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# An Evolutionarily Conserved Coiled-Coil Protein Implicated in Polycystic Kidney Disease Is Involved in Basal Body Duplication and Flagellar Biogenesis in *Trypanosoma brucei*†

Gareth W. Morgan,[1]‡ Paul W. Denny,[1]§ Sue Vaughan,[2] David Goulding,[1] Tim R. Jeffries,[1]¶ Deborah F. Smith,[1]‖ Keith Gull,[2] and Mark C. Field[1]*

*Department of Biological Sciences, Imperial College, London,[1] and Sir William Dunn School of Pathology, University of Oxford, Oxford,[2] United Kingdom*

Supplementary data: methods and Figures S1 and S2.

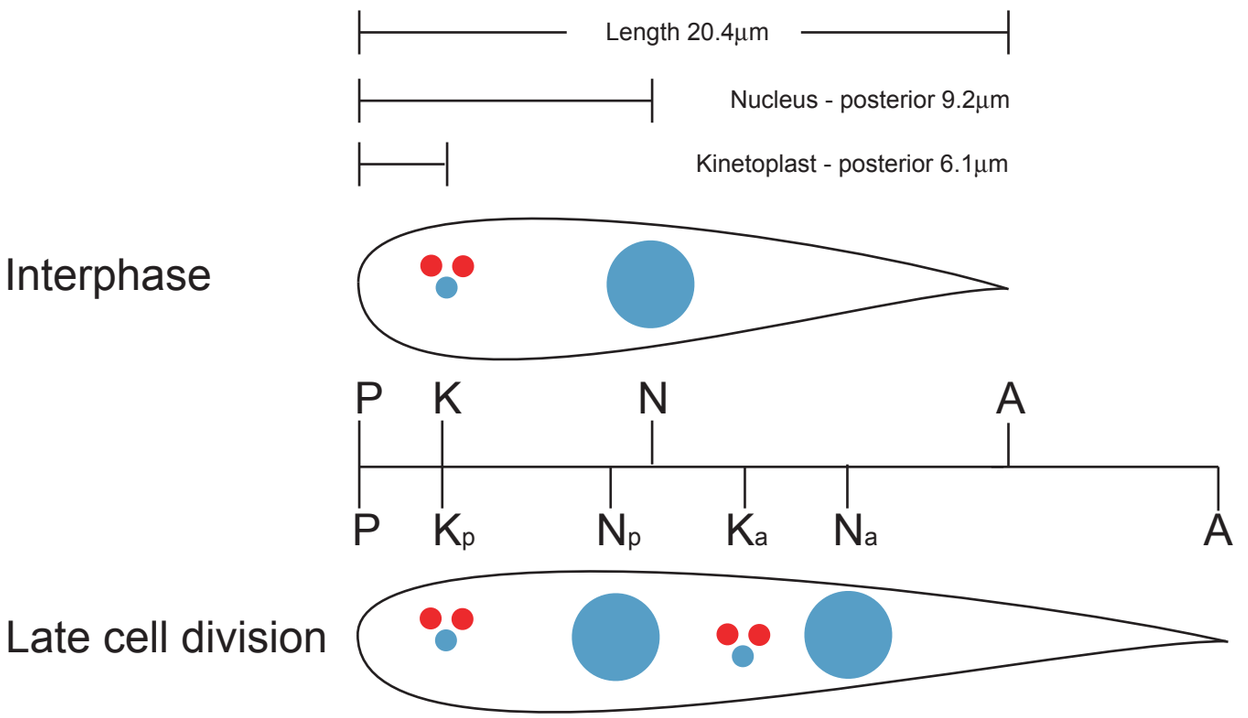
*Expression and location of TbLRTP:* Polyclonal antisera against recombinant TbLRTP-GST fusion protein were generated in rabbits and affinity purified. The specificity of the antibodies was demonstrated by immunoprobing *E. coli* lysates containing GST-fusion proteins of trypanosome TbDLP (Morgan *et al.*, 2004) or TbLRTP (Fig. S1A). Reactivity was obtained against a band at ~66kDa in the TbLRTP lysate only, consistent with the predicted molecular weight of TbLRTP-GST. Lower molecular weight reactivity was variable and likely due to non-specific proteolysis. In lysates prepared from two major trypanosome life stages anti-TbLRTP serum detected a single protein of ~43 kDa, whilst immunofluorescence localised TbLRTP to structures juxtaposed to the kinetoplast (K) and anterior regions towards the nucleus (N) (Fig. S1B). As the kinetoplast is physically attached to the basal body and flagellum the location of TbLRTP suggests an association with components of the flagellum and/or basal body (Robinson and Gull, 1991; Ogbadoyi *et al.*, 2003).

**Figure S1. TbLRTP protein is expressed in both major *T. brucei* life stages and is localised to the posterior region of the cell.** A: Left; affinity purified rabbit antibodies generated against a GST-TbLRTP fusion protein exhibit specific reactivity against TbLRTP as demonstrated by immunoprobing of lysates of *E.coli* expressing a dynamin-GST fusion protein or TbLRTP-GST fusion protein. Right; immunoprobing of lysates from 2 x $10^7$ cell equivalents of bloodstream (BSF) and procyclic form

(PCF) cells with anti-TbLRTP antibodies demonstrates approximately equivalent expression levels in the two life stages. Trypanosomal BiP (TbBiP) is used as a loading control. B: Immunofluorescence images of PFA fixed PCF or BSF cells with anti-TbLRTP□antibodies. The position of the nucleus (N) and kinetoplast (K) is visualised by DAPI staining. In PCF cells TbLRTP is juxtaposed to the kinetoplast and the nuclear region (upper panel). In BSF cells (lower panel) TbLRTP is localised to structures originating from the kinetoplast running to the posterior of the nuclear envelope, underlying the flagellum. Bars = 5 μm.

**Figure S2. Schematic of the cellular dimensions and cell cycle of trypanosomes.** A: Schematics of interphase trypanosome and a cell in late stages of cell division are shown, together with the positions of marker organelles. Distances from the posterior of the cell to the anterior tip (length) and to the kinetoplast or nucleus are shown, and are taken from the data presented in this report. P; posterior, K; kinetoplast (subscript p; posterior, a; anterior), N; nucleus (subscript p; posterior, a; anterior), A; anterior extremity. DNA-containing organelles are in blue, basal bodies in red. B: A full cell cycle is shown, with the basal bodies in red and the kinetoplast and nucleus in blue; the kinetoplast is the smaller structure close to the basal bodies. The kinetoplast/nucleus/basal body configurations are shown. During interphase there are two basal bodies, the old one originating from the previous but one cell division and the probasal body that arose during the last division. On entering cell division the basal bodies mature and replicate. At about the same time the kinetoplast enters S phase and elongates. Hence all cells that are of the 1K1N configuration should have either two or four basal bodies. Cell division is completed by segregation of a pair of basal bodies with each daughter kinetoplast, followed by nuclear division and finally organellar repositioning prior to the cleavage furrow dividing the cell into two daughter cells to complete cytokinesis and cell division.

A

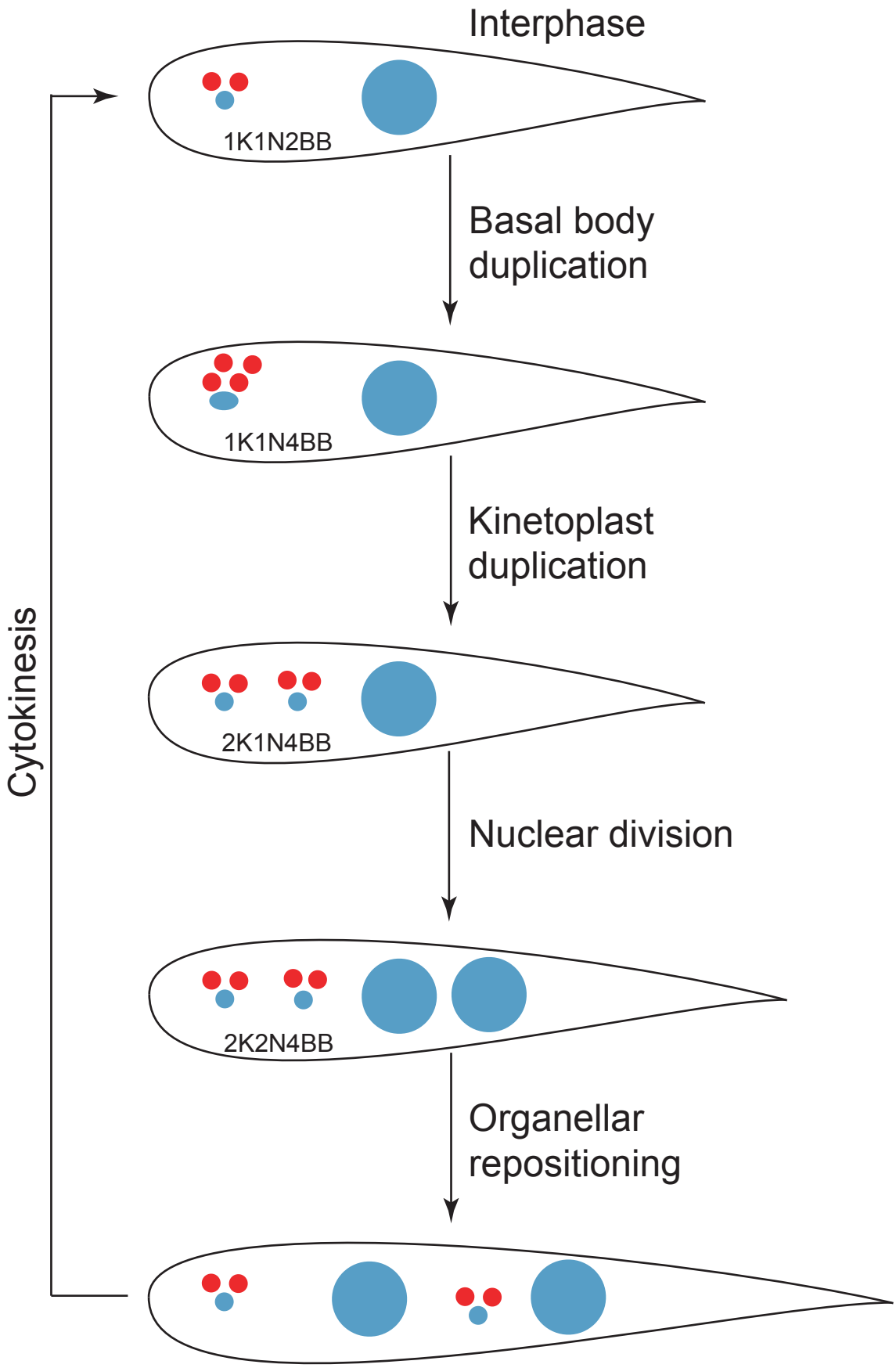



Kinetoplast (K)

Basal body

Nucleus (N)

B


Interphase
1K1N2BB
Basal body duplication
1K1N4BB
Kinetoplast duplication
2K1N4BB
Nuclear division
2K2N4BB
Organellar repositioning
Cytokinesis