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A Repetitive Protein Essential for the Flagellum Attachment Zone Filament Structure and Function in *Trypanosoma brucei*

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The flagellum is attached along the length of the cell body in the protozoan parasite *Trypanosoma brucei* and is a defining morphological feature of this parasite. The flagellum attachment zone (FAZ) is a complex structure and has been characterised morphologically as comprising a FAZ filament structure and the specialised microtubule quartet (MtQ) plus the specialised areas of flagellum: plasma membrane attachment. Unfortunately, we have no information as to the molecular identity of the FAZ filament components. Here, by screening an expression library with the monoclonal antibody L3B2 which identifies the FAZ filament we identify a novel repeat containing protein FAZ1. It is kinetoplastid-specific and provides the first molecular component of the FAZ filament. Knockdown of FAZ1 by RNA interference (RNAi) results in the assembly of a compromised FAZ and defects in flagellum attachment and cytokinesis in procyclic trypanosomes. The complexity of FAZ structure and assembly is revealed by the use of other monoclonal antibody markers illustrating that FAZ1 is only one protein of a complex structure. The cytokinesis defects provide further evidence for the role of an attached flagellum in cellular morphogenesis in these trypanosomes.

Key words: flagellum; flagellum attachment zone filament; FAZ; cytokinesis; cytoskeleton; *Trypanosoma brucei*.

Introduction

The protozoan parasite *Trypanosoma brucei* causes African sleeping sickness in humans and Nagana in cattle. The parasite is widespread in sub-Saharan Africa, accounting for ~50,000 human deaths annually and there is a high economic cost with the loss of domestic cattle. The parasite undergoes a complex life cycle; alternating between insect vector (the tsetse fly) and mammalian host. A distinguishing morphological feature of this parasite is the single flagellum which is attached along the length of the cell body with a small overhang that protrudes from the anterior end of the cell (Fig. 1A) (for review see Gull 1999). This feature is unusual as the flagella/cilia of most eukaryotic cells extend out freely into the surrounding medium. The importance of the flagellum in many aspects of this parasite's cellular morphogenesis, organelle positioning and pathogenicity, in addition to motility have become clear from recent studies (Baron et al.

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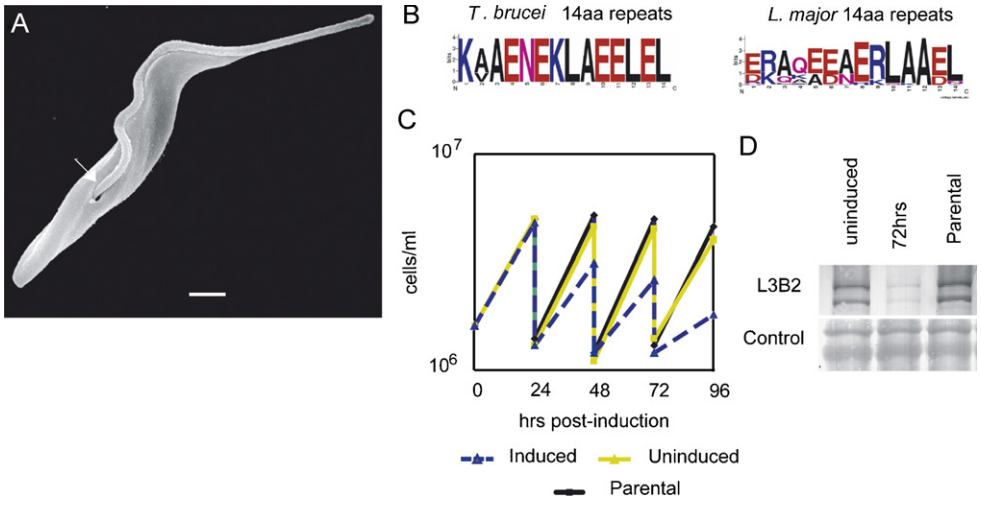

Figure 1. RNAi of FAZ1 affects growth in procyclic *T. brucei*; **A**: Scanning electron micrograph of a procyclic *T. brucei* cell. The single flagellum exits the flagellar pocket (arrow) at the posterior end of the cell and is attached along the length of the cell body (scale bar = 1 μm); **B**: Sequence logos illustrate the similarity of the 14aa repeats within the FAZ1 protein in *T. brucei* and *L. major*; **C**: Knockdown of FAZ1 by RNAi results in a growth effect after 24 h induction compared to the uninduced cells or the parental cell line; **D**: Western blotting with L3B2 antibody detects two bands >200 kDa in parental and non-induced, but at 72 h post-induction the level of protein detected is greatly reduced.

2007; Broadhead et al. 2006; Davidge et al. 2006; Kohl et al. 2003; Moreira-Leite et al. 2001; Robinson et al. 1995).

In contrast to other kinetoplastids, the trypanosomatids are characterised by their possession of this attached flagellum. The flagellum of *T. brucei* is composed of a classical 9+2 axoneme plus the paraflagellar rod (PFR), which is attached to the axoneme and is essential for motility of the parasite (Bastin et al. 1998). The flagellum attachment zone (FAZ) defines a complex structure that connects the flagellum to the long axis of the cell body via the FAZ filament and the microtubule quartet (MtQ) with associated membranous elements. The FAZ filament is located in a gap between sub-pellicular microtubules of the microtubule corset which underlies the plasma membrane. The MtQ is located immediately to the left of the FAZ filament when viewed from the posterior end of the cell and is associated with smooth endoplasmic reticulum (Sherwin and Gull 1989a). The MtQ is nucleated close to the basal body (at the proximal end of the flagellum) within the cytoplasm and has distinct biochemical characteristics such as resistance to salt (Robinson et al. 1995). A similar ultrastructure has also been well characterised in *T. cruzi* (Rocha et al. 2006) and, although epimastigote flagella are only partially attached, FAZ filament-like structures have been reported in the flagellar pocket areas of *Leishmania mexicana* (Weise et al. 2000) and *Crithidia fasciculata* (Brooks 1978).

The long slender cell shape of the parasite is maintained by the corset of sub-pellicular microtubules underlying the plasma membrane. Importantly, the MtQ is nucleated close to the basal bodies and therefore these microtubules are antiparallel (plus end at the anterior of the cell) to the main set of cortical microtubules (minus end at the anterior end of the cell). This sub-pellicular corset remains intact during the cell division cycle necessitating a very precise coordination between its duplication and the duplication and segregation of the many single copy organelles (Sherwin and Gull 1989b). The flagellar attachment zone is central to these processes. As the new flagellum extends out of the flagellar pocket at the posterior end of the cell, FAZ filament assembly lags slightly behind, so attaching the newly synthesised flagellum to the cell body as it follows a left-handed helical path (Kohl et al. 1999), guided by the flagella connector (Moreira-Leite et al. 2001). The flagella connector extends to a distance of ~0.6 of the old flagellum length and whilst the connector is effectively stationary, the new flagellum continues to extend in length and separation of the basal bodies (with attached mitochondrial DNA, termed the kinetoplast) of the old and new