Paraflagellar rod is vital for trypanosome motility

African trypanosomes are protozoan parasites that cause sleeping sickness in man. In addition to the axoneme, their flagellum contains a large structure called the paraflagellar rod (PFR) whose function is unknown. We used an antisense RNA approach to produce a specific molecular ablation of the PFR structure. The mutant cells are paralysed, demonstrating that the PFR has an essential role in cell motility.

A PFR is found exclusively in the flagellum of three evolutionary ancient groups of protists: kinetoplastids[1], euglenoids[2] and dinoflagellates[3]. Its function has been the subject of much conjecture[1,3] yet remains entirely cryptic. The major PFR components are two closely related proteins, PFR-A and PFR-C, sharing 60% amino acid sequence identity[1,4–6].

We cloned the near-full-length sequence of the *PFR-A* gene (nucleotides 78–1,758)[7] in the reverse orientation into the strong trypanosome expression vector pHD451 (provided by S. Biebinger and C. Clayton, Heidelberg)[8] and integrated into trypanosome DNA by homologous recombination[9–11]. Transformants where the antisense construct was targeted to the inverted rRNA spacer or to the procyclin promoter did not produce a particular phenotype and the decrease in the amount of PFR-A protein was marginal. However, in one transformant, cells grew normally but sedimented to the bottom of the well and seemed paralysed. This mutant was cloned twice by limiting dilution and named *snl*-1.

Southern blot analysis of *snl*-1 revealed that the plasmid was integrated within one of the two *PFR-A* loci (trypanosomes are diploid) owing to a rare homologous recombination event (not shown). Northern blot analysis (Fig. 1a) showed that only a tiny amount of *PFR-A* mRNA was present in the *snl*-1 compared with wild-type cells. Western blots (Fig. 1b) of total cell lysates probed with L13D6, a monoclonal antibody recognizing both PFR-A and PFR-C (gift from Linda Kohl, University of Manchester) showed two bands of roughly equal intensities (PFR-A and PFR-C) in the wild-type cells (lane 1) but only PFR-C in the mutant trypanosomes (lane 4). This implies that *PFR-C* gene expression was not modified by the presence of antisense construct against *PFR-A*.

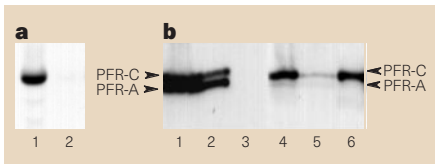

Figure 1 PFR-A expression is switched off in the *snl*-1 cell line. **a**, Northern blot analysis of extracted total RNA from wild-type (lane 1) and *snl*-1 (lane 2) trypanosomes hybridized with a *PFR-A* antisense RNA probe labelled with digoxigenin. Equal loading was confirmed by staining with ethidium bromide. **b**, Immunoblot analysis of wild-type (lanes 1–3) and *snl*-1 (lanes 4–6) trypanosomes. Protein samples were probed with the L13D6 antibody. Lanes 1 and 4, total cell extract; lanes 2 and 5, pellet (detergent insoluble fraction); lanes 3 and 6, supernatant (detergent-soluble fraction).

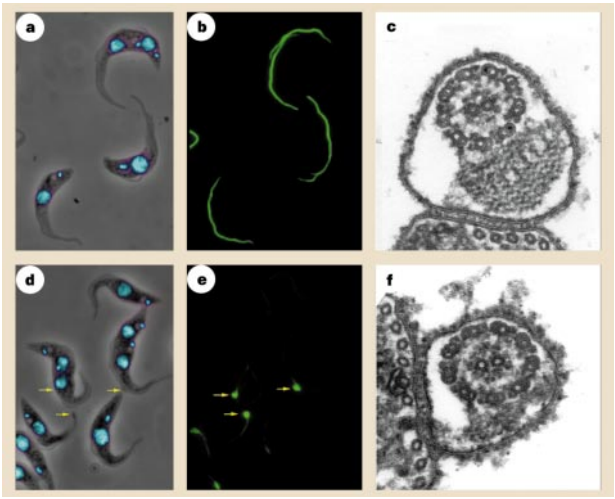

Figure 2 Morphological analysis of wild-type (**a**–**c**) and *snl*-1 cells (**d**–**f**). Cells were stained with 4,6-diamidino-2-phenylindole, a DNA-intercalating dye staining the nucleus and the kinetoplast (shown in blue), superimposed on the phase-contrast image (**a**,**d**). **b**,**e**, Immunofluorescence pattern with the L13D6 antibody. Arrows show the dilation of the distal tip of the flagellum. **c**,**f**, Electron microscopic cross-sections of the flagellum of wild-type (**c**) and *snl*-1 trypanosomes (**f**).

We extracted cells with detergent[12] to separate out the assembled cytoskeleton including the flagellum (Fig. 1b). In wild-type trypanosomes, the PFR proteins were found exclusively in the cytoskeleton (lane 2) and no soluble pool was detected (lane 3). In the *snl*-1 mutant, the PFR-C protein was shifted to the soluble fraction (lane 6) and only a small amount was left in the cytoskeleton (lane 5). Therefore, in the absence of PFR-A, the PFR-C protein did not assemble in a cytoskeletal structure.

In immunofluorescence[13], anti-PFR antibodies produced the expected bright signal in the flagellum of wild-type trypanosomes (Fig. 2a, b). This was markedly decreased in the *snl*-1 mutant (Fig. 2d, e). Under phase-contrast microscopy, many *snl*-1 cells exhibited a pronounced dilation of the distal tip of the flagellum (Fig. 2d, arrows) which stained strongly with the L13D6 antibody, indicating that non-assembled PFR-C entered the flagellum compartment and accumulated at the distal tip. Under the electron microscope we observed a major and specific ablation of the PFR in the *snl*-1 mutant (Fig. 2c, f). Cross-sections of the wild-type flagellum revealed the typical PFR structure (Fig. 2c), composed of three regions and specific attachment points to the axoneme[1]. In the *snl*-1 flagella, the intermediate and distal regions of the PFR were missing and only a fraction of the smaller proximal region remained (Fig. 2f).

Wild-type trypanosomes swim actively *in vivo* and *in vitro*, the flagellum beating from tip to base[14]. In the *snl*-1 mutants, although the polarity was not affected, the frequency and the amplitude of flagellar beating were drastically decreased. Motility was so compromised that the *snl*-1 cells sedimented rather than staying in suspension.

The paralysis of the *snl*-1 mutant reveals an essential role for the PFR in flagellum and cell motility in trypanosomes. Our approach of integrating an antisense construct into one homologous gene locus suggests that position within the genome might be critical for the success of this method in trypanosomes. This could also be indicative of antisense interference with early processing. Production of such novel cellular phenotypes will find wide application in studies of parasites because motility functions have been implicated in events such as parasite–host attachment, surface-antigen trafficking and endocytosis.

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