TFK1, a new basal body transition fibre protein that is essential for cytokinesis in *Trypanosoma brucei*

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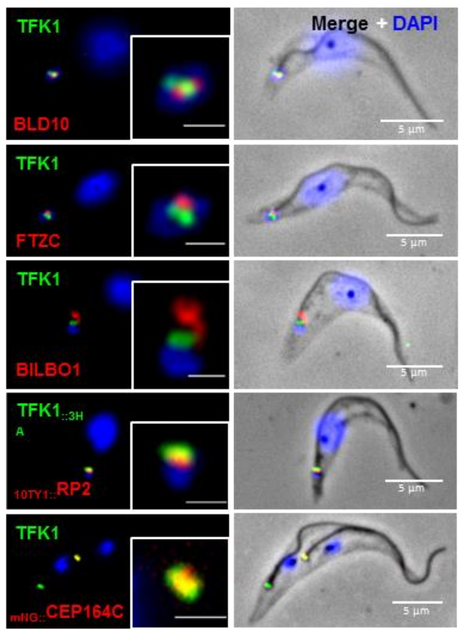
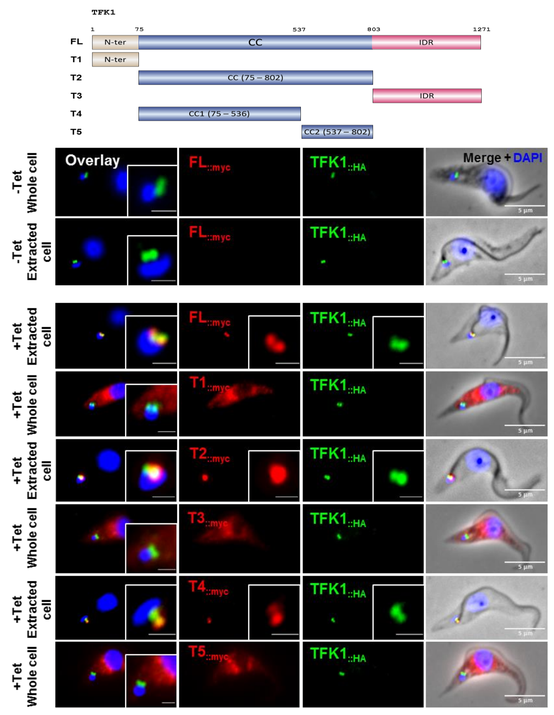
2. TFK1 is a transition fibre protein

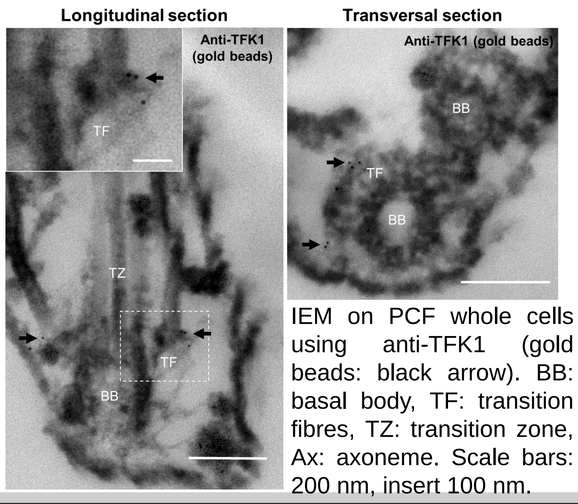
TFK1 partially localizes with the CEP164C transitional fiber marker protein



The TFK1 CC1 domain is sufficient to target to the transition fibre zone

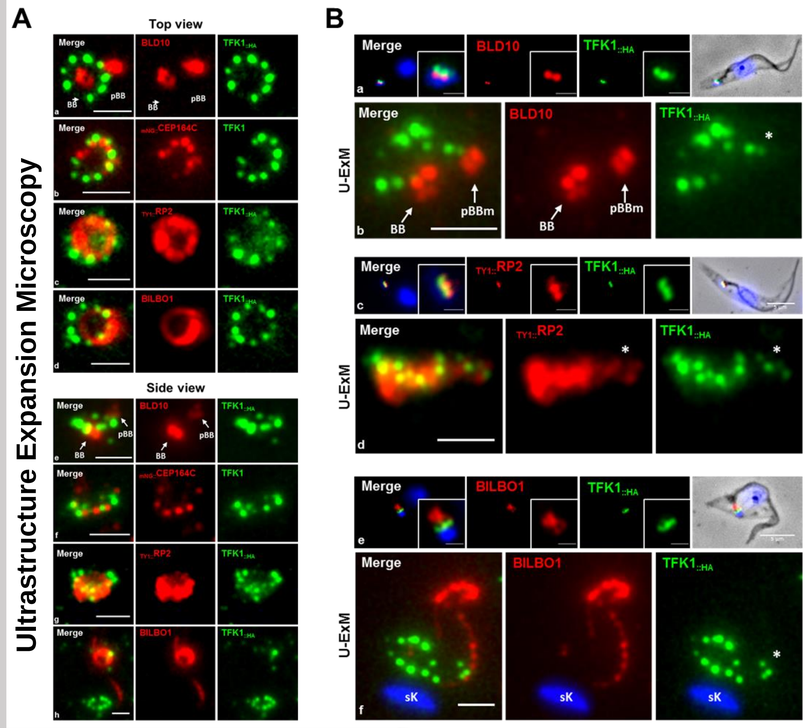


TFK1 is localized at the outskirt of the transition fibres



IEM on PCF whole cells using anti-TFK1 (gold beads: black arrow). BB: basal body, TF: transition fibres, TZ: transition zone, Ax: axoneme. Scale bars: 200 nm, insert 100 nm.

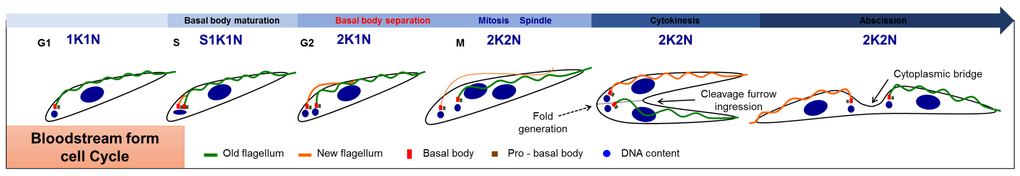
3. TFK1 displays nine circularly arranged dots located on the mature basal body



(A) U-ExM with anti-TFK1 in PCF whole cells (green), with BLD10 (red) (a), or CEP164C (red) (b), or RP2 (red) (c) or BILBO1 (red) (d) in G1 stage cells. Scale bars: 5 µm, inset 1 µm. (B) Immunofluorescence (a, c, e) and U-ExM (b, d, f) on detergent-extracted co-labelling with anti-TFK1 (green) with BLD10 (red) on BB and pro-BB maturation (pBBm) (a, b), or RP2 (red) (c, d), or BILBO1 (red) during pro-BB maturation in SK1N cells stage (Early kinetoplast S phase) (e, f). The labeling at the maturing pro-BB is indicated by an asterisk. Scale bars: 2 µm. Expansion factor: 4.8 fold.

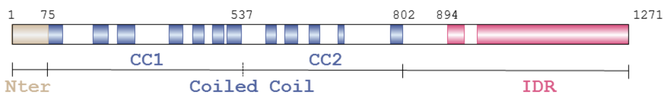
1. TFK1, the first Transition Fibres protein Kinetoplastid-specific

- *Trypanosoma brucei* is an unicellular flagellated parasite with a single flagellum emerging from a flagellar pocket
- Responsible for Human and Animal African Trypanosomiasis
- Two forms: the procyclic insect forms (PCF) in the vector insect midgut and the infectious bloodstream forms (BSF) upon transmission to the vertebrate host.



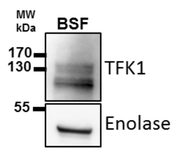
TFK1

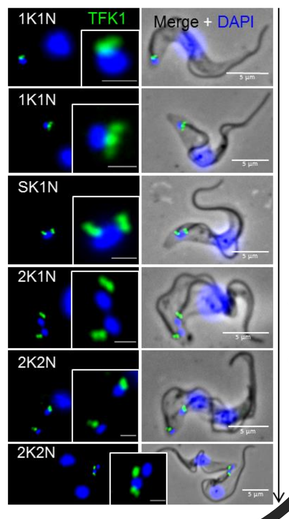
Transition Fibres protein Kinetoplastid specific-1



Nter, coiled-coil domains (CC) and C-terminal intrinsically disordered region (IDR).

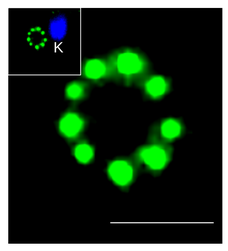
- Kinetoplastid-specific coiled-coil protein
- Cytoskeletal located at the basal body region



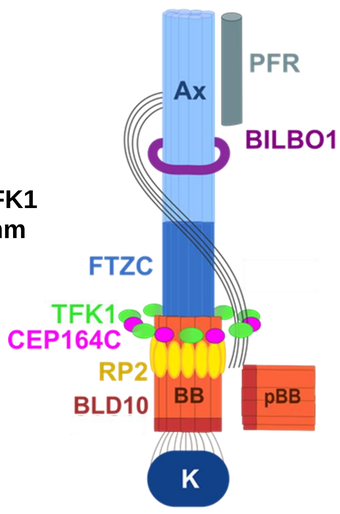


Immunofluorescence on detergent-extracted cells of TFK1 (green, anti-TFK1) across the cell cycle

Nine circular dots of TFK1 with a diameter ≈ 478 nm

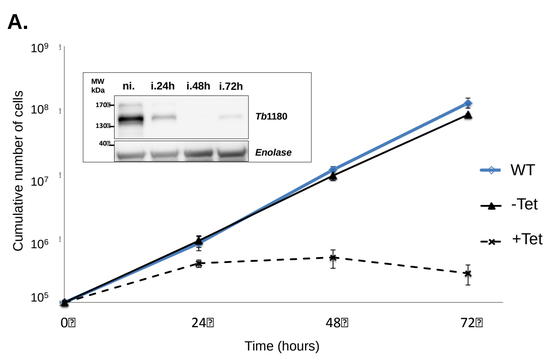


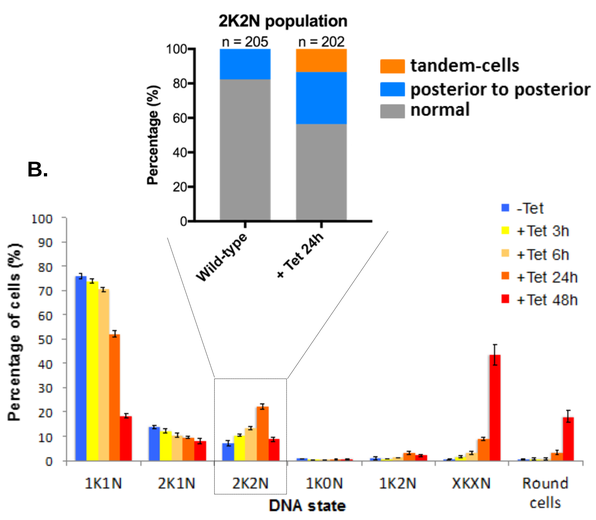
Top view of U-ExM of TFK1 composed of nine dots signal.



Scheme of the TFK1 localization with key basal body markers in *T. brucei.*

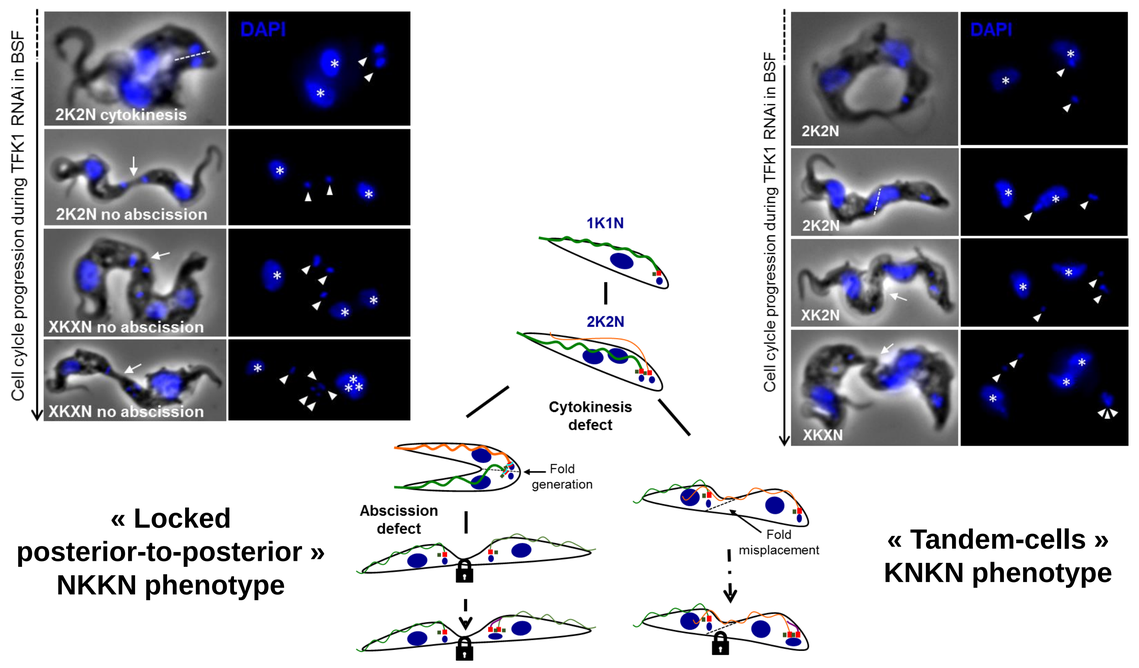
4. Bloodstream formTFK1 knockdown induces cell death …





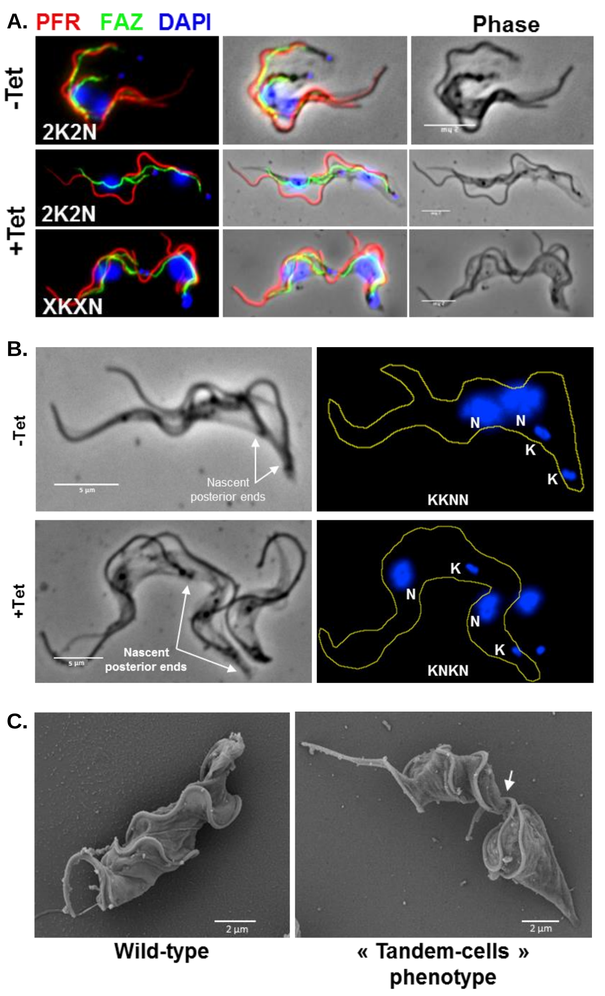
RNA interference knockdown of TFK1 in bloodstream form. (A) Cumulative growth curve of control (WT), RNAi cell line before induction (-Tet) and after induction (+Tet). Western blot to monitor the protein level of endogenously tagged $_{10TY::}$TFK1 with enolase as loading control. (B) Quantification of DNA state in control cells (WT) and RNAi induced cells (+Tet 3h, 6h, 24h and 48h).

… induces cytokinesis defects…



DAPI staining on whole cells after TFK1 RNAi knockdown showing cell cycle progression (position of division fold generation, dashed line; nucleus (asterisk); kinetoplast (arrowhead); fold (dashed line); cytoplasmic bridge (arrow)).

…and undescribed KNKN "Tandem-cells" phenotype



(A) Immunofluorescence on BSF cytoskeleton with FAZ and PFR labelling, (B) with DAPI staining and (C) SEM analysis of 2K2N cell, before (-Tet) and 24 h after (+Tet) TFK1 RNAi. Scale bars: 5 µm. The arrows indicate the cytoplasmic bridge.

New insight into the organisation of transition fibres

TFK1 is a kinetoplastid specific protein and a mature and maturing BB marker, localized on the transitional fibre. TFK1 is **the third component of the transition fibres region**, with TbRP2 and CEP164C, in *T. brucei*. Our high-resolution ultrastructure expansion microscopy data demonstrate that TFK1 is displayed in a typical radial arrangement in the **distal appendage matrix, as nine dense points between the molecules of CEP164C**.

TFK1 is essential for BSF, unlike PCF. Its depletion induces, on one hand, **previously undescribed cytokinesis defects** by the absence of furrow associated **with segregation of BBs similar to that of PCFs (KNKN)** and on the other hand, leads to **the blockage of abscission** during cytokinesis in BSFs.

What does TFK1 do?

Our results suggest an essential role of TFK1 in the **segregation and positioning of BBs to ensure the shape of subsequent daughter cells**. The role of TFK1 could be **extended to other forms of the trypanosome life cycle**, which exhibit different morphologies (trypomastigote and epimastigote) as well as a different positioning of the nucleus and basal bodies along the axis of the cell.

Acknowledgements

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