Polo-like kinase is required for Golgi and bilobe biogenesis in *Trypanosoma brucei*

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A bilobed structure marked by TbCentrin2 regulates Golgi duplication in the protozoan parasite *Trypanosoma brucei*. This structure must itself duplicate during the cell cycle for Golgi inheritance to proceed normally. We show here that duplication of the bilobed structure is dependent on the single polo-like kinase (PLK) homologue in *T. brucei* (TbPLK). Depletion of TbPLK leads to malformed bilobed structures, which is consistent with an inhibition of duplication and an increase in the number of dispersed Golgi structures with associated endoplasmic reticulum exit sites. These data suggest that the bilobe may act as a scaffold for the controlled assembly of the duplicating Golgi.

Introduction

Proper inheritance of a full complement of organelles is a characteristic feature of cell division. The inheritance process, known as biogenesis, proceeds in two distinct steps: duplication of the organelle followed by partitioning of the replicated structures. The biogenesis of the secretory pathway is a complex process that requires the orchestrated inheritance of multiple membrane-bound compartments (Shorter and Warren, 2002). This process has been extensively studied in mammalian cells, with a particular emphasis on the partitioning of duplicated Golgi into the two daughter cells (Munro, 2002). However, the complexity of the Golgi in higher eukaryotes, in which many stacks are assembled into a higher order Golgi ribbon (Ladinsky et al., 1999), has made it difficult to study the duplication process.

A variety of unicellular eukaryotes have simpler Golgi, including *Pichia pastoris*, *Toxoplasma gondii*, and *Trypanosoma brucei*, and these have been used to determine how the Golgi duplicates (Field et al., 2007; He, 2007). These organisms have fewer Golgi, with *P. pastoris* maintaining two to five separate organelles (Bevis et al., 2002), whereas *T. gondii* and *T. brucei* have only a single stack that duplicates during the cell cycle (Duszenko et al., 1988; Field et al., 2000; Pelletier et al., 2002; He et al., 2004). In *T. brucei*, the new Golgi is assembled at a fairly fixed distance from the old (He et al., 2004), and this location appears to be determined by a bilobed structure defined by the Ca^{2+}-binding protein TbCentrin2. One lobe of this structure is associated with the old Golgi and the other becomes associated with the new, which suggests that it marks the position of future growth. This bilobe itself undergoes duplication during the cell cycle, whereas the old bilobe remains associated with the old Golgi and the new bilobe with the new. This ensures that each daughter cell inherits one bilobe and one Golgi. The importance of this bilobed structure for Golgi biogenesis is confirmed by depletion of TbCentrin2 using RNAi, which leads to an inhibition of Golgi duplication (He et al., 2005).

Multiple kinases have been implicated in the partitioning of the Golgi during mitosis in mammalian cells (Shima et al., 1997, 1998) including Mek1 (Acharya et al., 1998; Feinstein and Linstedt, 2007), Erk1c (Shaul and Seger, 2006), Cdc2 (Lowe et al., 1998), and polo-like kinase (PLK; Sutterlin et al., 2001; Preisinger et al., 2005). Mammalian cells have four different PLK homologues that vary in their function and expression profiles (van de Weerdt and Medema, 2006). Their localization is mediated by a C-terminal domain known as the polo box, which binds to specific phosphorylated sequences in target proteins (Elia et al., 2003a,b). In addition to their role in Golgi partitioning, they have also been implicated in spindle pole assembly, centriole maturation, and cytokinesis (Barr et al., 2004; Sumara et al., 2004).

T. brucei contains a single PLK homologue that has also been implicated in cytokinesis (Kumar and Wang, 2006; Hammarton et al., 2007). Depletion of the kinase leads to growth arrest and an accumulation of cells with abnormal DNA content, which is consistent with results in mammalian cells. PLK may also be involved in the duplication of the basal bodies and the flagellum.

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Abbreviations used in this paper: ERES, ER exit site; FAZ, flagellum attachment zone; PLK, polo-like kinase.

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Given the role played by PLK in Golgi biogenesis in mammalian cells, we decided to investigate whether PLK in *T. brucei* might not only be involved in cytokinesis but also in Golgi biogenesis. Here, we show that the PLK homologue in *T. brucei* (TbPLK) plays a key role in the biogenesis of the bilobe, which in turn affects the biogenesis of the Golgi.

Results and discussion

T. brucei has a highly organized cellular anatomy, with most organelles present in a single copy, and the duplication of these organelles occurs at precise times during the cell cycle, which typically lasts 9 h (Fig. 1 A; Sherwin and Gull, 1989; He et al., 2004). The basal body (comprising two centrioles) nucleates the flagellum and is the first organelle to duplicate, and the new basal body initiates growth of the new flagellum. The Golgi, situated between the nucleus and the flagellar pocket, is next to undergo duplication, followed by duplication of the bilobe. Additional, smaller Golgi also appear at about this time. Bilobe duplication is followed by duplication and segregation of the kinetoplast (which contains all of the mitochondrial DNA) mediated by the separating basal bodies. Division of the nucleus is followed by cytokinesis, generating two daughters with the same complement of organelles.

The flagellum adheres to the cell body via the flagellum attachment zone (FAZ), and previous work by Kumar and Wang (2006) showed that TbPLK might be a marker for this structure. Because they used a tagged copy of TbPLK, we decided to repeat the results using affinity-purified antibodies raised against TbPLK. As shown in Fig. 1 B, we were able to find TbPLK at the growing tip of the new FAZ, which is similar to the localization found by Kumar and Wang (2006). The tip of the FAZ tracks the growth of the new flagellum as it grows from the posterior end of the cell to the anterior (Fig. 1 B, arrowheads).

Interestingly, earlier in the cell cycle, TbPLK was present in some cells near to the kinetoplast in a structure reminiscent of the bilobe (Fig. 1 B, b, arrowhead). To confirm this, cells were fixed and double labeled with antibodies to TbPLK and the monoclonal antibody 20H5. This antibody recognizes TbCentrins 1 and 2 and labels the bilobe, the basal bodies, and, occasionally, the flagellum (He et al., 2005). TbPLK partially colocalized with the bilobed structure (Fig. 1 C). At early times, TbPLK was concentrated at the basal bodies and the posterior lobe of the bilobe (Fig. 1 C, a–c). Once basal body replication had occurred, the TbPLK signal increased on the duplicating bilobe, which had assumed an amorphous shape (Fig. 1 C, d–f and g–i). Once bilobe duplication was complete, the TbPLK signal at the bilobe was lost as the kinase appeared at the growing tip of the new FAZ (Fig. 1 C, j–l and m–o). TbPLK did not colocalize with the Golgi at any stage, as shown using cells expressing the retention domain of a putative glycosyltransferase (GntB) fused to YFP (Fig. S1, available at http://www.jcb.org/cgi/content/full/jcb.200708082/DC1).

To confirm a role for TbPLK in bilobe function, a cell line was generated to allow depletion of this kinase. Previous work had shown that depletion of TbPLK leads to defects in cytokinesis and the accumulation of multinucleated cells (Kumar and Wang, 2006; Hammarton et al., 2007). To deplete PLK in *T. brucei*, we used an inducible, inheritable system for producing double-stranded RNA (Wirtz et al., 1999). Blotting with TbPLK antisera showed very efficient depletion after only 8 h of induction (Fig. S2 A, available at http://www.jcb.org/cgi/content/full/jcb.200708082/DC1). The growth of cells was monitored with or without the induction of TbPLK RNAi. There was no difference after 8 h, but at 20 h, the cells lacking TbPLK had ceased to divide and remained arrested for the length of the experiment (Fig. S2 B). Measurements of cell size showed that the TbPLK RNAi cells at later time points had increased to ∼150% the size of uninduced cells (from 4.5 to 6.4 μm; Fig. S2 C). This increase in size was evident by bright-field microscopy, which also showed that many of the cells had multiple detached flagella consistent with previously reported data (Fig. S2 D, arrowheads; Kumar and Wang, 2006). DAPI labeling of the TbPLK RNAi cells at different time points showed the accumulation of aberrant DNA (Fig. S2 E). 8 h after TbPLK RNAi induction, mitosis proceeded, but there was a slight decrease in cells in the 1N2K state (Fig. S2 E). At 16 h, cells began to accumulate in the 2N2K state, which suggests that there was some delay in cytokinesis. Aberrant 2N1K cells lacking a duplicated kinetoplast began to appear. At 24 h, a large number of cells were multinucleated along with an increased number of anucleate (0N1K) cells. Interestingly, nuclear duplication did not appear to be affected.

Cells depleted of TbPLK were then examined for bilobe morphology. Cells were observed 18 h after RNAi induction, a point when TbPLK depletion was evident but before the accumulation of multinucleated cells. Fixed cells were labeled with 20H5 and DAPI. A clear defect in bilobe morphology and number was evident in cells where TbPLK had been depleted. Fewer 1N1K cells had two bilobes (6% compared with 33% in control cells), which suggests a delay in the biogenesis of this structure (Fig. 2 A). Furthermore, 46% of the bilobes were malformed compared with 7% in control cells. In control cells where the kinetoplast had duplicated (1N2K), essentially all the cells had two bilobes (Fig. 2 B). In cells depleted of TbPLK, this dropped to 28%, the rest having one bilobe (20%) or malformed bilobes (51%). A similar pattern was seen in cells after karyokinesis (2N2K; Fig. 2 C). More than 96% of the control cells had two bilobes, and this dropped to 35% in cells depleted of PLK; the rest had one bilobe (11%) or malformed bilobes (53%). Examples of the bilobe structures used to assemble these data are shown in Fig. 2 D for control (a–c) and TbPLK-depleted cells (d–f). The malformed bilobes contained extra processes that gave them a disorganized appearance.

The 20H5 antibody used to identify the bilobe also detects basal bodies, making it possible that the observed defects in the TbPLK RNAi cells were caused by aberrant basal body duplication or maintenance. This seems unlikely, given previously reported results showing that basal body duplication was not affected after TbPLK depletion and that flagellar duplication, which depends on basal body maturation, proceeded normally (Kumar and Wang, 2006). However, recent work has suggested that 2N1K cells generated by PLK RNAi may have basal body defects (Hammarton et al., 2007). To check if this is the case in cells with normal DNA content, cells were depleted

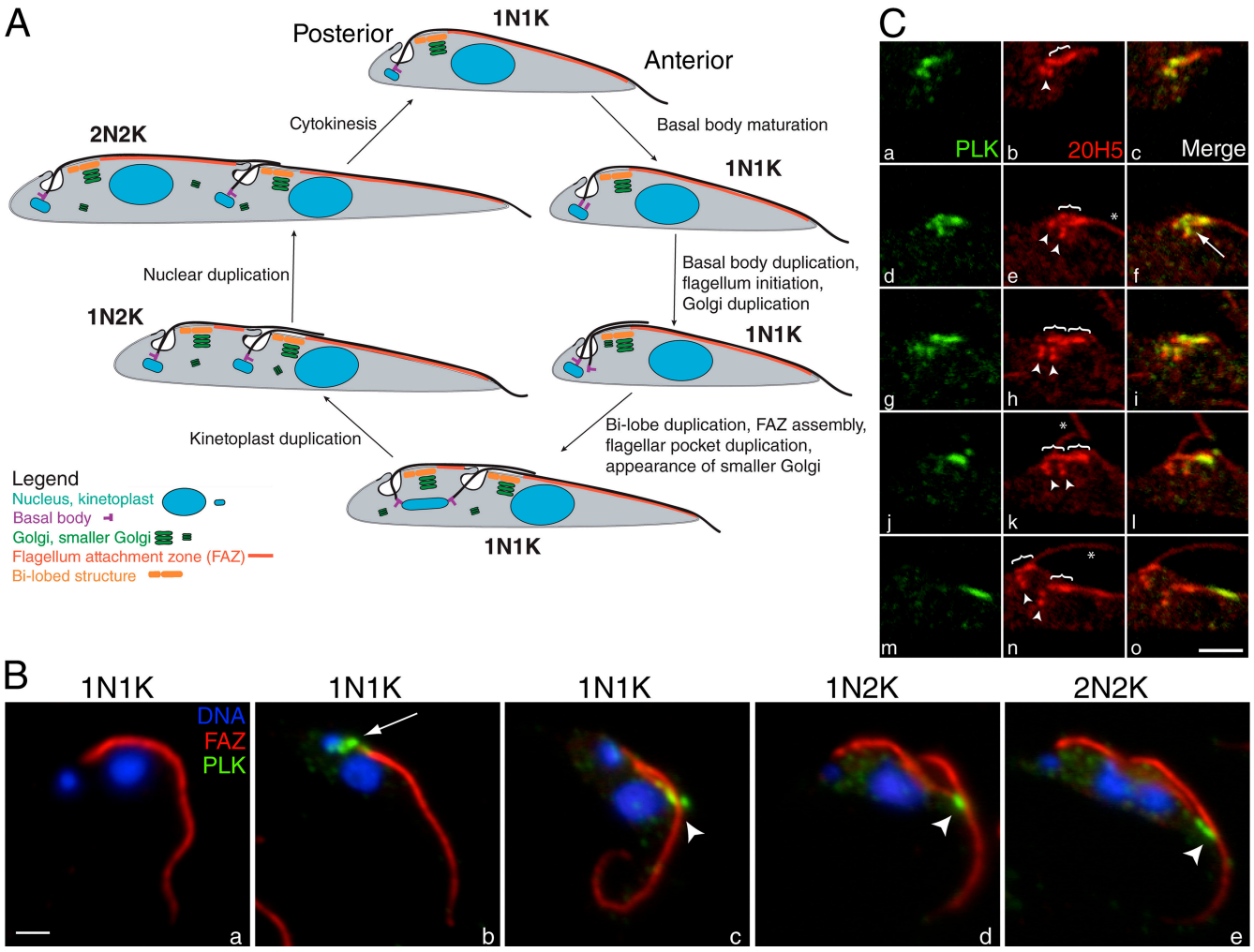

Figure 1. **TbPLK localizes to the bilobed structure during its duplication.** (A) Schematic representation of the *T. brucei* life cycle. See text for details. (B) TbPLK localized to a region near the kinetoplast and then proceeded along the growing tip of the new FAZ. Cells were fixed and labeled for the FAZ with the monoclonal antibody L3B2 (FAZ; red), TbPLK (PLK; green), and DNA (DNA; blue). TbPLK was not expressed in many 1N1K cells (a) and first appeared near the kinetoplast (b, arrow) then tracked along the growing tip of the new FAZ (c–e, arrowheads). (C) Duplication of the bilobe during the cell cycle. Cells were fixed and labeled with antibodies against TbPLK (PLK, green) and the monoclonal antibody 20H5 (red), which labels the bilobe (brackets), basal bodies (arrowheads), and, occasionally, the flagella (asterisks). The panels show single confocal sections of duplicating bilobes from early in the process until two fully formed structures are present. TbPLK was initially associated with the basal bodies and the more posterior lobe of the bilobe (a–c). Once the basal bodies had duplicated, the bilobe assumed a more amorphous shape, which likely represents its duplication phase (d–f). TbPLK occasionally bridged the basal bodies and the bilobe (arrow in f). The bilobe then took on an elongated shape and colocalized less with TbPLK (g–i). Once two bilobes were clearly visible, the TbPLK signal was concentrated at the anterior tip of the new bilobe (j–l). As the two bilobes separated, TbPLK was found at the growing tip of the new FAZ (m–o). Bars: (B) 2 μm; (C) 1 μm.

of TbPLK and labeled with 20H5. Basal bodies were labeled with YL1/2, which detects tyrosinated tubulin (Sherwin et al., 1987). The basal bodies in these cells were intact and appeared to be duplicating normally (Fig. 2 E). The malformed structures labeled by 20H5 were not labeled with YL1/2, which argues that it is the bilobes not the basal bodies that are affected by depletion of TbPLK.

Because the bilobe is important for Golgi duplication, cells depleted of TbPLK were next analyzed for the presence of Golgi using antibodies against the *T. brucei* homologue of the Golgi matrix protein GRASP (He et al., 2004). Under normal conditions, the Golgi duplicates before the kinetoplast followed by the appearance of some smaller Golgi, most of which disappear before cytokinesis (Fig. 1 A; He et al., 2004). 1N1K cells usually contain either one or two Golgi, with a few cells containing three or four Golgi (Fig. 3 A). Upon TbPLK RNAi induction, the number of cells having one or two Golgi diminished, whereas those with three or more tripled (from 20 to 64%, Fig. 3 A). Cells with five or more Golgi, which were not observed in the uninduced sample, comprised almost 20% of the population (Fig. 3 A). At the 1N2K stage, TbPLK depletion led to a decrease in cells with two Golgi (from 52% in the uninduced cells to 16%; Fig. 3 B) and a concomitant increase in cells with three or more Golgi (from 48% to 80%; Fig. 3 B). As with the 1N1K state, cells with five or more Golgi became more common (from 2% in the uninduced cells to 16%; Fig. 3 B). The difference in Golgi number was less evident at the 2N2K stage because of the additional Golgi that appeared before cytokinesis (Fig. 1 A). However, the PLK-depleted cells were much more likely to have six or

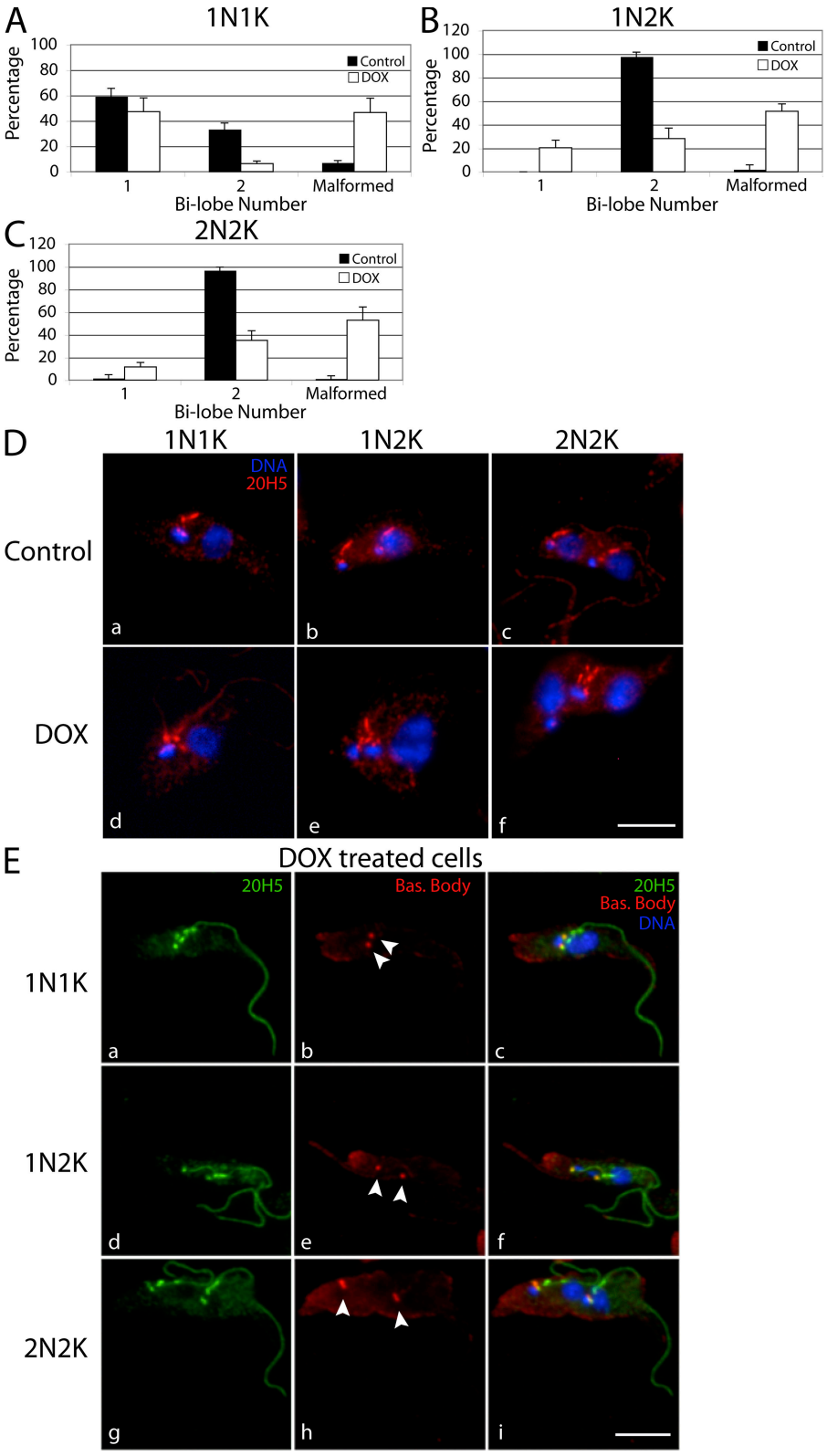

Figure 2. **TbPLK depletion caused defects in bilobe duplication.** (A–C) TbPLK RNAi cells were treated with ethanol (control) or doxycycline (DOX) for 18 h and then labeled for DNA with DAPI and the bilobed structure (and basal bodies) with 20H5. The number and state of the bilobe in cells with normal DNA content were quantitated in three independent experiments and expressed as the mean ± SEM. (n = 621 for control cells and 663 cells for DOX). (D) Examples of cells from the datasets in A–C (DNA, blue; bilobe, red) showing normal duplication of the bilobe (a–c) and malformed bilobes (d–f). (E) The perturbed 20H5 staining was not the product of aberrant basal body production. TbPLK RNAi cells were induced for 18 h and then labeled with 20H5 (green) and YL1/2 (bas. body; red), which detects tyrosinated tubulin present on the basal bodies. The usual number of basal bodies (arrowheads) were present at all cell cycle stages. Bars, 3 µm.

more Golgi (from 8% in the uninduced cells to 36% in the induced cells; Fig. 3 C). Examples of the Golgi used to assemble these data are shown in Fig. 3 D. The figure shows the normal progression (Fig. 3 D, top) and examples of the high numbers of Golgi that can be found in cells depleted of TbPLK (Fig. 3 D, bottom).

In *T. brucei*, the ER exit site (ERES) and Golgi form a functional unit that duplicates and is inherited together (He et al., 2004). Even the smaller Golgi that appear and then largely disappear before cytokinesis have associated ERES (He et al., 2004). To test if TbPLK depletion leads to disruption of this association, the COPII component Sec13, a marker of the ERES

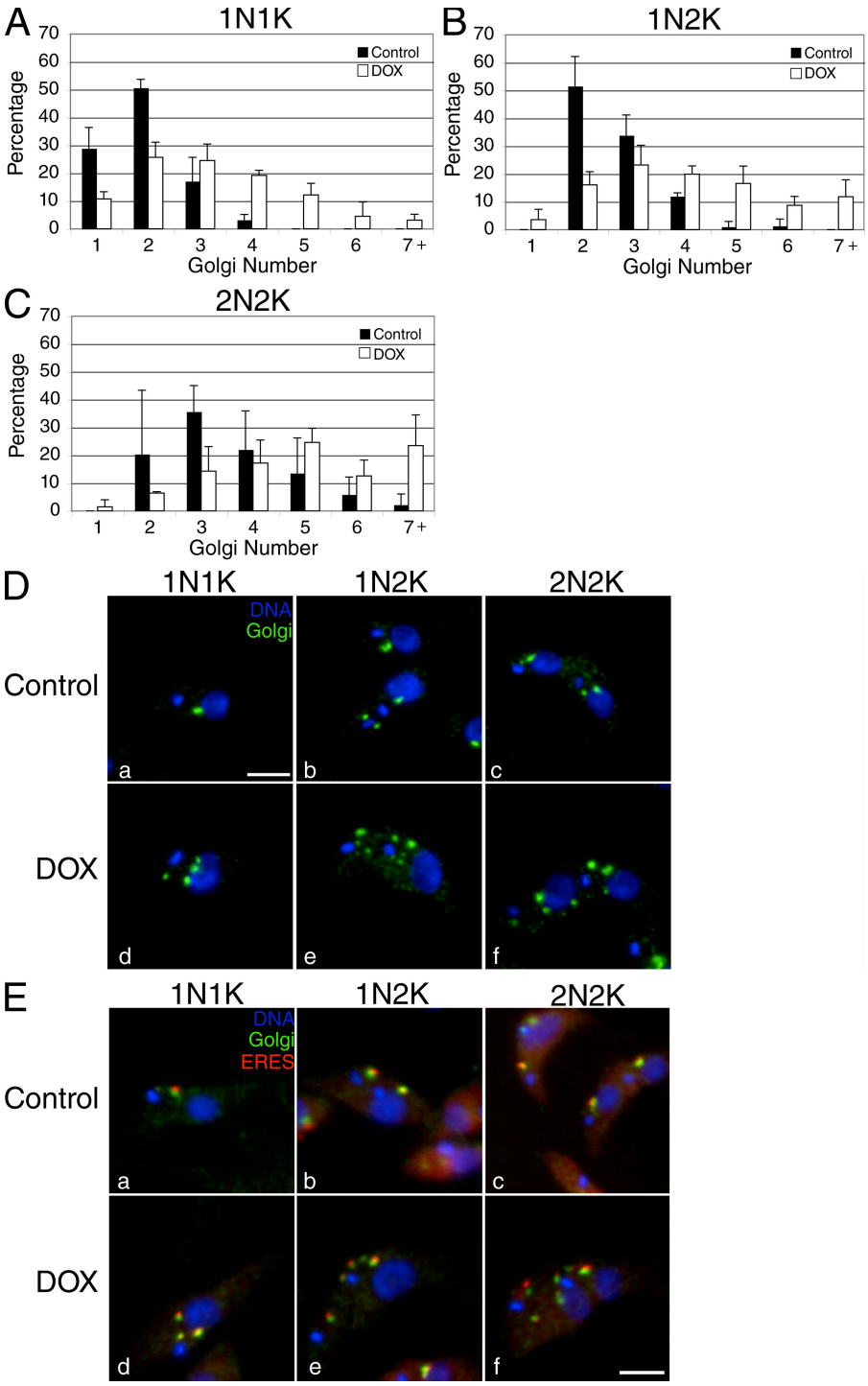

Figure 3. **TbPLK depletion causes an increase in Golgi and ERES number.** (A–C) Cells containing the TbPLK RNAi construct were treated with ethanol (control) or doxycycline (DOX) for 18 h and then labeled for DNA (DAPI) and the Golgi (anti-TbGRASP). The number of Golgi in individual cells with normal DNA content was quantitated in three independent experiments and expressed as the mean ± SEM. (n = 621 for control cells and 663 cells for DOX). (D) Examples of cells from the datasets in A–C, (DNA, blue; Golgi, green) showing normal duplication of the Golgi and the appearance of one or two extra Golgi (a–c) and examples of cells with very high numbers of Golgi after TbPLK depletion (d–f; up to nine Golgi). (E) Cells expressing the TbPLK RNAi construct and Sec13-YFP (ERES, red) were treated as in D. Note that the pairing of the ERES and Golgi observed at all stages of the cell cycle in control cells (a–c) was not disrupted in the cells where TbPLK had been depleted (d–f) even though the cells have more Golgi and ERES. Bars, 3 μm.

in *T. brucei* (He et al., 2004), was fused to YFP and stably expressed in the TbPLK RNAi background (Fig. 3 E). All of the Golgi in control cells were partnered with an ERES (Fig. 3 E, a–c), and this did not change after depletion of TbPLK, even in cells containing high numbers of Golgi (Fig. 3 E, d–f). This suggests that the association between these organelles is not dependent on the presence of TbPLK.

Having shown that TbPLK depletion causes defects in bilobe duplication and Golgi number, we sought to show that these two observations were directly connected. In other words, that cells with malformed bilobes also had abnormal Golgi numbers. Cells were depleted of TbPLK followed by labeling with 20H5 and antibodies against TbGRASP. Results were plotted as the number of Golgi and bilobes (and malformed bilobes) contained within each cell (Fig. 4 A). Cells that had malformed bilobes were much more prevalent in cells depleted of TbPLK (52% vs. 6% in the uninduced control; Fig. 4 A), and these cells were much more likely to have three or more Golgi (44% vs. 2% in the uninduced control; Fig. 4 A). Examples of the control and induced cells used to assemble these data are shown in Fig. 4 B.

A

Bi-lobe Number	Golgi Number		
CTRL	1	2	3+
1	18	21	6
2	1	24	24
MF	1	3	2

B

Bi-lobe Number	Golgi Number		
DOX	1	2	3+
1	4	9	19
2	0	2	13
MF	2	6	44

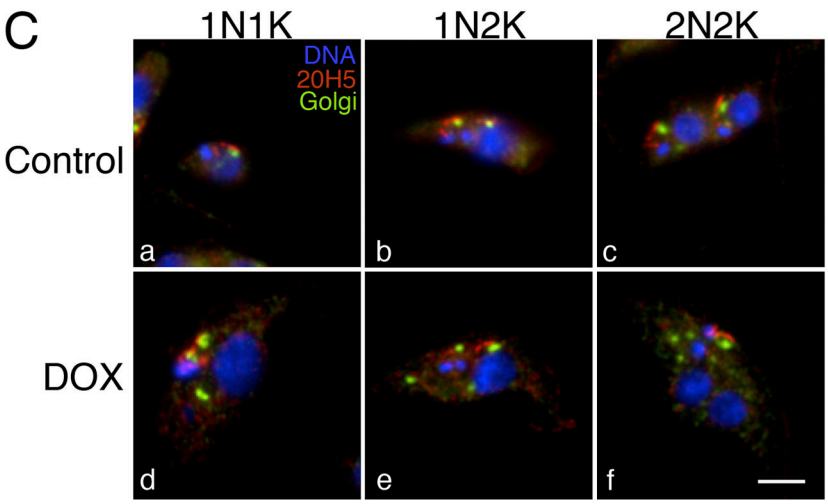

Figure 4. **TbPLK depletion simultaneously affects bilobe duplication and Golgi number.** (A) Cells containing the TbPLK RNAi construct were treated with ethanol (CTRL) or doxycycline (DOX) for 18 h and then labeled for DNA (DAPI), the bilobed structure (20H5), and the Golgi (anti-TbGRASP). The number and state of the bilobed structure and the number of Golgi in individual cells was quantitated. The results from three separate experiments were tabulated to show the number of Golgi present in cells with different numbers and type of bilobe. The numbers are presented as the percentage with a total of 621 cells for the control and 868 cells for the DOX sample. MF, malformed. (B) Examples of cells from the datasets in A (DNA, blue; bilobe, red; Golgi, green) showing bilobe and Golgi duplication, both normal (a–c) and abnormal (d–f). Cells with abnormal bilobes had abnormal Golgi numbers. Bar, 3 μm.

This result links proper duplication of the bilobe structure with Golgi number.

The localization of TbPLK, along with the effects of its depletion, suggests that the kinase might phosphorylate bilobe resident proteins that are essential for its duplication. At present, the only proteins known to localize to the bilobe are TbCentrin2 (He et al., 2005) and TbCentrin4 (Selvapandiyan et al., 2007). To test if either of these proteins could serve as a TbPLK substrate, they were expressed in *E. coli* and subjected to ^{32}P kinase assays using bead-immobilized YFP-TbPLK (Fig. 5, A and B; Hammarton et al., 2007). Other kinase substrates were included, such as α- and β-casein as well as TbGRASP, whose mammalian homologue GRASP65 is phosphorylated by PLK1 (Lin et al., 2000). YFP-TbPLK phosphorylated α casein but showed minimal activity on β-casein, which is consistent with previously published work (Fig. 5 B; Hammarton et al., 2007). The kinase showed minimal activity toward TbGRASP and TbCentrin4 but avidly phosphorylated TbCentrin2, which suggests that this isoform might be a substrate for TbPLK in vivo (Fig. 5 B). Experiments are underway to determine whether TbCentrin2 is phosphorylated by TbPLK in vivo and whether this phosphorylation is necessary for bilobe duplication.

Depletion of TbCentrin2 inhibits Golgi duplication (He et al., 2005), so if TbPLK works through TbCentrin2, one might expect that depletion of TbPLK would have the same effect. In fact, the opposite was found, with the number of Golgi increasing during the cell cycle. Although normal cells would typically have up to four Golgi (old, new, and two smaller Golgi), cells depleted of TbPLK frequently had seven or more Golgi. It is difficult to say at present whether these additional Golgi represent an increase in the number of smaller Golgi that are normally seen later in the cell cycle. What is clear is that each is partnered with an ERES so that they likely function to transport cargo proteins. Furthermore, most of these additional Golgi do not appear to be associated with the malformed bilobes. One possibility is that TbPLK has other targets on the bilobe and/or

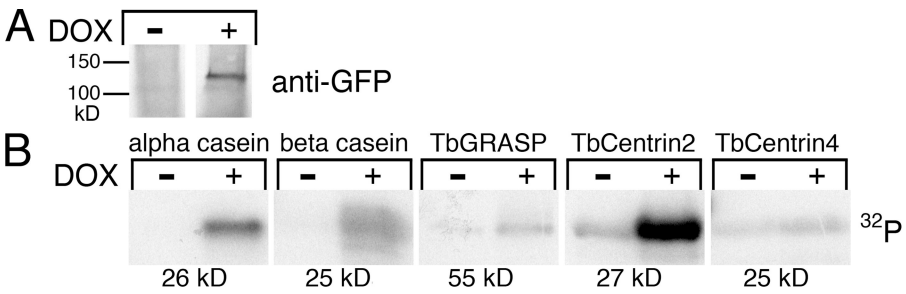

Figure 5. **YFP-TbPLK phosphorylates TbCentrin2 in vitro.** (A) Cells expressing inducible YFP-TbPLK were treated with (+) or without (−) doxycycline for 12 h, lysed, and incubated with rabbit anti-GFP immobilized on beads, and bound proteins were fractionated by SDS-PAGE and probed using mouse anti-GFP antibodies. A protein of the correct molecular weight for YFP-TbPLK was present only in the induced cells. (B) Immobilized YFP-TbPLK was incubated with the indicated proteins and γ-^{32}P ATP followed by fractionation on SDS-PAGE and autoradiography.

the Golgi that mediates association of the two. If the bilobe acts as the site where a new Golgi is constructed, then the malformed bilobe that results from TbPLK depletion might no longer be able to act as this site. In this instance, new Golgi will be made de novo, independently of the bilobe (just as the smaller Golgi are). The increased number is more difficult to explain. One possibility relates to the size of the TbPLK-depleted cells. They are bigger and so could perhaps accommodate more Golgi. Alternatively, it is interesting that the extra Golgi seen in normal cells are not associated with the bilobe and are smaller. Perhaps the bilobe also regulates the size of the growing Golgi and, without a functional bilobe, more, smaller Golgi are made instead. The function of the bilobe would then be to ensure that the Golgi is of a sufficient size to service the needs of each newly formed daughter cell, something of particular importance for parasitic organisms such as *T. brucei*, which have to evade the defense mechanisms of the host. A test of this idea will require will detailed information concerning the structure and composition of the bilobe.

Materials and methods

The 29-13 (Wirtz et al., 1999) cell line expressing the tetracycline repressor and T7 RNA polymerase was supplied by G. Cross (The Rockefeller University, New York, NY). The cells were cultured in Cunningham's media supplemented with 15% tetracycline-free FBS, 15 µg/ml neomycin, and 50 µg/ml hygromycin at 27°C. An antibody against TbGRASP has been described previously (He et al., 2004). The 20H5 anti-centrin monoclonal antibody was provided by J. Salisbury (Mayo Clinic, Rochester, MN). The anti-FAZ monoclonal antibody L3B2 was provided by K. Gull (Oxford University, Oxford, UK). The YL1/2 monoclonal antibody was obtained from Millipore.

To generate antibodies against PLK, a C-terminal fragment of the protein corresponding to the polo box domain (aa 468–768) was expressed in *E. coli* as a His_6-fusion. After purification, this fragment was used to immunize rabbits, whose serum was subsequently affinity purified using a GST-tagged form of the polo box domain to yield specific antibodies toward PLK.

To deplete PLK, RNAit (Redmond et al., 2003) was used to identify a suitable fragment of the PLK gene corresponding to nt 1,691–2,166, which was subsequently amplified from 29-13 genomic DNA and cloned into pZJM (Wang et al., 2000). The RNAi construct was stably integrated into 29-13 cells and individual clones were isolated using limiting dilution. The expression of double-stranded RNA was initiated by the addition of 10 µg/ml doxycycline. The Sec13-YFP construct has been described previously (He et al., 2004) and was stably expressed in the PLK RNAi cell line. For the GntB-YFP construct, the first 56 amino acids of the glycosyltransferase, comprising the Golgi localization sequence, were fused to the N terminus of YFP. All constructs were verified by DNA sequencing.

For cell counting and size analysis, a cell and particle counter (Z2 Coulter Counter; Beckman Coulter) was used. Events that fell between 3 and 10 microns were analyzed.

For Western blotting, 3×10^6 cells were used for each time point. The cells were lysed directly in SDS loading buffer and the lysates were separated using 4–20% acrylamide gels. The separated proteins were transferred to nitrocellulose and probed using the anti-TbPLK antisera. To confirm equivalent loading, the blot was stripped and reprobed with antibodies against α-tubulin. Horseradish peroxidise–labelled secondary antibodies, ECL reagent, and film (Kodak) were used for detection.

The TbPLK isolation and subsequent kinase assays were adapted from a previously published protocol (Hammarton et al., 2007) to a cell line expressing inducible YFP-TbPLK. Lysates from induced and uninduced cells were immunoprecipitated with anti-GFP and incubated with recombinantly expressed TbCentrin2, TbCentrin4, and TbGRASP. α- and β-casein were also included as substrates. The reactions were fractionated on acrylamide gels, which were dried and exposed to film.

For immunofluorescence, cells were taken from culture and washed once with PBS. The cells were then adhered to glass coverslips and immersed for 9 min in −20°C methanol. The cells were rehydrated in PBS and blocked overnight in blocking buffer (3% BSA in PBS). Primary antibodies were diluted in blocking buffer and applied to the coverslips for 1 h at room temperature. The coverslips were then washed three times in PBS, placed in blocking buffer for 30 min, and incubated with secondary antibodies for 1 h at room temperature. Alexa 594–conjugated chicken and Alexa 488–conjugated goat secondary antibodies (Invitrogen) were used for detection. For quantitation purposes, cells were imaged using a microscope (Axioskop 2) equipped with an Apotome system for z sectioning, a motorized stage, a camera (Axiocam), and a Plan Apochromat 63× NA 1.4 oil lens (all from Carl Zeiss, Inc.). The tiling function was used to generate large montaged datasets, usually spanning four x-y fields and eight z sections, which were exported as TIFFs and assembled in ImageJ using Image5D (rsb.info.nih.gov.ij).

For the colocalization studies, a confocal microscope (LSM510) was used with a Plan Apochromat 63× NA 1.4 oil lens. Images were acquired with a zoom setting of 2, x-y resolution of 0.08 µm per pixel, and z step size of 0.2 µm. The pinhole in the 488-nm channel was set to 1 Airy unit, corresponding to an optical section thickness of 0.7 µm. For multichannel imaging, the pinholes of the non-488-nm channels were set to produce identical optical section thickness in all channels while keeping the pinhole diameter at 1 Airy unit in the 488-nm channel. Individual channels were converted to TIFF images and exported and assembled in Photoshop CS3 (Adobe).

Online supplemental material

Fig. S1 shows that TbPLK localizes to the bilobed structure before Golgi duplication. Fig. S2 contains a full characterization of the TbPLK RNAi cell line, including Western blots of TbPLK depletion, growth curves, and DNA counts in the presence and absence of RNAi induction. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200708082/DC1.

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