An Aurora Kinase Homologue Is Involved in Regulating Both Mitosis and Cytokinesis in *Trypanosoma brucei* 

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The chromosomal passenger protein aurora kinases have been implicated in regulating chromosome segregation and cell division. Three aurora kinase homologues were identified (TbAUK1, -2 and -3) in the *Trypanosoma* Genomic Data Base, and their expressions in the procyclic form of *Trypanosoma brucei* were knocked down individually by using the RNA interference technique. Only a knockdown of TbAUK1 arrested the cells in G2/M phase with each cell showing an extended posterior end, two kinetoplasts, and an enlarged nucleus, apparently the result of an inhibited kinetoplast multiplication and a failed mitosis. There is no mitotic spindle structure in the TbAUK1-depleted cell. The two kinetoplasts moved apart from each other but stopped just before cytokinesis, suggesting that cytokinesis was blocked in its early phase. Overexpression of TbAUK1 in the cells resulted in little change in cell growth. By immunofluorescence, TbAUK1 was primarily localized to the nucleus in interphase and to the mitotic spindle during apparent metaphase and anaphase. Thus, differing from other eukaryotes, TbAUK1 has an apparent triple function in coupling mitosis and kinetoplast replication with cytokinesis in *T. brucei*. *T. brucei* polo-like kinase, previously identified as the initiator of cytokinesis (11), was either depleted or overexpressed in the TbAUK1-deficient cells. A dominant TbAUK1-depleted phenotype was demonstrated in both cases, suggesting that TbAUK1 plays an essential role in cytokinesis that cannot be affected by changes in the level of *T. brucei* polo-like kinase. To our knowledge, this is the first time that the function of an aurora B-like kinase is a prerequisite for polo-like kinase action in initiating cytokinesis. TbAUK1 is also the first identified protein that couples both mitosis and kinetoplast replication with cytokinesis in the trypanosome.

During cell cycle progression in eukaryotes, mitosis is a critical phase in ensuring that the two daughter cells inherit the same genetic background upon subsequent cell division. Although it represents the shortest phase in the metazoan cell cycle, mitosis is when the cell undergoes the most rapid and dramatic structural reorganizations. The interphase microtubule network de-polymerizes between the end of G2 phase and the beginning of prophase (1). The centrosomes, which have duplicated during S phase, migrate around the nucleus and form a spindle, whereas chromatin condenses upon the entry into prophase. After spindle formation and nuclear envelope breakdown, the kinetochores capture the plus ends of microtubules and align the chromosomes at the metaphase plate. Two identical sets of chromosomes then begin to separate by decreasing the length of kinetochore fibers in anaphase A (2). Further separation of the chromosomes takes place by departing the spindle poles in anaphase B, which is followed by cytokinesis initiation and cell division.

These mitotic events in metazoa are tightly controlled by a number of regulatory proteins. Among them, the aurora kinases have received much attention recently for their roles in regulating both chromosome dynamics and cytokinesis (3, 4). Aurora kinases consist of a family of serine/threonine kinases whose multiple roles are well conserved throughout evolution within each subfamily (5). A single homologue was found in budding and fission yeasts. A deletion of the homologue (Ipl1) from budding yeast showed abnormal ploidy, suggesting a role in regulating chromosome segregation and cytokinesis for the missing protein (6). Three chromosome passenger proteins, aurora A, B, and C, were identified in mammals (7, 9, 10). Aurora A and B are essential for mitosis and have distinct and varying localizations in mammalian cells (6). Aurora A begins to accumulate on the centrosomes during S phase and becomes heavily concentrated at the spindle poles along the spindle microtubules by mitosis. Aurora B localizes first to chromosomes in the prophase and then concentrates at the centromeres during the prometaphase and metaphase. It plays a crucial function by departing from the chromosomes at the onset of anaphase and relocating to the central spindle, where it has another role in initiating cytokinesis (11). Consistent with their localizations, aurora A regulates spindle assembly, and aurora B controls chromosome segregation and cytokinesis initiation (4). Aurora C was found in testis and certain tumor cell lines and localized to the spindle poles during late mitosis (8).

*Trypanosoma brucei* is the causative agent of sleeping sickness in human and nagana in cattle in sub-Saharan Africa and also among the most ancient and evolutionarily divergent eukaryotes with many unique biological features (12, 13). The most apparent is the single mitochondrion in each cell, and the arrangement of mitochondrial DNA in a large disk-like structure, the kinetoplast. Replication of the kinetoplast, which is closely associated with the flagellum basal body, and its segregation depends on the flagellum-mediated separation of replicated basal bodies, representing initiation of cytokinesis (22). The kinetoplast cycle alone can apparently drive up to 20% of the procytic form cells into cell division without completing mitosis, a phenomenon not
observed in the bloodstream form (16) or any other eukaryotes (20, 23). Furthermore, a homologue of polo-like kinase in T. brucei (TbPLK)\(^2\) is localized in the potential flagellum attachment zones and apparently initiates only cytokinesis without an apparent role in mitosis as is the case with the polo-like kinases in other eukaryotes (24). It further illustrates that the regulatory networks that correlate mitosis and cytokinesis are different in T. brucei.

In this study, we identified three aurora kinase homologues in T. brucei. However, only one of them is apparently involved in cell cycle regulation. This aurora kinase, TbAUK1, localizes in the nucleus, is concentrated in the central spindle during mitosis, and plays an essential role in forming mitotic spindles. It controls mitosis, kinetoplast replication, as well as initiation of cytokinesis. Overexpression and depletion of TbPLK in aurora-deficient cells showed a consistently dominant TbAUK1-depleted phenotype, suggesting an essential role of TbAUK1 in regulating cytokinesis in trypanosomes.

MATERIALS AND METHODS

Cell Cultures—Procyclic form T. brucei strain 29-13 cells (25) were cultivated at 26 °C in Cunningham's medium (26) supplemented with 10% fetal bovine serum (Atlanta Biological, Lawrenceville, GA). G-418 (15 μg/ml) and hygromycin B (50 μg/ml) were included in the culture medium to maintain the T7-RNA polymerase and tetracycline-repressing gene constructs in the cells.

RNA Interference (RNAi)—Partial cDNA fragments (250–550 bp) of three aurora kinase homologues (designated TbAUK1, -2, and -3; Trypanosome Genomic Data Base accession numbers Tb11.01.0330, Tb03.28C22.460, and Tb09.160.0570) were amplified by PCR using pairs of gene-specific primers and ligated into the pZJM vector by NotI for integration into the T. brucei cDNA spacer region. Transfection of strain 29-13 with the linearized DNA constructs by electroporation was performed essentially as described previously (20). The transfectants were selected under 2.5 μg/ml phleomycin with individual cells cloned by limiting dilutions on agarose plates (28). The stable transfectants thus selected were grown in the culture medium containing phleomycin.

Transcription of the DNA insert was induced by adding 1 μg/ml tetracycline to the medium to switch on the T7 promoter. The double-stranded RNA thus synthesized is expected to lead to specific degradation of its corresponding mRNA in T. brucei (29, 30). To evaluate the effect of each RNAi on cell proliferation, cell numbers were counted at different time intervals using a hemocytometer.

Semi-quantitative RT-PCR—Total RNA was extracted from T. brucei cells using the TRizol reagent (Amersham Biosciences) and treated with DNase I (Roche Applied Science) to digest the remaining DNA. RT-PCR was then performed using the one-step RT-PCR kit (Invitrogen) and a pair of gene-specific primers that differs from the primer pair used in generating the original RNAi construct.

Fluorescence-activated Cell Sorting (FACS) Analysis—Cell samples for FACS were prepared and analyzed as described previously (20). The propidium iodide (PI)-stained cell samples from FACS analysis were subsequently examined with an Olympus phase-contrast and fluorescence microscope to tabulate the numbers of nuclei and kinetoplasts in individual cells and the numbers of cells with different morphologies in populations of more than 200 cells in each sample.

Immunofluorescence Microscopy—For immunofluorescence experiments, cells were harvested, washed three times with phosphate-buffered saline (137 mM NaCl, 8 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4, pH 7.4), and fixed with cold methanol at ~20 °C for 20 min (16). The fixed cells were washed and blocked in a blocking buffer (1% bovine serum albumin and 0.1% Triton X-100 in phosphate-buffered saline) at room temperature for 60 min and incubated with the primary antibody at room temperature for another 60 min. The following primary antibodies were used: YL1/2 (Chemicon, Temecula, CA; rat monoclonal antibody against yeast tyrosinated α-tubulin, used at 1:400 dilution); ROD1 (from Dr. Keith Gull, Oxford University; mouse monoclonal antibody against two paraglial rod proteins; used without dilution); NotI for integration into the T. brucei cDNA spacer region. Transfection of strain 29-13 with the linearized DNA constructs by electroporation was performed essentially as described previously (20). The transfectants were selected under 2.5 μg/ml phleomycin with individual cells cloned by limiting dilutions on agarose plates (28). The stable transfectants thus selected were grown in the culture medium containing phleomycin.

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KMX-1 antibody against mitotic spindles (from Dr. Keith Gull, Oxford University, mouse monoclonal antibody against Physarum polycephalum amoebal tubulin protein); and the HA probe for HA3 tag (sc-7392 RTIC; Santa Cruz Biotechnology). The fluorescein isothiocyanate-conjugated anti-rat IgG antibody (diluted 1:400; Sigma), Cy3-conjugated anti-mouse IgG antibody (diluted 1:300; Sigma), or Alexa Fluor 488 (A-21422; Molecular Probes) was then applied, and the cells were incubated at room temperature for another 60 min. Slides were mounted in Vectashield in the presence of 1 μg/ml 4,6-diamino-2-phenylindole (DAPI) and examined with a fluorescence microscope with the facility of confocal microscopy.

Overexpression of TbAUK1-3HA in Procyclic Form T. brucei—The full-length gene of TbAUK1 was amplified by PCR using primers that had a HindIII site at the 5′-end and a BamHI site at the 3′-end of the gene. The reverse primer also contained the sequence of a triple hemagglutinin tag (HA tag) to the C terminus of TbAUK1 in-frame with the gene sequence. The PCR-amplified TbAUK1-3HA was cloned into the HindIII/BamHI-digested pLew100 vector (25), which places the expression of the inserted gene under the control of a tetracycline-inducible T7 promoter. The cloned construct was linearized with NotI and transfected into the procyclic form T. brucei 29-13 cells (25). Stable transfectants were selected under 2.5 μg/ml phleomycin. Expression of TbAUK1-3HA was induced by adding 0.1 μg/ml tetracycline to the culture medium.

Overexpression and Knockdown of TbPLK in TbAUK1-depleted Procyclic Form T. brucei—For overexpression, a modified pLew100 vector containing a full-length TbPLK gene with a GFP tag at the C terminus (24) was transfected into the RNAi TbAUK1-depleted procyclic form cells by electroporation as described previously (20). For double knockdowns, a pZJM plasmid containing the conjugated cDNA fragments of TbPLK and TbAUK1 was linearized and introduced into the procyclic form cells (20). The transfected cells were selected under 5 μg/ml puromycin. The stable transfectant thus obtained was then induced with 1 μg/ml tetracycline to switch on the T7 promoter, to initiate both the TbAUK1 RNAi and the TbPLK overexpression or the double knockdown simultaneously. After a 1-day induction, the cells were collected, and the levels of TbPLK-GFP, TbPLK, and TbAUK1 mRNAs were examined by semi-quantitative RT-PCR and the overexpressed protein.
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by Western blotting. The cell sample after a 1-day induction was also analyzed by FACSscan, and the morphology of cells was examined by epifluorescence microscopy as described previously.

RESULTS

Identification of Aurora Homologues in Trypanosome Genomic Data Base—Three aurora kinase homologues were identified in the Trypanosome Genomic Data Base and assigned TbAUK1, -2, and -3. They showed 30–40% sequence identity and 50–60% sequence similarity with the human and yeast aurora kinases except for TbAUK3, which has a longer sequence at the C terminus than the others. All three homologues have a signature motif (DFGWSXXXXXRTXCGTXXDYL-PPE) in the activation loop specific for aurora kinases in the catalytic domain (7). They also possess the D (destruction)-box (LXXXPXXR-XXLXXXXHPW) near the C terminus that is common to all aurora kinases (Fig. 1). But none of the three trypanosome auroras has the A-box domain, which is a specific structural feature of mammalian aurora A. Thus, none of the three enzymes could be a close homologue of aurora A. A careful alignment of the protein sequences suggests that all the three auroras are close homologues of aurora B kinase and the yeast aurora Ipl1 (Fig. 1)

RNAi of the Expression of Aurora Homologues in Procyclic Form T. brucei—We employed the RNAi technique to knock down expression of the three aurora homologue genes (TbAUK1, TbAUK2, and TbAUK3) in the procyclic form T. brucei. A 250–500-bp DNA fragment corresponding to a unique sequence from the coding region of each gene with no significant sequence identity among the rest of the genomic sequences in the Trypanosome Genomic Data Base was amplified by PCR, cloned into the RNAi vector pZIM, linearized, and transfected into the cells (see “Materials and Methods”).

The effects of RNAi on expression of the three TbAUK genes were examined by semi-quantitative RT-PCR analysis (Fig. 2A, insets, and supplemental Fig. 1). In all cases, levels of the targeted mRNAs diminished significantly after 3 days of RNAi.

The potential effects of the aurora RNAi were monitored by daily counting of the numbers of transfected cells after tetracycline induction (Fig. 2A and supplemental Fig. 1). The results indicated that a knockdown of TbAUK1 led to a significant inhibition of the increase in cells such that the cell number after 4 days was only 12% of that of the uninduced control. A knockdown of TbAUK2 or TbAUK3, however, did not have any detectable effect on the increase of cell number (supplemental Fig. 1).

Effect of Aurora Depletion on Cell Cycle Progression—FACS analysis of the cellular DNA content indicated that after 16 h of RNAi on expression of TbAUK1, there were a significant enrichment of G2/M phase cells and a dramatic reduction in G1 cells (Fig. 2B). This apparent arrest of cell cycle progression at G2/M is more immediate than that from knocking down the cdc2-related kinases in our previous studies (20, 23). There was also the emergence of a sub-G1 peak, which could represent the apoptotic cells resulting from the drastic cell cycle arrest. After a 24-h knockdown, the G1 phase and S phase cell populations decreased from ~45 to 13% and 42 to 18%, respectively, whereas the G2/M phase cells increased from 13 to 69% (Fig. 2B). Depletion of TbAUK2 or TbAUK3 produced virtually no detectable shift in the cell population (supplemental Figs. 1 and 2), suggesting that TbAUK1 is the critical kinase involved in regulating entrance into mitosis during cell cycle progression of T. brucei, whereas function of the other two homologues in mitosis, if any, is not easily identified at present. All the subsequent studies were thus focused on TbAUK1.

Unusual Morphologies of the TbAUK1-deficient Cells—The PI-stained TbAUK1-depleted procyclic form cells were examined with a fluorescence microscope to tabulate the population of cells with one nucleus + one kinetoplast (1N1K), one nucleus + two kinetoplasts (1N2K), two nuclei + two kinetoplasts (2N2K), or no nucleus + one kinetoplast (0N1K, the zoids). After 8 h of RNAi induction, there was no obvious difference between the induced and the uninduced cells (Fig. 3A). After a 24-h RNAi induction, however, there was a significant decrease in the 1N1K population from 75 to 17%, which was accompanied by an increased population of 1N2K cells from 12–53% (Fig. 3A). This dramatic conversion of 1N1K cells to 1N2K cells within such a short time likely represents a blocked mitosis un inhibited single round of kinetoplast segregation, which is known to occur ahead of mitosis (17). But this round of kinetoplast segregation is not followed by another round of kinetoplast replication, indicating inhibition of further kinetoplast replication by depleting TbAUK1. Furthermore, cytokinesis initiation leading to large numbers of zoids and 1N1K cells, as we have observed in the G2/M cells through knocking down CycB2 or CRK3 (18,
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Although T. brucei chromosomes do not condense during mitosis (35), our recent efforts in immunostaining T. brucei with the KMX-1 antibody (see "Materials and Methods") indicated that by knocking down the expression of anaphase-promoting complex subunit APC1 or CDC27, the procyclic form was apparently arrested in metaphase, and the bloodstream form was likely stopped in late anaphase (36). This antibody has thus enabled us to identify the mitotic spindle structures in T. brucei during metaphase and anaphase. When cells in the current experiment were stained with KMX-1, essentially all the 1N1K cells in both control and the RNAi populations were without such a structure as anticipated (Fig. 4). Among the 1N2K cells in the control, which constituted only 12% of the total, about one-half of the population possessed the mitotic spindle structure, and the other half did not (Fig. 4A). These spindle-less cells were probably caught at a time between kinetoplast replication and mitotic initiation (17). In the TbAUK1-depleted cells 24 h into RNAi, however, the 1N2K cells constituted 53% of the total population (see Fig. 3A), but none were found to possess a mitotic spindle (Fig. 4B). Apparently, the failure of these cells in completing the mitotic process can be attributed to the failure in forming mitotic spindle. We consider this a clear indication that TbAUK1 functions in forming the mitotic spindle during mitosis of the procyclic form T. brucei.

Localization of TbAUK1 in Procyclic Form T. brucei—Overexpression of TbAUK1 mRNA in the transfected procyclic form cells was examined by RT-PCR. The results, presented in Fig. 5A, showed a significantly enhanced level of TbAUK1 mRNA from the 1.71-fold expression over the endogenous level (because of apparent leaky expression in the absence of tetracycline) to 4.37-fold after tetracycline induction for 24 h. The level of TbAUK1-3HA protein, estimated with the HA probe and initiated cytokinesis in the procyclic form (35), our recent efforts in immunostaining T. brucei with the KMX-1 antibody (see "Materials and Methods") indicated that by knocking down the expression of anaphase-promoting complex subunit APC1 or CDC27, the procyclic form was apparently arrested in metaphase, and the bloodstream form was likely stopped in late anaphase (36). This antibody has thus enabled us to identify the mitotic spindle structures in T. brucei during metaphase and anaphase. When cells in the current experiment were stained with KMX-1, essentially all the 1N1K cells in both control and the RNAi populations were without such a structure as anticipated (Fig. 4). Among the 1N2K cells in the control, which constituted only 12% of the total, about one-half of the population possessed the mitotic spindle structure, and the other half did not (Fig. 4A). These spindle-less cells were probably caught at a time between kinetoplast replication and mitotic initiation (17). In the TbAUK1-depleted cells 24 h into RNAi, however, the 1N2K cells constituted 53% of the total population (see Fig. 3A), but none were found to possess a mitotic spindle (Fig. 4B). Apparently, the failure of these cells in completing the mitotic process can be attributed to the failure in forming mitotic spindle. We consider this a clear indication that TbAUK1 functions in forming the mitotic spindle during mitosis of the procyclic form T. brucei.

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Localization of TbAUK1-3HA in the transfected cells during different mitotic phases was monitored by immunostaining the cells using the HA probe and KMX-1. The results showed that TbAUK1-3HA is localized inside the nucleus and apparently filled the central portion of the nucleus during the interphase (Fig. 6, top panel). This localization is shifted to the short spindle structure during the apparent metaphase (Fig. 6, 2nd panel) and becomes distributed along the elongated spindle or centralized in its midzone during the anaphase (Fig. 6, 3rd and bottom panel). This localization and trans-localization during mitosis are similar to that of aurora B kinase in mammalian cells (11), and support the previous notion that TbAUK1 plays an important function in the formation of the mitotic spindle.

Overexpression of TbPLK in TbAUK1-depleted Cells Does Not Alter the Phenotype of the Latter—In the procyclic form, TbPLK also plays a critical role in initiating cytokinesis (24). But in contrast to the other known polo-like kinases, TbPLK is apparently not involved in regulating the G2/M transition, metaphase-anaphase transition, anaphase release, or mitotic exit in trypanosome. A knockdown of TbPLK from the procyclic form T. brucei resulted in arresting the cell number at 8-fold of the initial count, with most of the cells filled with multiple nuclei, kinetoplasts, basal bodies, and full-length flagella (24). TbPLK is thus controlling primarily cytokinesis initiation. We wanted to learn how TbAUK1, a connector between mitosis, kinetoplast replication, and cytokinesis, and TbPLK, an initiator of only cytokinesis, could function together and whether they act sequentially or in parallel in controlling cytokinesis initiation. To answer this question, TbPLK tagged with GFP at the C terminus was first overexpressed in the wild type procyclic form T. brucei, and the cell growth was found unaffected (data not shown) as was demonstrated previously in a similar experiment (24). When the same tagged TbPLK was overexpressed in the TbAUK1-depleted procyclic form T. brucei cells, however, there was a significant depression in cell number similar to that observed in cells only depleted of TbAUK1 in Fig. 2A (data not shown). The TbPLK mRNA expression was monitored 1 day after tetracycline induction with semi-quantitative RT-PCR. The results (Fig. 7A) indicate a 1.98-fold leaky expression over the endogenous level and a 3.64-fold enhancement upon tetracycline induction of the cells.
Fig. 7B. TbAUK1 depletion thus has a dominant effect on the cells unaffected by the increased level of TbPLK.

The doubly transfected cells were also stained with DAPI and examined under a fluorescence microscope after a 1-day induction. There was a decrease in the 1N1K population from 75 to 29% and a corresponding accumulation of 1N2K cells from 12 to 48% (and an increase in the zoid population from 1 to 10%) during the initial 24 h (Fig. 8A). These changes are again very similar to those obtained from knocking down TbAUK1 alone (Fig. 8A). For the TbPLK overexpressing cells without knocking down TbAUK1, the only noticeable change was an apparent conversion of some 1N1K cells into zoids constituting 13% of the cell population shift (24). The 10% increase of zoids in the double mutant may thus reflect such an effect from overexpressing TbPLK (Fig. 8A). However, the overall profile of morphological distribution of the double transfectant is that of the TbAUK1-depleted cells, once again consistent with the dominant effect of TbAUK1 depletion. The 1N2K cells of the double mutant possess also an elongated posterior end, an enlarged and extended nucleus, and two well separated kinetoplasts (Fig. 8B) similar to those obtained by knocking down TbAUK1 alone (see Fig. 2).

Cells Depleted of Both TbAUK1 and TbPLK Show a Dominant TbAUK1-depleted Phenotype—To further investigate the relationship between TbAUK1 and TbPLK in controlling mitosis and cytokinesis in T. brucei, we knocked down the expression of both TbAUK1 and TbPLK simultaneously in the procyclic form. The inhibitory effects of RNAi on TbAUK1 and TbPLK gene expressions were verified by semi-quantitative RT-PCR analysis (Fig. 9A, inset), showing that the levels of TbAUK1 and TbPLK mRNAs are both diminished significantly after the induction of RNAi for only a day.

The effects of this double knockdown on cell growth were monitored by daily counting of the number of transfected cells in culture (Fig. 9A). The results indicate that the double knockdown essentially stopped cell growth within the 1st day upon RNAi induction, and the cell count began to drop gradually after 1 day. This is a more dramatic inhibitory effect than that from knocking down TbAUK1 alone (compare Figs. 9A and 2A). A knockdown of TbPLK alone in the procyclic form allowed the cells to grow almost normally for the ensuing 3 days before shutting off the growth completely (24). A synergistic inhibitory effect on cell growth thus appears to take place from knocking down both gene expressions. The slow decrease of cell number in the ensuing days could reflect gradual cell death caused by a shut down of both mitosis and cytokinesis.

FACScan analysis of the DNA content in double knockdown cells indicated that, after 1 day of RNAi induction, the population of G1 phase and S phase cells decreased from 51 to 21% and 35 to 18%, respectively, whereas the population of G2/M phase cells increased significantly from 14 to 61% (Fig. 9B). These data are once again highly similar to the TbAUK1-deficient cells (compare Fig. 9, B with C, and Fig. 2B), whereas the TbPLK-depleted cells are known to quickly convert to cells of 4C, 8C, or an even higher DNA content within 1 day (24). The double knockdown cells thus assume the same FACScan profile as the TbAUK1 single knockdown cells.

The morphology of DAPI-stained double knockdown cells was also examined with a fluorescence microscope. A decrease of 1N1K population from 75 to 26% and a corresponding accumulation of 1N2K cells from 12 to 51% were observed after 1 day of tetracycline induction (Fig. 10A). The 1N2K cells showed an elongated posterior end, an enlarged and extended nucleus, and two well separated kinetoplasts (Fig. 10B)
similar to those found in the TbAUK1-only knockdown cells (Fig. 3B). These are again very different from the TbPLK knockdown cells, which have a steady decrease in the 1N1K population and a corresponding increase in cells with multiple nuclei and multiple kinetoplasts (1N2K) within a day of RNAi induction (24). Apparently, the double knockdown controls the initiation of cytokinesis but plays no apparent role in controling mitosis or kinetoplast replication (24, 20) rather loose association among mitosis, kinetoplast replication, and the drive of the latter by kinetoplast segregation (18, 20). These distinctive functions of the two kinases may explain why TbAUK1 has a dominant effect in cell cycle regulation over TbPLK.

Cytokinesis in metazoa initiates in anaphase, beginning with furrow ingression, and ultimately cleaves the dividing cell into two daughter cells. An aurora B complex has emerged as a critical coordinator between mitosis and cytokinesis in vertebrates, Caenorhabditis elegans, Drosophila, and fission yeast (37), although its precise role has not yet been clarified. Inhibition of aurora B kinase is known to result in defective cytokinesis similar to that from depleting MKLP1, a kinesin-like protein that stabilizes and bundles overlapping anti-parallel microtubules in the midzone (38). Aurora B complex is known for its role of recruiting CeMKLP1/ZEN-4 in C. elegans (39). Coincidentally, one of the critical functions of the polo-like kinases among the eukaryotes other than T. brucei is to also regulate bipolar spindle formation by interacting with and regulating numerous microtubule-associated proteins, including MKLP1 (40). Here the functions of polo-like kinase and aurora B kinase appear to overlap. A close homologue of MKLP1 (Tb927.5.2090) has been identified in the Trypanosome Genomic Data Base and may turn out to be a substrate for both TbPLK and TbAUK1 for cytokinesis initiation. However, the apparent lack of involvement of TbPLK in mitotic control and the apparent dominance of TbAUK1 action over TbPLK complicate the situation. The septum initiation network for cytokinesis in fission yeast is initiated by the polo-like kinase (Plk1) without an apparent involvement of aurora B kinase (41). Therefore, the role of aurora B in promoting cytokinesis in fission yeast is probably by a pathway separate from the septum initiation network. It is thus interesting that TbAUK1 action should dominate that of TbPLK in T. brucei, suggesting an upstream position of TbAUK1 from TbPLK and a somewhat distinct mechanism of cytokinesis regulation in this organism.

This unique mechanism may be partly attributed to the sole function of TbPLK in initiating cytokinesis and the triple roles of TbAUK1. The rather loose association among mitosis, kinetoplast replication, and cytokinesis and the drive of the latter by kinetoplast segregation (18, 20) further reflect the important function of TbAUK1 in linking mitosis and kinetoplast replication with cytokinesis. The confinement of only two widely separated kinetoplasts in a TbAUK1-depleted cell indicates that kinetoplast replication is also positively regulated by this protein (17).
This inhibition of two separated kinetoplasts from replicating, observed for the first time in the trypanosomes, indicates a coordination between nuclear division and kinetoplast replication. However, the continued progression of mitosis and kinetoplast segregation when cytokinesis is blocked by TbPLK depletion also convincingly indicates that a blocked cytokinesis does not exert a feedback inhibition on either mitosis or kinetoplast segregation (24). Thus, the TbAUK1 function in promoting mitosis is apparently accompanied by its signaling of cytokinesis initiation after the two replicated kinetoplasts become separated, whereas TbPLK may act downstream from that signal.

The cell cycle regulation in the bloodstream form of T. brucei is known to differ significantly from that in the procyclic form. A mitotic arrest in the bloodstream form still allows re-entries into new G1 phases and continued nuclear DNA synthesis resulting in multiple nuclear aggregates (20). Cytokinesis is totally stopped by the mitotic arrest, which leads to no zoid formation except for cells filled with multiple kinetoplasts (16). A recent knockdown of TbAUK1 from the bloodstream form produced cells with multiple nuclear aggregates as well as multiple kinetoplasts, basal bodies, and flagella in a rounded cell shape.3 These apparently distinctive roles played by TbAUK1 in the two forms of trypanosome may provide a good opportunity to dissect the different mechanisms of cell cycle regulation in the two forms.

The single aurora B homologue, Ip11, in budding yeast monitors tension at kinetochores, interacts with the inner centromere protein Pic1, and mediates chromosome segregation (42). It promotes chromosome bi-orientation by destabilizing the attachment of microtubules on mono-oriented kinetochores (43). This is made possible by the formation of a complex among Ip11, Sli15 (INCENP), and Bir1 (survivin) that can sense the tension generated by the attachment of sister kinetochores to opposite spindle poles by interacting with the Dam1 outer kinetochore microtubule-binding complex and the Ndc80 complex (42). Several Ip11 targets have been identified in the Dam1 complex (Dam1, Ask1, Spc34, and Spc19) and the Ndc80 complex (3). Another key inner kinetochore component Ndc10p has

3 Z. Li and C. C. Wang, unpublished data.
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![FIGURE 10. Morphological phenotypes of the double knockdown cells. The cells from 1 day of double knockdown were stained with DAPI and examined with a fluorescence microscope. A, quantification of cells with different numbers of nuclei (N) and kinetoplasts (K). Data are presented as the mean percentages ± S.E. of the total populations counted (>200 cells in each of three independent experiments). B, the double knockdown cells mostly in the 1N2K state.](image)

been also reported to be a likely Ipl1p target (44). However, a recent search of the Trypanosome Genomic Data Base indicated that none of the above-mentioned proteins has a close homologue in the trypanosome. It is thus difficult to postulate at present whether TbAUK1 regulates mitosis by a similar mechanism involving structurally distinctive proteins or whether it acts by a different mechanism in trypanosomes.

In conclusion, we have identified in the procyclic form *T. brucei* an aurora kinase B homologue, which controls both mitosis and cytokinesis initiation. The irony lies in the fact that cytokinesis in the trypanosomes is not under rigid regulation of mitosis. But TbAUK1 is capable of playing a role in completing mitosis and initiating cytokinesis after the completion of the kinetoplast cycle. In the mutants depleted of CycB2 or CRK3, the mitotically arrested cells are still capable of completing cytokinesis and cell division, whereas the TbAUK1-depleted cells cannot. Just how TbAUK1 coordinates mitosis with kinetoplast segregation and cytokinesis initiation for a smooth cell cycle progression in the procyclic form remains a major challenge in future studies.

Acknowledgments—We thank Professor W. Zacheus Cande of the University of California, Berkeley, for the valuable and stimulating discussions with us during the progress of this work and for most helpful comments and suggestions on the manuscript. We are also grateful to Dr. Lee Douglas of the University of California, Berkeley, for critical reading of the manuscript and the many useful suggestions. We also thank Dr. Keith Gull of Oxford University for the generous gifts of ROD1 and KMX-1 antibodies.

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