Protist, Vol. 160, 576–590, November 2009
http://www.elsevier.de/protis
Published online date 29 July 2009

Protist

ORIGINAL PAPER

Cell Morphogenesis of *Trypanosoma brucei* Requires the Paralogous, Differentially Expressed Calpain-related Proteins CAP5.5 and CAP5.5V

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Submitted April 3, 2009; Accepted May 16, 2009
Monitoring Editor: Larry Simpson

Proteins from the calpain super-family are involved in developmentally- and environmentally-regulated re-modelling of the eukaryotic cytoskeleton and the dynamic organisation of signal transduction cascades. In trypanosomatid parasites, calpain-related gene families are unusually large, but we have little insight into the functional roles played by these molecules during trypanosomatid lifecycles. Here we report that CAP5.5, a cytoskeletal calpain-related protein subject to strict stage-specific expression in the sleeping sickness parasite *Trypanosoma brucei*, is essential and required for correct cell morphogenesis of procyclic (tsetse mid-gut stage) *T. brucei*. Striking consequences of *CAP5.5* RNA interference are the loss of protein from the posterior cell-end, organelle mis-positioning giving rise to aberrant cytokinesis, and disorganisation of the sub-pellicular microtubules that define trypanosome cell shape. We further report that the stage-specificity of *CAP5.5* expression can be explained by the presence of a paralogue, *CAP5.5V*, which is required for cell morphogenesis in bloodstream *T. brucei*; RNAi against this paralogous protein results in a qualitatively similar phenotype to that described for procyclic *CAP5.5* RNAi mutants. By comparison to recently described phenotypes for other procyclic trypanosome RNAi mutants, likely functions for CAP5.5 and CAP5.5V are discussed.

Key words: calpain; cytoskeleton; microtubule; procyclic; trypomastigote; trypanosome.

Introduction

Cytoskeletal re-modelling is a critical feature in any cell division cycle. In eukaryotes, cytoskeletal re-modelling is also often central to cell motility and the dynamic organisation of signal transduction cascades. In animals, molecules that are often central to cytoskeletal organisation and signalling include calpains – a family of Ca^{2+}-regulated cysteine proteases – and calpain-related proteins (Franco and Huttenlocher 2005; Goll et al. 2003; Lebart and Benyamin 2006). Calpain-related proteins have been identified in fungi, protists, and plants too, where they are involved in developmental and environmentally-regulated processes (Croall and Ersfeld 2007;

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doi:10.1016/j.protis.2009.05.003

Denison et al. 1995; Futai et al. 1999; Li et al. 2004; Rosenthal 2004; Wang et al. 2003). However, calpain-related gene families in non-animal taxa are generally small, and often limited to a single gene (e.g. *DEK1* in *Arabidopsis thaliana* (Wang et al. 2003)). Thus, notwithstanding the challenges posed by the need for survival or growth within several distinctive environments in both mammalian host and insect vector, our report of unusually large calpain-related gene families in three rather different trypanosomatid parasites, *Trypanosoma brucei*, *T. cruzi*, and *Leishmania major* (Ersfeld et al. 2005), was both surprising and intriguing.

The trypanosomatids are a group of flagellate parasites and include several major human, livestock and plant pathogens. Most members of this parasite family undergo complex lifecycles, requiring differentiation into multiple, morphologically distinct forms in very different environments within a mammalian host and an insect vector. *Trypanosoma brucei*, the focus of this study, is the causal agent of human African sleeping sickness and livestock trypanosomiasis; it is transmitted between mammals by tsetse flies and seven morphologically distinct forms of the parasite are currently recognised (Sharma et al. 2008; Van Den Abbeele et al. 1999; Vickerman 1969, 1985). Of these different morphological forms two, the procyclic (tsetse mid-gut) and (pathogenic) long-slender bloodstream trypomastigote forms, can be cultured in vitro and subjected to genetic manipulation in the laboratory. Different trypanosomatid morphologies are classified according to several structural parameters, including the position of the nucleus and the kinetoplast (the name given to the unique and intricate mitochondrial genome structure in trypanosomatids) within the cell body (Gull 1999). However, trypanosome cell shape is always determined by the patterning of an elaborate, microtubule-based cytoskeleton (e.g. Gull 1999; Kohl et al. 2003; Matthews et al. 1995; Robinson et al. 1995; Sherwin and Gull 1989a, 1989b; Sherwin et al. 1987). This subpellicular cytoskeleton consists of a corset-like monolayer of evenly-spaced microtubules, in which neighbouring microtubules are cross-linked to one another and the plasma membrane by various microtubule-associated proteins (MAPs). During each cell division cycle new subpellicular microtubules are extended and intercalated between older microtubules assembled in previous cell cycles (Sherwin and Gull 1989b).

A number of MAPs have been identified from *T. brucei* and other trypanosomatids (Baines and Gull 2008; Hertz-Fowler et al. 2001; Vedrenne et al. 2002), but the molecular mechanisms which orchestrate cytoskeletal re-modelling during the cell cycle or differentiation, and the semi-conservative inheritance of subpellicular microtubules between the progeny at cytokinesis are largely unknown. In this context, we have very little insight into why expansion of the calpain-related gene family has occurred or of function in these important and evolutionarily divergent (Burki et al. 2008; Hampl et al. 2009) parasites. Intriguingly, the first biochemically identified *T. brucei* calpain-related protein, CAP5.5,[1] is a cytoskeletal protein in procyclic trypomastigotes which is evenly distributed across the sub-pellicular microbtuble corset in detergent-extracted cells (Hertz-Fowler et al. 2001). CAP5.5 is also subject to strict stage-specific regulation: protein is expressed in procyclic trypomastigotes, and classically provides a late stage marker for bloodstream-procyclic trypomastigote differentiation when detected using the anti-CAP5.5 monoclonal antibody (Matthews and Gull 1994). Interestingly, N-terminal myristoylation and palmitoylation of CAP5.5 suggests the protein interfaces with the plasma membrane, as well as subpellicular microtubules, but degeneracy within the putative active-site of the calpain-related domain raises uncertainty as to whether CAP5.5 is an active protease (Hertz-Fowler et al. 2001), and recombinant expression of native protein has been unsuccessful. Thus, several functions can be envisaged for CAP5.5: it could be a mere structural component of the procyclic cytoskeleton, it might be a protease required for cytoskeletal re-modelling, or perhaps the subpellicular microtubules provide a platform to facilitate organisation of a CAP5.5-dependent signalling cascade. Here, we report the results from RNAi experiments that selectively targeted either *CAP5.5* or a recently identified paralog, which we call *CAP5.5V* (*CAP5.5 variant*).

[1]CAP5.5 (Tb927.4.3950; encoding cytoskeletal-associated-protein 5.5) was previously named CALP1 by Hertz-Fowler et al. (2001), and then revised to TbCALP4.1CAP5.5 by Ersfeld et al. (2005). However, in the publicly available *T. brucei* genome sequence the original name of CAP5.5 is retained. Applying the nomenclature suggested by Ersfeld et al. (2005) CAP5.5V (Tb927.8.8330) would formally be recognised as TbCALP8.1-CAP5.5V.CAP5.5 (Tb927.4.3950; encoding cytoskeletal-associated-protein 5.5) was previously named CALP1 by Hertz-Fowler et al. (2001), and then revised to TbCALP4.1CAP5.5 by Ersfeld et al. (2005). However, in the publicly available *T. brucei* genome sequence the original name of CAP5.5 is retained. Applying the nomenclature suggested by Ersfeld et al. (2005) CAP5.5V (Tb927.8.8330) would formally be recognised as TbCALP8.1CAP5.5V.