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Actin-depolymerizing factor, ADF/cofilin, is essentially required in assembly of Leishmania flagellum

Summary

ADF/cofilins are ubiquitous actin dynamics-regulating proteins that have been mainly implicated in actin-based cell motility. Trypanosomatids, e.g. Leishmania and Trypanosoma, which mediate their motility through flagellum, also contain a putative ADF/cofilin homologue, but its role in flagellar motility remains largely unexplored. We have investigated the role of this protein in assembly and motility of the Leishmania flagellum after knocking out the ADF/cofilin gene by targeted gene replacement. The resultant mutants were completely immotile, short and stumpy, and had reduced flagellar length and severely impaired beat. In addition, the assembly of the paraflagellar rod was lost, vesicle-like structures were seen throughout the length of the flagellum and the state and distribution of actin were altered. However, epistomal complementation of the gene restored normal morphology and flagellar function. These results for the first time indicate that the actin dynamics-regulating protein ADF/cofilin plays a critical role in assembly and motility of the eukaryotic flagellum.

Introduction

Eukaryotic cilia and flagella are highly conserved organelles that are required in diverse motile and sensory functions (Afzelius, 2004; Snell et al., 2004; Pan et al., 2005; Quarriby and Parker, 2005). The flagellum of trypanosomatids, such as Trypanosoma and Leishmania, is a unique multifunctional organelle that plays critical roles in cell motility, chemotaxis, cell signalling and host cell invasion (Landfear and Ignatushchenko, 2001; Gull, 2003; Hill, 2003; Kohl, 2003) and is comprised of two main components, the axoneme and paraflagellar rod (PFR). The axoneme is composed of around 250 proteins arranged in a core structure of nine peripheral microtubule doublets surrounding two single microtubules (Ralston and Hill, 2008). In addition, these organisms contain a unique crystalline lattice structure, called PFR, running along the axoneme under the flagellar membrane (Maga and Lebowitz, 1999). While the canonical 9 + 2 axoneme structure powers beating in most eukaryotic flagella (Ralston and Hill, 2008), PFR has been implicated in flagellar motility and waveform generation (Maga and Lebowitz, 1999). A number of studies have shown that microtubule-based dynein and kinesin motors play pivotal role in dynamic assembly and motility of the trypanosomatid flagellum (Blainneau et al., 2007; Absalon et al., 2008; Ralston and Hill, 2008). However, despite their presence in the flagellum of various organisms including Leishmania (Muto et al., 1994; Yanagisawa and Kamiya, 2001; Sahasrabuddhe et al., 2004; Minoura, 2005), the role of actin and actin-binding proteins (Ross et al., 2008) still remains largely unexplored.

Actin is a ubiquitous cytoskeletal protein that exists in filamentous (F-) and monomeric (G-) states. In its filamentous state, actin forms a complex network with the help of a variety of actin-binding proteins (Sheterline and Sparrow, 1994). The dynamics of this network is regulated by a specific group of actin-binding proteins of which actin-depolymerizing factor ADF/cofilin constitutes an important component (Ono, 2007). ADF/cofilins are essentially present in all eukaryotic cells and have been suggested to play a key role in actin-based cell motility (Pollard and Borisy, 2003).

Leishmania are an important group of parasites that cause a spectrum of human diseases including 'kala-azar', and have been exploited as a model system to explore the mechanisms that regulate flagellar assembly and motility in eukaryotic organisms. Here we show that deletion of the ADF/cofilin gene in Leishmania results in immotile cells with reduced flagellar length and severely impaired beat. Additionally, the PFR is not made, vesicle-like structures appear throughout the flagellum and actin distribution is markedly altered. These results strongly suggest that the actin dynamics-regulating protein, ADF/cofilin, plays a crucial role in flagellar assembly and motility.