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Chair of ZOOLOGY 1

Characterisation of the two myosins of *T. brucei*

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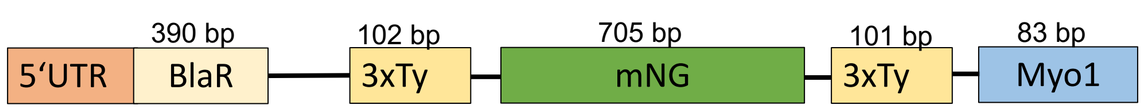
Abstract: Trypanosomes have a very reduced actomyosin system, and *Trypanosoma brucei* expresses only a single actin gene and two myosin genes. The retention of this rudimentary actomyosin system alongside the trypanosomes' massive investment in their microtubule system suggests that the actomyosin system performs certain functions that the microtubule cytoskeleton cannot recapitulate. Both of the *T. brucei* myosins have received little attention to date. The data here represent the first steps in a full characterisation of these two proteins in bloodstream form *T. brucei*.

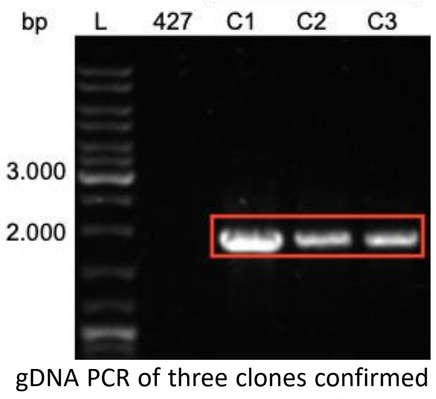
1. Generation of an *mNG::TbMYO1* cell line



Schematic view of the TbMyo1 in situ tagging construct. The wildtype strain 427 was transfected with the PCR tagging product. The tagging construct encoded a blasticidin resistance gene, a 3xTy1 tag, the mNG gene followed by a 3xTy1 tag.

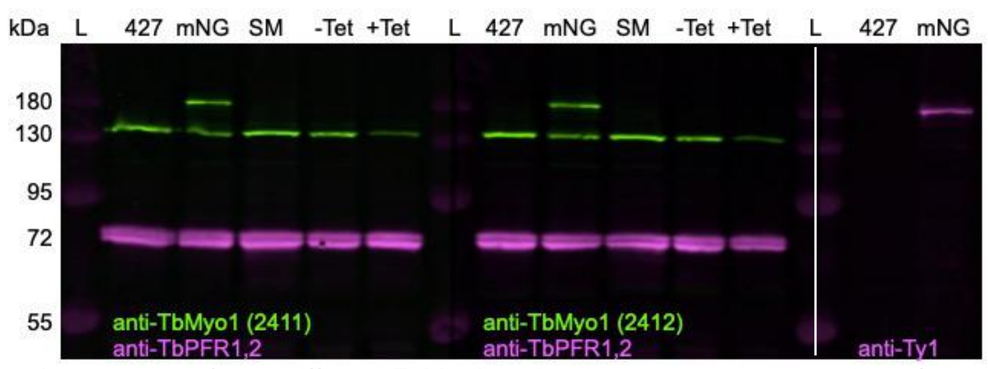
2. Validation of the TbMyo1 antibodies and localisation of the protein

A



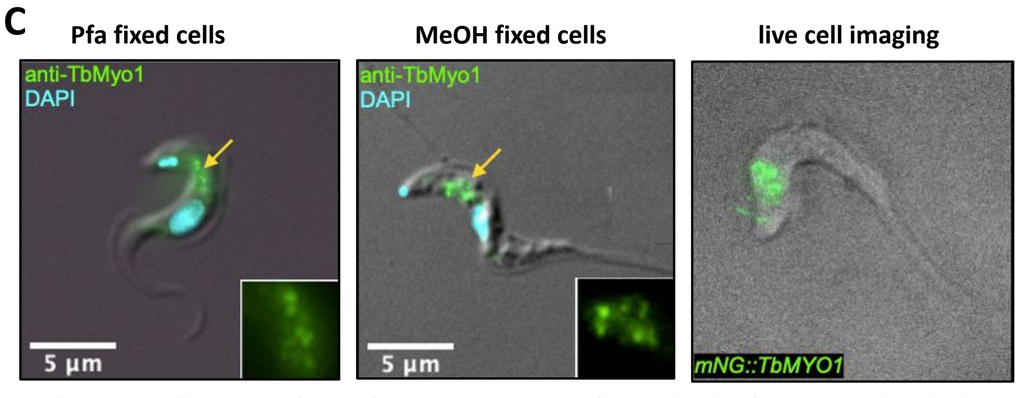
gDNA PCR of three clones confirmed the integration of the mNG tag at the Tb*MYO1* locus (size: 1733 bp)

B



Immunoblot of two different TbMyo1 antibodies showed the detection of the TbMyo1 protein in different cells; the depletion could also be confirmed -> both antibodies are specific to the TbMyo1 protein.

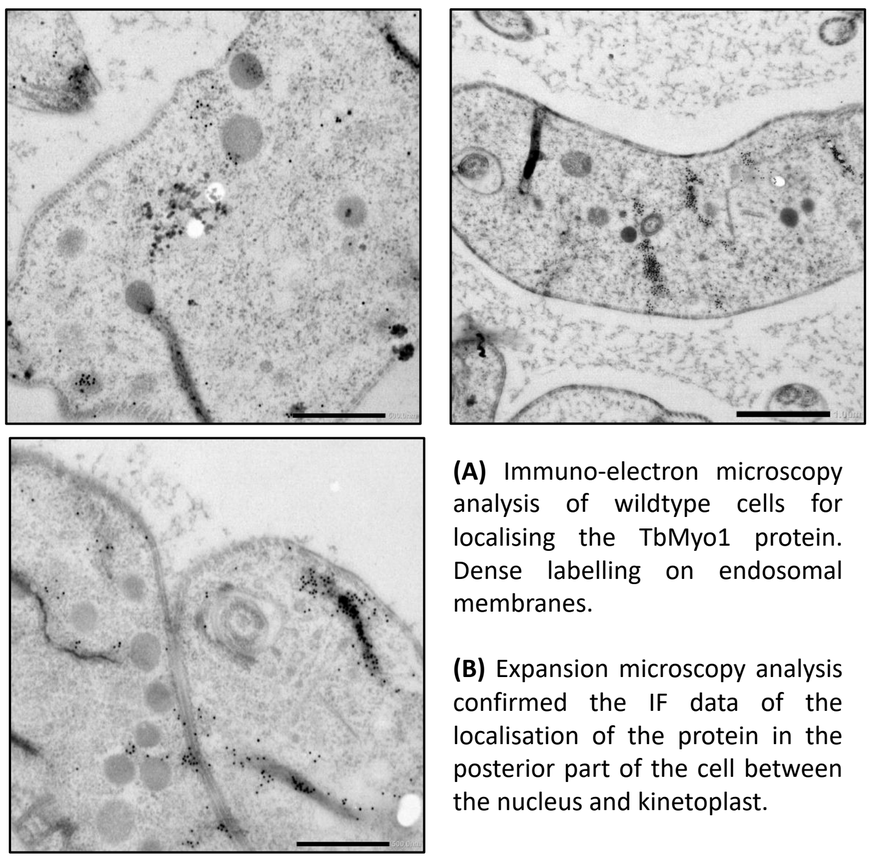
C



Confirmation of the specificity of the antibodies in pfa and MeOH fixed cells. The TbMyo1 localises between the nucleus and the kinetoplast. Live cell imaging also validated the localisation using the *mNG::TbMYO1* cell line.

3. Ultrastructural analysis and biochemical localisation of the TbMyo1 protein

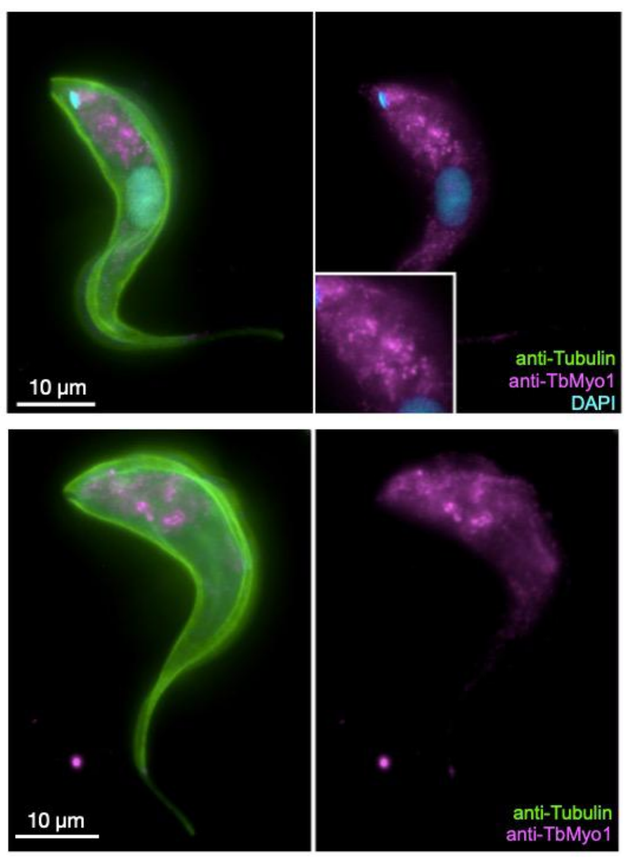
A



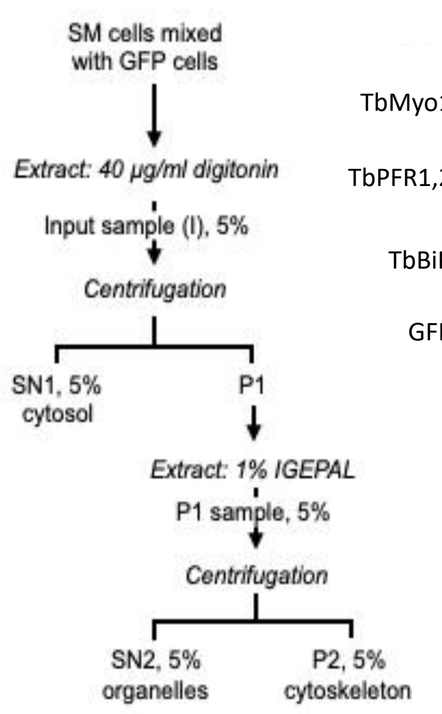
(A) Immuno-electron microscopy analysis of wildtype cells for localising the TbMyo1 protein. Dense labelling on endosomal membranes.

(B) Expansion microscopy analysis confirmed the IF data of the localisation of the protein in the posterior part of the cell between the nucleus and kinetoplast.

B

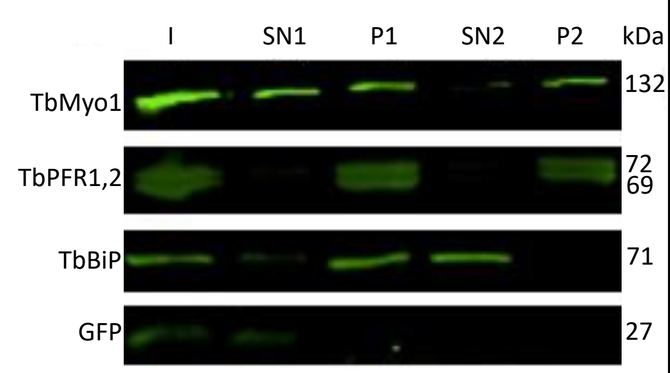


C



Schematic view of the two-step fractionation.

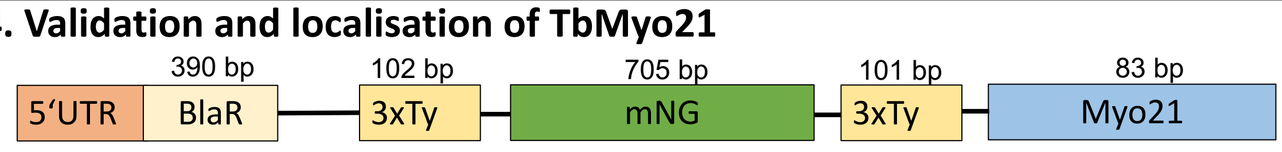
E



The two-step fractionation indicates TbMyo1 being present in the cytosol (SN1), organelle (SN2) and cytoskeleton (P2) fraction. Distribution between SN2 and P2 is almost at 50%. TbPFR1,2 is only present in the cytoskeleton fraction. TbBiP (ER marker) is only in the organelle fraction. The GFP of the GFP expressing cells is in the cytosol fraction.

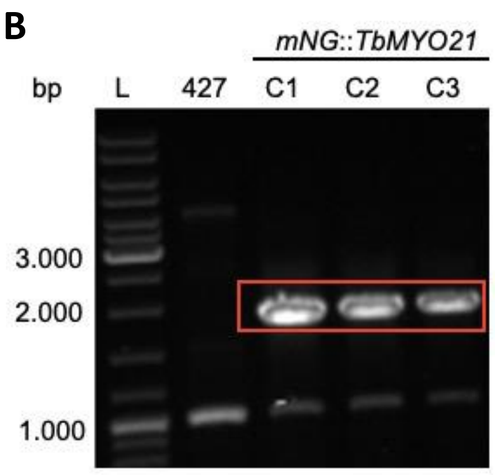
4. Validation and localisation of TbMyo21

A



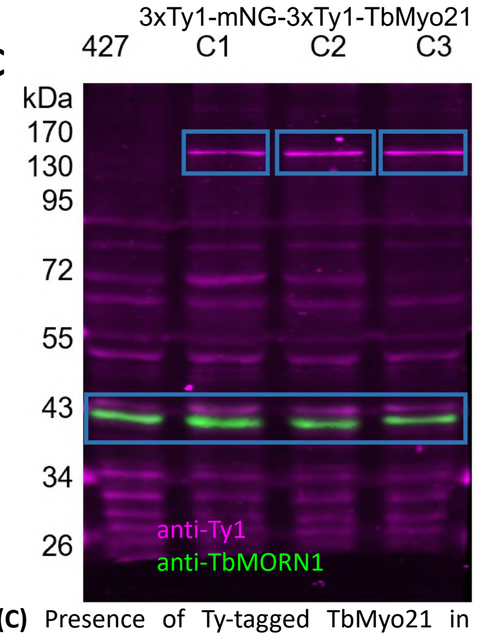
(A) Schematic view of the TbMyo21 in situ tagging construct. The wildtype strain 427 was transfected with the shown PCR tagging product.

B



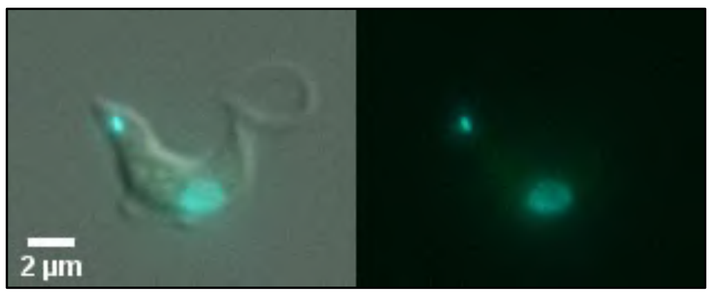
(B) gDNA PCR of three clones confirmed the integration of the mNG-tag at the TbMYO21 locus (size: 1884 bp).

C

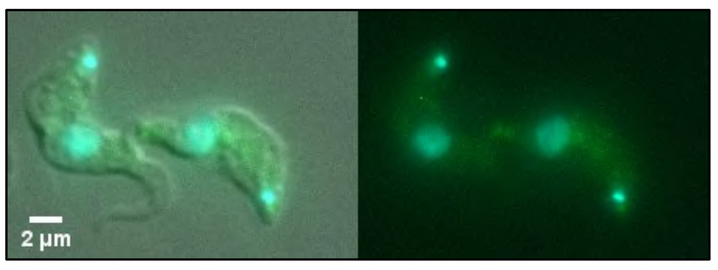


(C) Presence of Ty-tagged TbMyo21 in mNG-clones. Absence of Ty-tag in wildtype Tb427. TbMORN1 as loading and detection control.

D1

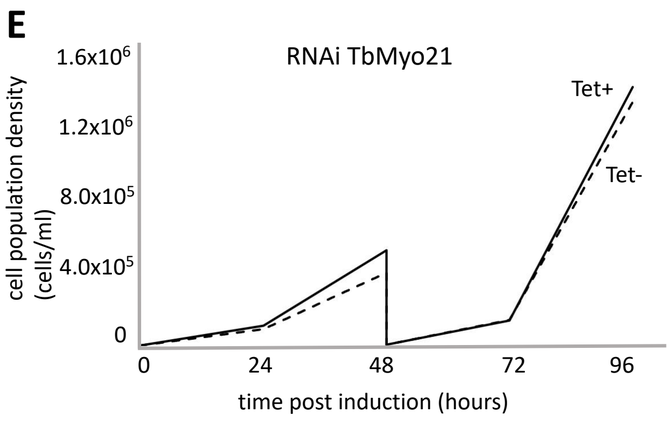


D2



(D) Pfa-fixed mNG-TbMyo21 cells. **(D1)** Autofluorescence of WT 427 in comparison to **(D2)** mNG-tagged TbMyo21 cells. **(D2)** The mNG-signal in the whole cell was very weak, so no specific localisation of TbMyo21 protein could be detected.

E



(E) The depletion of TbMyo21 protein did not influence the cell viability of bloodstream form *T.brucei*, but had a small impact on the growth of the induced cells.

Outlook for TbMyo21 localisation:

- Immunofluorescence of TbMyo21 with anti-Myo21 antibodies
- Reverse transcription PCR of TbMyo21

5. Summary

TbMyo1 was localised to the posterior part of bloodstream form *T. brucei* cells using two different antibodies and an mNG tag.
Ultrastructural analysis using immuno-electron microscopy and expansion microscopy suggested that TbMyo1 is present on endosomes and vesicles.
Biochemical fractionation indicated that there are cytosolic, organelle-associated and cytoskeleton-associated pools of TbMyo1.
The expression level of TbMyo21 in bloodstream form *T. brucei* appears to be much lower than that of TbMyo1.
The exact localisation of TbMyo21 remains unclear. Depletion of TbMyo21 has a mild effect on population growth.

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