Formins and VASPs may co-operate in the formation of filopodia

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Abstract

Filopodia are finger-like cell protrusions composed of parallel arrays of actin filaments, which elongate through actin polymerization at their tips. These highly dynamic structures seem to be used by many cell types as sensing organs to explore environmental cues and have been implicated in cell motility as well as in cell–substrate adhesion. Formins are highly conserved multidomain proteins that play important roles in the nucleation of actin and the formation of linear actin filaments, yet their role in filopodia formation has remained poorly defined. The *Dictyostelium* diaphanous-related formin dDia2 is strongly enriched in filopodia tips. Genetic and biochemical analysis revealed that this protein is important for cell migration and cell adhesion, but most importantly for the formation of filopodia. Recently, we have identified the *Dictyostelium* VASP (vasodilator-stimulated phosphoprotein) orthologue as a binding partner of dDia2 and provide evidence for a co-operative role of both proteins in filopodia formation.

What are filopodia?

Filopodia are thin protrusions of the cell membrane that are filled with tight parallel bundles of linear actin filaments [1–3]. These long and highly dynamic structures can extend and retract at a rate of approx. 10 μm/min. An average filopodium is thought to contain 10–30 linear actin filaments with their fast growing barbed ends located at the filopodial tip [4]. The diameter of filopodia adds up to only a few hundred nanometres but they can reach a length of several micrometres.

Filopodia can be observed in many cell types, where they play a role in a variety of cellular processes. Filopodia have been most intensely studied in motile cells such as keratinocytes, fibroblasts and in the neurite growth cones, where they emerge from flat sheets of dense actin-meshworks of the leading edge [3]. In these cells filopodia appear to fulfil a sensory role [5]. They seem to be used for the exploration of other cell surfaces or the extracellular matrix, thereby identifying targets for adhesion or sensing guidance cues during neuronal development. In line with this function, filopodia are involved in long-range cell–cell communication during development where interaction of migrating epithelial sheets is required such as in *Drosophila* embryogenesis [1,6]. Filopodia can also be seen on the apical surface of a variety of cultured cell lines as well as in *Dictyostelium* [7,8]. In *Dictyostelium*, apical filopodia seem to be involved in the adhesion to bacteria or yeast particles before phagocytosis [9], whereas their role in most other cell types is still largely unknown.

How do cells make filopodia?

Filopodia formation was closely linked to a number of actin-associated proteins enriched in filopodia including the actin-bundling protein fascin [10], talin [7,11], myosin VII and X [9,12], SCAR (suppressor of cAMP receptor)/WAVE [WASP (Wiskott–Aldrich syndrome protein)-family verprolin homology protein] [13], IRSp53 (insulin receptor substrate 53) [14,15] as well as Ena (*Drosophila* enabled)/VASP (vasodilator-stimulated phosphoprotein) [3,16–18]. Ena/VASPs were proposed to associate with barbed ends of actin filaments, to protect them from capping protein and to induce growth of filopodial actin filaments [3,18,19]. However, it still remains unclear how most of these proteins precisely contribute to filopodia formation.

Two models have been proposed to explain the formation of either lamellipodia or filopodia at the molecular level [3]. The dendritic nucleation model suggests that lamellipodia arise when new actin filaments form as branches on existing actin filaments, under the control of the Arp2/3 (actin-related protein 2/3) complex. The filaments push the membrane outwards as they elongate by polymerization, until the growing ends are capped. Capping proteins bind tightly to barbed ends, thereby blocking filament growth. The result is a lamellipodial actin network of short, stiff and branched filaments that can exert force against the membrane. According to the convergent elongation model of filopodium formation, a tip complex of proteins recruits filaments of the lamellipodial network to become a filopodium. The tip complex includes Ena/VASPs, which were proposed to promote the growth of long, unbranched filaments by inhibiting the

Key words: actin, dDia2, *Dictyostelium*, filopodium, formin, vasodilator-stimulated phosphoprotein (VASP).

Abbreviations used: Arp2/3, actin-related protein 2/3; DAD, diaphanous-autoregulatory domain; DRF, diaphanous-related formin; Ena, *Drosophila* enabled; FH, formin homology; GBD, GTPase-binding domain; GFP, green fluorescent protein; TRITC, tetramethylrhodamine β-isothiocyanate; VASP, vasodilator-stimulated phosphoprotein.

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capping process [19]. Ena/VASPs might also be required to recruit other proteins to further stabilize and organize the actin filaments into bundles as the filopodium grows. However, despite its function as a potent nucleator of actin, the Arp2/3 complex leads to the formation of branched actin filaments, remains bound to the mother filament and does not associate with filopodia [20]. This implies that another actin-nucleator could be required for creating parallel filopodial actin filaments that grow at their tips. Formins, which constitute the second major class of actin nucleators of eukaryotic cells, possess the biochemical properties for generating linear actin filaments that are prominent in actin cables, stress fibres and filopodia [21]. Furthermore, mammalian mDia2 as well as *Dictyostelium* dDia2 localize to filopodial tips and were therefore potential candidates to be involved in nucleation of filopodial actin filaments [22–24].

Formins

The protein family of formins is conserved throughout a wide range of species, including *Dictyostelium*, plants, *Caenorhabditis*, *Drosophila*, mice and humans. These multidomain proteins are defined by a highly conserved FH2 (formin homology 2) domain that confers actin-nucleation activity [25]. On the N-terminal side, the FH2 domain is usually flanked by a proline-rich FH1 domain that binds profilin, SH3 (Src homology 3) domains and WW domains (protein–protein interaction domain containing two conserved tryptophan residues) [26,27]. The conserved FH2 domains from various species nucleate new actin filaments, probably by stabilizing an actin dimer [28], and remain tightly bound to the barbed ends of the growing filaments [24,29]. Formins have been found to act as processive motors and were shown to produce piconewton forces during the insertional assembly of actin filament barbed ends [30,31].

DRFs (diaphanous-related formins) constitute a conserved subfamily of regulated formins that act as effectors of Rho family GTPases [32]. In these proteins the FH1 and FH2 domains are flanked by an N-terminal GBD (GTPase-binding domain) and a C-terminal DAD (diaphanous-autoregulatory domain). Binding of an activated Rho-GTPase to the GBD reduces an inhibitory intramolecular interaction between GBD and DAD and leads to an activated state [33]. Many DRFs also contain an FH3 domain between GBD and FH1 that is important for subcellular localization [34].

The *Dictyostelium* formin dDia2 interacts with VASP and is required for the formation of filopodia

The characterization of the *Dictyostelium* formin dDia2 and the identification of VASP as a dDia2-binding protein provided deeper insight into the molecular mechanism of filopodia formation in this model organism [24]. dDia2 is a DRF most closely related to human mDia1/mDia2 and to human Daam 1 (dishevelled-associated activator of morphogenesis). It interacts specifically with profilin II and is an effector of the small GTPase Rac, which in turn was previously shown to be part of a signalling pathway required for filopodium formation in *Dictyostelium* [35]. A recombinant FH1FH2 fragment of dDia2 promotes actin assembly, removes capping protein from capped filament ends and prevents depolymerization of F-actin in a concentration-dependent manner [24]. *In vivo* dDia2 is strongly enriched at the tips of substrate-attached and free filopodia and remains at the tips of growing and retracting filopodia, suggesting that it is persistently associated with the growing barbed ends of filopodial actin filaments (Figure 1). Mutants lacking dDia2 are severely impaired in the formation of filopodia, resulting in significantly fewer and shorter filopodia (Figure 2). The expression of GFP (green fluorescent protein)-tagged dDia2 restores filopodium formation in the null mutant, demonstrating a specific requirement for dDia2 in this process. Conversely, the overexpression of dDia2 leads to longer and more stable filopodia in comparison with wild-type cells (Figure 2). Thus dDia2 expression and filopodia formation are directly correlated. In addition to its important role in the establishment and maintenance of filopodia, dDia2 is also involved in the regulation of cell migration and cell–substrate adhesion (Figure 3). In comparison with wild-type cells, dDia2-null mutants show a slightly reduced contact area and an enhanced random motility on a glass surface [24]. dDia2-overexpressing cells show a strongly increased contact area and a reduced motile behaviour. These findings demonstrate an inverse correlation between dDia2 expression and cell motility and revealed that filopodia are not essential for motility of *Dictyostelium* amoebae.

Other key players for the formation of filopodia in mammalian cells and *Dictyostelium* are members of the Ena/VASP family [3,17], but their precise functions and interactions with other proteins in filopodia, however, remain controversial. Consistent with a crucial function of VASP, *Dictyostelium* mutants lacking VASPs are severely impaired in filopodium formation [17]. Interestingly, dDia2 interacts

Figure 1 | The formin dDia2 is localized at the tips of filopodia

A *Dictyostelium* cell expressing GFP-tagged full-length dDia2 was fixed and labelled with anti-GFP antibodies to visualize dDia2 (green) and with TRITC (tetramethylrhodamine β-isothiocyanate) phalloidin to visualize F-actin (red). The merged image of a part of the cell shows strongest dDia2 enrichment at the distal tips of filopodial actin filaments. The distribution of endogenous dDia2 is similar [24]. Scale bar, 2 μm. The brightest point projection of 11 slices in the *z*-direction, each 0.2 μm apart, is shown.

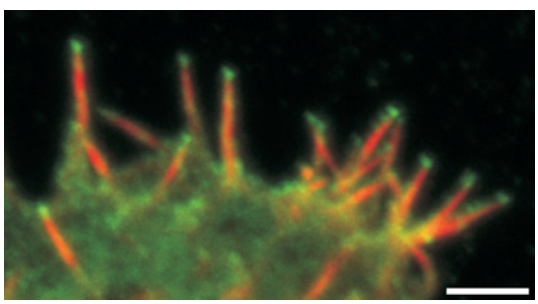


Figure 2 | **The expression of dDia2 directly correlates with filopodia formation**

Cell morphology and F-actin organization in wild-type cells and mutants that lack or overexpress dDia2. The cells were fixed and labelled with TRITC-phalloidin to visualize F-actin. Three-dimensional reconstructions were computed from confocal sections. dDia2-null mutants are strongly impaired in filopodia formation, whereas GFP–dDia2-overexpressing cells form excess filopodia in comparison with the wild-type. Scale bar, 5 μm.

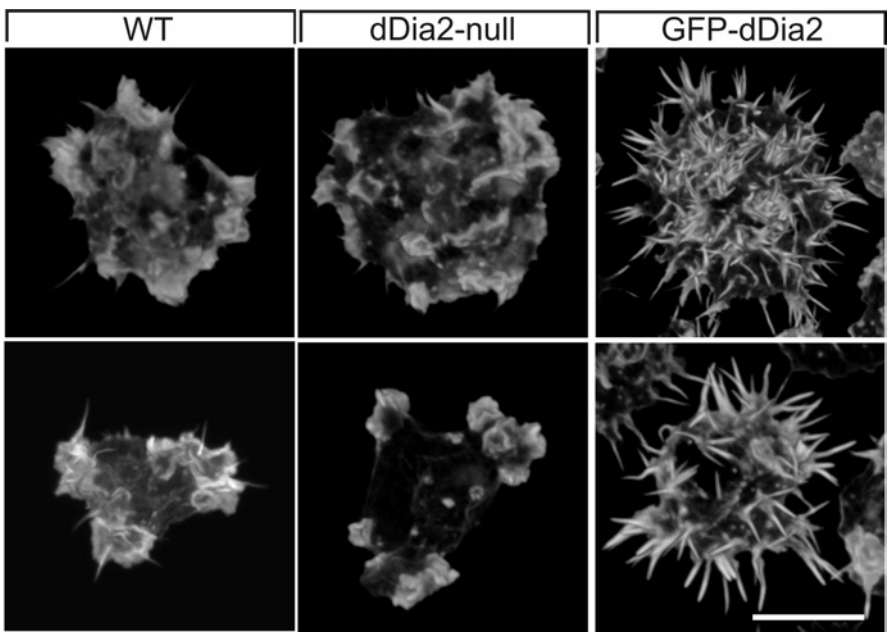
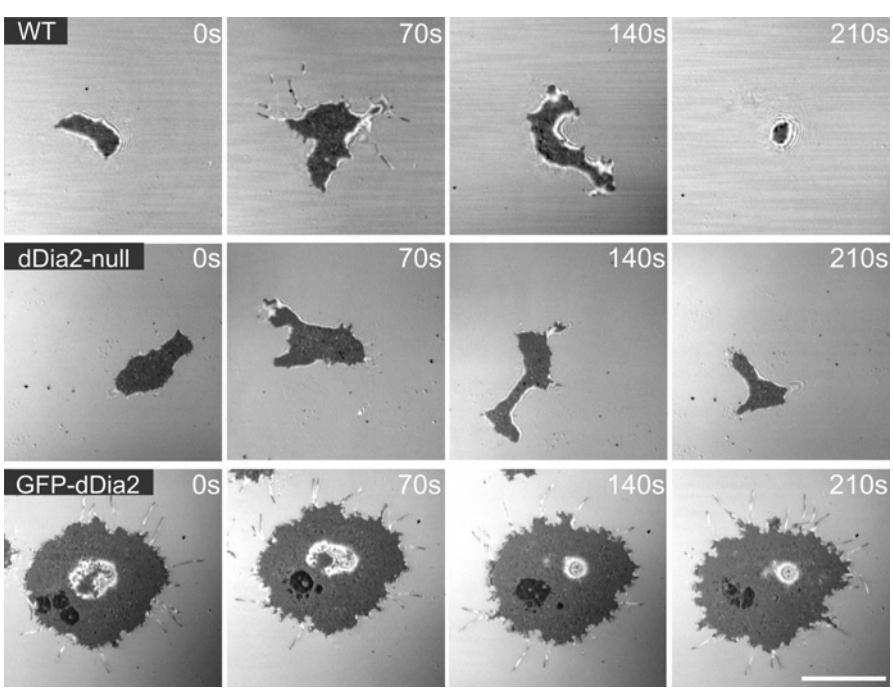


Figure 3 | **Strong cell–substrate adhesion in dDia2-overexpressing cells**

Reflection interference contrast microscopy micrographs (RICM) of a wild-type (WT) and a dDia2 mutant cell migrating on a glass surface in nutrient medium. The dark areas indicate where the cells are in close proximity to the substratum. The shown images are representative frames from time-lapse series. Scale bar, 10 μm.



directly with VASP through its FH2 domain. Recombinant *Dictyostelium* VASP nucleates and bundles actin filaments, but, in contrast with dDia2 FH1FH2, it does not appear to compete with capping proteins for binding to barbed ends. This suggests that VASP plays another role in filopodium formation. Clues will be obtained from rescue experiments

of VASP-null mutants with different VASP constructs. The interaction of VASP with dDia2, and its bundling capability suggest an attractive role for the co-operation of both proteins in filopodia formation. Actin filaments formed by dDia2 in the filopodial tip complex could be stabilized by bundling through VASP while they are being formed.

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