, cells arrested in G1 phase via a combined knockdown of CycE1, CRK1, and CRK2 (38), and those trapped in G2/M phase through depleting a mitotic cyclin CycB2/CYC6 (15) or CRK3 (37) failed to generate zoids. The cells arrested in G2/M were blocked from cytokinesis. But in ~5% of the cells, the kinetoplast continues to replicate, and the nucleus under G2/M arrest cannot stop from reentering a new G1 phase, thus resulting in cells with multiple kinetoplasts and multinuclear aggregates. Furthermore, these cells also possessed multiple basal bodies and multiple flagella (15, 37), suggesting a normal progression of the kinetoplast/basal body/flagellum cycle in the mitotically and cytokinetically arrested cells. Thus, cytokinesis initiation in the bloodstream form appears to be dependent on the completion of mitosis and apparently is unaffected by the kinetoplast cycle, which is the opposite from what was observed in the procyclic form. These distinctive mechanisms of cell cycle control in two developmental stages of the same organism may constitute an intriguing biological phenomenon facilitating an understanding of the link between mitosis and cytokinesis.

Aurora-B kinase is a chromosome passenger protein that regulates chromatin condensation, chromosome segregation, and cytokinesis (4). Its depletion from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (6, 22, 27), *Caenorhabditis elegans* (34), *Drosophila melanogaster* (13), and mammals (18, 36) leads to defects in chromosome segregation and cytokinesis but does not prevent continuous nuclear DNA synthesis, thus resulting in polyploidy. The multicellular eukaryotic organisms also possess an aurora-A kinase, which concentrates at the centrosomes and regulates spindle formation (8). The single aurora-like kinase Ark1 in *S. pombe* regulates spindle formation, chromosome segregation, and cytokinesis, thus playing roles that are separately executed by distinct aurora-A and aurora-B kinases in multicellular organisms (27).

† Supplemental material for this article may be found at http://ec.asm.org.
We have recently identified three aurora-like kinase homologues (TbAUK1, 2, and 3) in *T. brucei*, but only TbAUK1 played an essential function in the procyclic form (39). It displayed the subcellular distribution pattern of a chromosome passenger protein, and its knockdown resulted in defects in spindle formation, chromosome segregation, and cytokinesis with the cells arrested with one elongated nucleus, two segregated kinetoplasts and basal bodies, and two full-length flagella without apparent morphological change (39). Like Ark1 in *S. pombe*, TbAUK1 may thus play both the roles of aurora-A and aurora-B kinases in the procyclic form.

In the present study, we showed that an RNAi silencing of TbAUK1 or overexpression of an inactive TbAUK1-K58R mutant in the bloodstream form also resulted in defective spindle formation, chromosome segregation, and cytokinesis. But the typical cellular architecture was also lost, and further nuclear DNA synthesis and organelle replications continued, thus producing giant polyploid cells with multiple kinetoplasts, basal bodies, flagella, and nucleoli. This is the first time to our knowledge that an aurora-like kinase homologue was found to function differently between two developmental stages of the same organism. Further investigations into the molecular mechanisms precipitating these distinctions are warranted.

**MATERIALS AND METHODS**

**Trypanosome cell cultures.** The bloodstream form of *T. brucei* strain 90-13 (43) was grown at 37°C with 5% CO2 supplied in the HMI-9 medium containing 10% fetal bovine serum (Atlanta Biological) and 10% serum plus (JRH). The cells were then fixed in cold methanol at 20°C for 30 min and rehydrated by incubation with the wash buffer (0.1% Triton X-100 in PBS) three times. The fluorescein isothiocyanate (FITC)-conjugated goat anti-rat immunoglobulin G (IgG) (diluted 1:400), Cy3-conjugated goat anti-mouse IgG (diluted 1:400), or FITC-conjugated goat anti-mouse IgG (used at 1:400) antibodies were applied to the cells, and the cells were incubated with the secondary antibodies at room temperature for another hour. After the cells were washed three more times with the wash buffer, they were stained with 1.0 μg/ml of 4,6-diamidino-2-phenylindole (DAPI) at room temperature, and the slides were mounted in Vectashield mounting medium and examined with a fluorescence microscope.

**In vitro kinase assay.** Full-length coding sequences of TbAUK1, TbAUK1-K58R, and *T. brucei* histone H3 were each cloned into pGEM-4T-3 (Amersham), expressed in Escherichia coli BL21 cells and purified through glutathione Sepharose 4B beads. TbAUK1 (0.5 μg) or TbAUK1-K58R (0.5 μg) was incubated with 2 μg histone H3 in the kinase buffer (10 mM HEPES, pH 7.5, 50 mM NaCl, 10 mM MgCl2, 1 mM dithiothreitol) containing 1 μCi [γ-32P]ATP (Amersham) and incubated at room temperature for 1 h. Proteins were separated on a 12% sodium dodecyl sulfate-polyacrylamide gel. The gel was dried and exposed in a phosphorimager.

**Overexpression of TbAUK1 and TbAUK1-K58R in both forms of *T. brucei*.** The full-length coding sequence of TbAUK1 was amplified by PCR and cloned with the N terminus ligated to the triple-hemagglutinin tag (three-HA tag) sequence of the modified pLew100 vector (39, 43), which places expression of TbAUK1 under the control of a tetracycline-inducible promoter. To generate the TbAUK1-K58R mutant, site-directed mutagenesis (Stratagene) was performed to change the conserved lysine residue at position 58 of TbAUK1 to arginine with pLew100-TbAUK1-3HA plasmid as the template. The resulting construct was designated pLew100-TbAUK1-K58R-3HA. The two constructs were each linearized with NotI digestion and transfected into the bloodstream form of *T. brucei* 90-13 cells (43). Stable transfectants were each selected with 2.5 μg/ml of tetracycline and cloned on agarose plate (5). Expression of TbAUK1-3HA or TbAUK1-K58R-3HA was induced by adding 0.1 μg/ml of tetracycline to the culture medium, and the cells were examined by Western blotting (23) with anti-HA MAb (Sigma; diluted 1:10,000).

The bloodstream-form cells harboring pLew100-TbAUK1-3HA or pLew100-TbAUK1-K58R-3HA construct were each in vitro differentiated into the procyclic form by adding 5 mM citrate and 5 mM cis-aconitate to the culture medium, and the cells were examined by Western blotting (23) with anti-HA MAb (Sigma; diluted 1:10,000).

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**RESULTS**

TbAUK1 is essential for proliferation of bloodstream-form *T. brucei*. We performed RNAi to silence each of the three TbAUK1, 2 and 3 homologues in the bloodstream-form *T. brucei* and showed by RT-PCR that the levels of TbAUK1 (Fig. 1A, inset), 2, and 3 (see Fig. SIA inserts in the supplemental material) mRNAs were all reduced significantly. Cell growth was inhibited dramatically upon TbAUK1 knockdown (Fig. 1A) but depressed only slightly when TbAUK2 was depleted, whereas TbAUK3 depletion exerted no apparent effect on cell growth (see Fig. SIA in the supplemental material). These results indicate that, as for the procyclic form (39), only TbAUK1 is essential for proliferation of the bloodstream form of *T. brucei*.

TbAUK1 is required for chromosome segregation in the bloodstream form. Flow cytometry was performed on the TbAUK1 RNAi-induced cells, and the data (Fig. 1) showed a dramatic decrease of cells with 2C DNA content accompanied by an enrichment of cells with 4C and 8C DNA contents. Further DNA synthesis was apparently continuing in the growth-arrested cells. This differs from the procyclic form, in which TbAUK1 deficiency arrested the cells with one elongated nucleus and two kinetoplasts (1N2K) without further DNA synthesis (39). Depletion of TbAUK2 and TbAUK3 from the bloodstream form showed no detectable effect on cell
cycle progression (see Fig. S1B in the supplemental material). Our further investigations were thus focused on TbAUK1.

Timed samples following TbAUK1 RNAi were classified by the numbers of nuclei and kinetoplasts in each cell. The results showed that cells with one nucleus and one kinetoplast (1N1K) decreased from \( \sim 75\% \) to \( \sim 10\% \) in the population after 48 h, while the 1N2K cells increased from \( \sim 10\% \) to \( \sim 45\% \) within the first 16 h but dropped to \( \sim 25\% \) after 48 h (Fig. 1C). About 90% of the 1N2K cells contained an enlarged nucleus with some chromosomes in a state of aberrant segregation (Fig. 1E,
Cells containing two kinetoplasts and two nuclei (2N2K) decreased from ~10% to ~5% during the 48 h of RNAi (Fig. 1C). In some cases, the two nuclei were connected by a thin thread of DNA (Fig. 1E, arrowhead), resembling the chromatin bridging phenotype in the *Drosophila* aurora-B kinase RNAi-induced cells (13). Cells with multiple kinetoplasts (more than two) and a bulky nuclear aggregate (designated XNMK) emerged during the late phase of RNAi and increased to ~60% of the population after 48 h (Fig. 1C). These cells were of a gigantic size, at least severalfold larger than the wild type. They had lost the original morphology and become round (Fig. 1D). They contained multiple kinetoplasts and a large amount of nuclear DNA that was often disorganized and fragmented in each cell (Fig. 1E). These cells were apparently polyploid, suggesting the occurrence of additional rounds of nuclear DNA synthesis in the absence of chromosome segregation. The presence of multiple kinetoplasts also indicated a continuous progression of the kinetoplast cycle while cytokinesis was blocked.

These observations differ significantly from those on the TbAUK1-depleted procyclic form of *T. brucei* (39). In those cells, the decrease in 1N1K cells is also closely accompanied by the increase of 1N2K cells, but the latter are not further transformed into XNMK cells. The single nucleus in a 1N2K cell is elongated but does not extend beyond the size of two segregated nuclei. The two kinetoplasts remain segregated and do not further multiply. Apparently, in the procyclic form, mitotic arrest due to defective chromosome segregation does not allow reentry into G1 phase for further nuclear DNA synthesis, and a blocked cytokinesis is coupled with an inhibited kinetoplast cycle (39).

The potential effect of TbAUK1 depletion on the formation of mitotic spindles was examined by immunostaining with the KMX-1 antibody. Among the control 1N2K cells, about 52% of the population possesses an oval-shaped spindle in an apparent metaphase (Fig. 2B), whereas ~55% of the 2N2K cells in apparent anaphase had the spindle stretched, with the two nuclei moving toward the spindle poles (Fig. 2A). Among ~28% of the 2N2K cells in apparent telophase, the spindle turned into a single line lying between two well-segregated nuclei (Fig. 2A). In the TbAUK1-depleted cells, however, no spindle-like structure could be observed in the 1N2K (336 cells examined) and XNMK (379 cells examined) cells (Fig. 2A and B). Among the 295 2N2K cells examined, ~12% demonstrated a single-line spindle structure, suggesting that it could have been formed prior to RNAi. The gradual decline of 2N2K population during the 48 h of RNAi appears to support this elucidation (Fig. 1C). TbAUK1 thus plays an essential role in chromosome segregation in the bloodstream form. Since the morphology of a trypanosome cell is maintained primarily by an array of subpellicular microtubules, we tested whether TbAUK1 depletion affects microtubules in the enlarged posterior end of the bloodstream form cells. Cells stained with TAT-1 and C3B9 antibodies to label α-tubulin and acetylated α-tubulin, respectively, were examined. The results showed that the enlarged posterior ends in the TbAUK1-deficient cells were more intensely stained by both antibodies than the anterior ends were (Fig. 3A and B), suggesting accumulation of microtubules at the posterior location.
**TbAUK1 depletion does not prevent multiplication of kinetoplast, basal body, and flagellum in the bloodstream form.** In view of the continuous kinetoplast replication in the TbAUK1-depleted bloodstream-form cells (Fig. 1C and E), we further examined the replication of basal body and flagellum, which are known to play essential roles in cytokinesis initiation, cell division, and morphogenesis of the trypanosome. The cells were stained with YL1/2 and ROD1 antibodies for basal bodies and flagella, respectively (Fig. 3C). The basal bodies were shown as two distinctive fluorescent dots at the posterior ends of the control 1N2K and 2N2K cells. The two full-length flagella were extended in close parallel along the dorsal side of cell body (Fig. 3C). In the TbAUK1-depleted 1N2K and 2N2K cells, two well-defined basal bodies each associated with a kinetoplast were also shown. However, the two flagella were widely separated from each other in the posterior portion of the cell, though each was still associated with a well-defined basal body (Fig. 3C). Multiple basal bodies and flagella were seen in the XNMX cells, while close associations among kinetoplasts, basal bodies, and flagella were still maintained (Fig. 3C). TbAUK1 depletion thus does not prevent multiplication of kinetoplasts and basal bodies, nor does it affect the synthesis of new flagella in the bloodstream form.

**Overexpression of an inactive TbAUK1 mutant in the bloodstream form.** The lysine residue in the subdomain II of aurora-B kinase from *S. pombe* (22, 27), *Drosophila* (13), *C. elegans* (34) and humans (18, 36) plays an essential role in the catalytic function and is well conserved (16). Mutation of this residue to arginine abolishes the kinase activity. This residue is also conserved in TbAUK1 at position 58 and may also play the same essential function. We performed site-directed mutagenesis and cloned the full-length sequence encoding the K58R mutant of TbAUK1. Aurora-B kinases are known to phosphorylate histone H3 (8). A homologue of histone H3 has been identified in the trypanosome genome database (Tb927.1.2530) and cloned. Recombinant TbAUK1, TbAUK1-K58R, and his-

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**FIG. 3.** (A and B) Effect of TbAUK1 depletion on the cytoplasmic microtubule dynamics in the bloodstream form. Control and TbAUK1-depleted bloodstream-form cells were fixed in 4% paraformaldehyde, permeabilized with cold methanol, and immunostained with TAT-1 antibody against α-tubulin (A) or C3B9 antibody against acetylated α-tubulin (B). Bars, 2 μm. (C) Effect of TbAUK1 depletion on replication and segregation of the organelles. Control and TbAUK1-deficient cells were fixed with cold methanol and processed for indirect immunofluorescence assay. Nuclei and kinetoplasts were stained with DAPI, and tyrosinated α-tubulin was decorated with rat MAb YL1/2 with fluorescein isothiocyanate (FITC)-conjugated anti-rat IgG as the secondary antibody (panel YL1/2). The paraflagellar rod in the flagellum was immunostained with mouse MAb ROD1 with Cy3-conjugated anti-mouse IgG as the secondary antibody (ROD1 panels). In the DAPI images, the kinetoplast DNA appears as small discrete dots. The brightly stained dots in the YL1/2 images are basal bodies and are indicated by arrows. Bar, 2 μm.
TbAUK1 and TbAUK1-K58R were each tagged with a triple-hemagglutinin (three-HA) tag at the C terminus, overexpressed in the bloodstream-form cells by tetracycline induction (43), and monitored by semiquantitative RT-PCR (see Fig. S2A in the supplemental material) and by immunoblotting with anti-HA antibody (Fig. 5A, insets). The results from RT-PCR showed significantly enhanced levels of TbAUK1 and TbAUK1-K58R mRNAs up to fivefold above the endogenous level after tetracycline induction for 24 h. Abundant TbAUK1-3HA and TbAUK1-K58R-3HA proteins were also detected in Western blots after tetracycline induction. The overexpression of wild-type TbAUK1 had no apparent effect on cell growth (Fig. 5A), whereas TbAUK1-K58R overproduction demonstrated severe growth inhibition similar to that from depleting TbAUK1 (Fig. 5A). Thus, the K58R mutant exerts a dominant-negative effect on the cell.

Similar to that from TbAUK1 depletion, overexpression of
TbAUK1-K58R enriched the cells with 4C and 8C DNA content (Fig. 5B), the 1N1K cells diminished and 1N2K cells increased and then declined, whereas XNMK cells with large patches of nuclear DNA and multiple kinetoplasts were increased and then declined, whereas XNMK cells with large patches of nuclear DNA and multiple kinetoplasts were increased and then declined.

In the bloodstream form, TbAUK1-K58R was associated with the large patches of nuclear DNA aggregates (Fig. 6). Since no apparent chromosome segregation has occurred after multiple rounds of nuclear DNA synthesis in the absence of new mitotic spindle formation, location of TbAUK1-K58R to the spindle midzone was not observed in these polyploid cells. No spindle structure was visible among the large numbers of newly formed chromosomes (Fig. 2A), but the mutant enzyme remained a chromosome passenger, suggesting that the K58R mutation in TbAUK1 has not affected its subcellular localization.

**The kinase activity of TbAUK1 is essential for both forms of T. brucei.** The effects from overexpressing TbAUK1 and TbAUK1-K58R were also investigated in the procyclic form derived from in vitro differentiation of the bloodstream-form cells harboring the overexpression constructs. Overexpression of the HA-tagged proteins was confirmed by immunoblotting (Fig. 5C, inserts). While overexpression of TbAUK1 had little effect on cell growth (Fig. 5C) or cell cycle progression (Fig. 5D) as indicated previously (39), the overexpressed TbAUK1-K58R caused significant growth inhibition (Fig. 5C) and mitotic arrest (Fig. 5D). An enrichment of 1N2K cells with two well-segregated kinetoplasts/basal bodies (see Fig. S3A in the supplemental material) were observed. No XNMK cells were detectable in the population, and there was no apparent morphological change among the cells (see Fig. S3B in the supplemental material). All these results are similar to those from TbAUK1-depleted trypanosome (39) and suggest that the TbAUK1 activity is essential for both bloodstream and procyclic forms of T. brucei.

**Distinction in nucleolar segregation between the TbAUK1-deficient bloodstream and procyclic forms.** Though chromosome segregation is defective in both forms of TbAUK1-ablated T. brucei, nuclear DNA synthesis still continues in the bloodstream form. We thus further stained the trypanosome cells with the anti-nucleolus monoclonal antibody L1C6 (26) to monitor the segregation of nucleoli in the two forms of TbAUK1-depleted trypanosome. This antibody is known to stain the nucleolus in one bright spot per nucleus but stains in a bright bar during early anaphase of procyclic-form T. brucei (9, 26). In the control bloodstream-form cells, a single bright dot was detected at an eccentric position within the nuclei of 1N1K cells (Fig. 7A; also see Fig. S2C in the supplemental material).

**Subcellular localization of TbAUK1 in the bloodstream form.** TbAUK1-3HA displayed a dynamic subcellular distribution during the cell cycle (Fig. 6). In 1N1K and 1N2K cells during interphase or prophase (26, 33), TbAUK1 was distributed throughout the nucleus in an apparent association with the chromatin (Fig. 6). However, in 2N2K cells during anaphase or telophase (26, 33), the protein is translocated to and concentrated at the midzone between the two segregated nuclei (Fig. 6), similar to the profile observed in the procyclic form (39). In the morphologically altered 1N2K cells, the overexpressed TbAUK1-K58R was mainly localized in the extended nucleus but also associated with the aberrantly segregated chromosomes (Fig. 6, arrow). In some 2N2K cells, the mutant enzyme was localized to the midzone like the wild-type TbAUK1 (Fig. 6), suggesting that a spindle was likely formed prior to the overexpression of TbAUK1-K58R. In the XNMK cells, TbAUK1-K58R was associated with the large patches of nuclear DNA aggregates (Fig. 6). Since no apparent chromosome segregation has occurred after multiple rounds of nuclear DNA synthesis in the absence of new mitotic spindle formation, location of TbAUK1-K58R to the spindle midzone was not observed in these polyploid cells. No spindle structure was visible among the large numbers of newly formed chromosomes (Fig. 2A), but the mutant enzyme remained a chromosome passenger, suggesting that the K58R mutation in TbAUK1 has not affected its subcellular localization.
forms, implying yet another distinction in the mechanisms of cell cycle regulation between the two forms of Trypanosoma brucei.

**DISCUSSION**

In the present study, we showed that RNAi silencing of the trypanosome aurora-B kinase homologue TbAUK1 or overexpression of the inactive TbAUK1-K58R mutant in the bloodstream-form *T. brucei* led to identical defects in mitotic spindle assembly, chromosome segregation, and cytokinesis as well as distorted morphology. We also showed that overexpression of TbAUK1-K58R mutant in procyclic-form *T. brucei* resulted in completely blocked mitosis and inhibited cytokinesis, identical to the phenotypes of TbAUK1-depleted procyclic form (39). These outcomes verified that the inactive mutant protein can exert a transdominant negative effect (10, 20) over the endogenous wild-type TbAUK1 in both forms of trypanosome and provide the same results as those from RNAi knockdown of TbAUK1, thus confirming the outcome from the current investigation.

Although the functions of TbAUK1 in the two forms of *T. brucei* are in general agreement with the dual roles of aurora-B kinase in other eukaryotes, i.e., chromosomal segregation and cytokinesis regulation (2, 4, 8, 12), its apparent additional involvement in mitotic spindle assembly suggests that TbAUK1 also performs the function of an aurora-A kinase (8). This multifunction resembles that of the single aurora-like homologue in *S. pombe*, Ark1, which is known to possess both aurora-A and -B functions as well (27). It is probable that, unlike metazoans, unicellular eukaryotes may have a simpler mechanism in regulating cell cycle progression that two aurora functions can reside in the same protein.

Many aspects of TbAUK1 functions in the two forms of *T. brucei* differ so widely that it could become a useful tool for further understanding the different mechanisms of cell cycle regulations and the changing link between mitosis and cytokinesis in the two developmental stages of trypanosomes. TbAUK1 deficiency enriches both forms in the 1N2K state in the early phase, with the two kinetoplasts widely segregated in the procyclic form but not in the bloodstream form, where the two kinetoplasts are known to stay closely together at the posterior end of the cell during cytokinesis (25). The single elongated nucleus shares a similar appearance in the two forms of cells during the early phase of TbAUK1 depletion, but the nucleus in the bloodstream form contains two segregated nucleoli, whereas the nucleus in the procyclic form contains only one, somewhat enlarged, nucleolus.

TbAUK1 deficiency leads to mitotic arrest with a complete cessation of further DNA synthesis in the procyclic form. The numbers of kinetoplasts, basal bodies, and flagella are each confined to two per cell. Replication of the basal body and kinetoplast is known to reach completion ahead of the onset of mitosis in the cell cycle of the procyclic form (45). Cytokinesis begins with the segregation of two kinetoplasts, with one staying at the mid-potion of the cell and the other migrating to the posterior end (31). This kinetoplast segregation, which does not occur in the bloodstream form, is followed by migration of one of the two newly divided nuclei in between the two separated kinetoplasts before cytokinesis initiation (31). With TbAUK1 depletion or TbAUK1-K58R overexpression, there was no new nucleus moving in between the two kinetoplasts, and the cells arrested at the cytokinetic initiation were mostly in the 1N2K form. The puzzle lies in why multiple kinetoplasts/basal bodies/flagella are not formed in the cytokinetically arrested procyclic form as in the bloodstream form. An additional control(s) could be operational in the procyclic form to prevent the mitotically and cytokinetically arrested cells from undergoing further rounds of organelle replication. Either the blocked cytokinesis or the arrested mitosis or both could exert an inhibitory effect on the further replication of kinetoplast, basal body, and flagellum. However, the fact that these organelles are replicated at least once in the cytokinetically arrested cells indicates that cytokinesis is not under the sole control of kinetoplast cycle but, rather, TbAUK1.

The mitotic defect in the bloodstream form is, however, more closely associated with the turning off of cytokinesis and cell division, but reentries into G2 phase, nuclear DNA synthesis, nucleolus replication, and organelle multiplication continue. After 48 h of TbAUK1 deficiency, the majority of cells become polyploid with large amounts of DNA forming nuclear aggregates with multiple nucleoli, kinetoplasts, basal bodies, and flagella. These cells are apparently not subject to checkpoint arrest and are capable of undertaking multiple rounds of

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**FIG. 7.** Effect of TbAUK1 deficiency from RNAi on nucleolar segregation in the bloodstream-form and procyclic-form cells. RNAi-induced cells for 24 h were fixed in cold methanol and processed for indirect immunofluorescence. Nuclei and kinetoplasts were stained with DAPI, and the nucleolus was immunostained with mouse MAb L1C6. Bars, 2 μm.

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polyploidy. This is, ironically, consistent with the aurora-B mutant phenotypes in yeasts (6, 22, 27) and in metazoans, such as C. elegans (34), Drosophila (13), and mammals (18, 36), suggesting a well-conserved role of aurora-B kinase in evolutionarily divergent organisms. However, it leaves the lack of polyploidy in the TbAUK1-deficient procyclic form unexplained. Perhaps the mitotic arrest in this form has prevented activation of certain G₁ phase genes for G₁ reentry. Alternatively, TbAUK1 deficiency in the bloodstream form may over-ride the spindle checkpoint and thus induce premature mitotic exit that leads to a G₁ reentry.

Multiple kinetoplasts, basal bodies, and flagella are formed in the polyploid bloodstream-form cells deficient in TbAUK1, suggesting the lack of TbAUK1 involvement with the progression of kinetoplast cycle. Meanwhile, the continued progression of the latter in the absence of cytokinesis also shows a lack of connection between the kinetoplast cycle and cytokinesis. This is again in contrast to that in the procyclic form in which zoid production is driven by the kinetoplast cycle.

Bloodstream-form cells with large nuclear aggregates, multiple kinetoplasts, basal bodies, and flagella were also observed among some of the mitotic cyclin CycB2/CYC6 (15; our unpublished data) or CRK3 (37) knockdown bloodstream-form cells. A small percentage (~5%) of XNMK cells without apparent morphological change was observed a few days after RNAi induction. Like the case in TbAUK1 deficiency, no zoid was detectable in these knockdown cells. An ablation of CycB2/CYC6 or CRK3 from the procyclic form, however, arrested the cells in G₂/M phase but generated zoids up to ~20% of the cell population (15, 24, 37). Cytokinesis initiation and cell division in the procyclic form are thus not totally dependent on the completion of mitosis. In contrast to the arrested kinetoplast cycle under TbAUK1 depletion, the kinetoplast cycle in the procyclic form continues to drive cytokinesis in the absence of CycB2/CYC6 or CRK3. Thus, TbAUK1 deficiency has the additional effect in blocking the kinetoplast cycle, which may in turn inhibit cytokinesis and cell division in the procyclic form. This effect is, however, not shown in the bloodstream form. One possible explanation could be that migration of one of the two replicated kinetoplasts to the mid-section of the cell prior to cytokinesis initiation, which occurs in the procyclic form but not the bloodstream form, could be prevented from sending the signal for cytokinesis initiation in the procyclic form by TbAUK1 depletion.

Another intriguing observation from the present study is the grossly distorted cell morphology in the bloodstream form when TbAUK1 is deficient. It argues for an essential role of TbAUK1, either directly or indirectly, in maintaining cell morphology in the bloodstream-form cells. Since the expansion of cell volume and the rounding of the cell can be attributed to excessive microtubule synthesis at the posterior end, TbAUK1 could exert a negative regulatory function on microtubule synthesis during morphogenesis. A similar observation was reported in Xenopus cells in which inhibition of aurora-B kinase leads to alterations in mitotic microtubule dynamics with an elaboration of astral arrays, a marked decrease in kinetochore fiber microtubules, and an increase in the stability of some cytoplasmic microtubules (17). Once again, the question is why the same function of TbAUK1 is not demonstrated in the procyclic form. The basic cell architectures of all forms of trypanosome cells are similar, but fundamental differences do exist between the different life cycle forms. For example, the positioning of one kinetoplast between the two nuclei in the procyclic form requires that the new flagellum of the procyclic-form cell be tethered at its tip to the lateral aspect of the old flagellum by a novel structure termed the flagellar connector, which is not present in the bloodstream form (3). Just precisely how TbAUK1 is involved in maintaining cell shape in the bloodstream form of trypanosomes remains elusive at this moment.

Roles of the aurora-B kinase in regulating chromosome segregation in yeasts and metazoans have been well documented (2, 8, 12). Aurora-B forms the chromosomal passenger complex with three additional proteins: INCENP, Survivin, and Borealin/Dasra, and its activity is enhanced by INCENP, the substrate activator of aurora-B kinase (11, 32, 40). To ensure the fidelity of chromosome segregation, the chromosomal passenger complex regulates kinetochore biorientation by monitoring and/or correcting microtubule-kinetochore attachments. Although aurora-B kinase-mediated phosphorylation of the Dam1 outer-kinetochore microtubule-binding complex and the Ndc80 complex (2) increases, this phosphorylation weakens the interaction between the Dam1 and Ndc80 complexes and facilitates formation of bipolar connections between chromosomes and the spindle (35). On the other hand, the chromosomal passenger complex also regulates the spindle checkpoint by maintaining the association of the checkpoint proteins Mad2 and BubR1 with the kinetochore (21) and the subsequent formation of a complex containing Mad2 and Mad3 (28). However, the recent genome sequencing of T. brucei revealed no close homologue of the Dam1 and Ndc80 complexes (1). In addition, many of the kinetochore components are also missing from the trypanosome genome, and only a single checkpoint protein Mad2 homologue is detected (1). These observations raise an interesting question of how TbAUK1 regulates chromosome segregation in trypanosomes in the absence of the above-mentioned machinery. Whether TbAUK1 functions through a similar mechanism involving structurally distinct proteins or acts by a different mechanism deserves further investigation.

In summary, the functions of TbAUK1 in the bloodstream form of T. brucei have revealed several distinctions in the mechanisms of cell cycle regulation from the procyclic form. The polyploidy, dissociation of the kinetoplast cycle from mitosis-cytokinesis, and gross distortion of cell morphology caused by TbAUK1 deficiency in the bloodstream form but not the procyclic form may provide a good opportunity for an in-depth dissection of the molecular basis coordinating mitosis with cytokinesis in the trypanosome.

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