mDia1-3 in mammalian filopodia

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mDia proteins are members of the formin family of actin nucleating proteins that polymerize linear actin filaments. Such filaments form the core of thin, tubular, membrane-bound cell surface protrusions known as filopodia, which are a major feature of mammalian cell morphology. Filopodia are dynamic structures that help cells sense environmental cues, and play a role in cell migration, axon guidance, angiogenesis and other processes. RhoGTPases bind to and control the activity of mDia proteins, and several other binding partners of the three mDia1 isoforms—mDia1, mDia2 and mDia3—have been documented. Two independent pathways controlling mammalian filopodium formation have emerged, with one driven by the RhoGTPase Cdc42, and the other by Rif. While mDia2 has been the main formin implicated in forming filopodia, mDia1 has recently surfaced as the key formin utilized by both the Cdc42 and Rif pathways to drive filopodial protrusion.

mDia Domain Organization and Interacting Proteins

When cells migrate, they extend dynamic membrane-bound actin-rich tubular protrusions known as filopodia.[1] Formins are a family of large multi-domain proteins that nucleate and polymerize actin to form linear actin filaments like those found within filopodia.[2] In this review we will focus on the formins mDia1–3 and their role in filopodium formation. Formins function as dimers and nucleate actin by means of a formin homology 2 (FH2) domain that binds globular actin monomers. Interaction of the adjacent formin homology 1 (FH1) domain with profilin effectively recruits actin monomers to the formin dimer, facilitating the polymerization process.[3] A subset of formins, known as Diaphanous-related formins (Drfs), bind to and are regulated by RhoGTPases.[3] Drfs are rendered inactive by interaction of a C-terminal Diaphanous autoregulatory domain (DAD) with an N-terminal Diaphanous inhibitory domain (DID) (**Fig. 1**). The binding of a RhoGTPase to the N-terminal GTPase binding domain (GBD) contributes to disruption of this autoinhibitory interaction, which results in the activation of the Drf.[2] In the case of the Drf FHOD1, phosphorylation of C-terminal serine and threonine residues by ROCK also overcomes the autoinhibition.[4] Other domains found in Drfs include a a dimerization domain (DD) and a coiled coil (CC) region, and some groups believe that the DD and DID together constitute a loosely defined formin homology 3 (FH3) domain.[3] An N-terminal phospholipid-binding basic domain (BD) has also been identified in the Drfs mDia1 and mDia2. mDia1 has three stretches of basic amino acids in this domain, which allows it to localize to the plasma membrane, while mDia2 has two.[5] In contrast, the Drf DAAM1 has only one stretch of basic amino acids in its N-terminal, and the distribution of constitutively active DAAM1 is restricted to the cytoplasm.[6] This might explain why only mDia1 and mDia2 have been implicated in the formation of membrane-bound cell surface protrusions like filopodia and lamellipodia,[7-9] while mDia3—lacking this BD—has only been reported to generate cytoplasmic actin structures.[10] Several RhoGTPases and other proteins have been reported to bind to mDia1-3. These are summarized in **Figure 1**.

mDia Proteins in Actin-Based Cellular Structures

mDia1-3 form several types of actin-based cellular structures. Within the cytoplasm, mDia1 gives rise to stress fibers,[11,12] and mDia2 drives the actin dynamics that power vesicle movement[13] and creates the actin scaffold for constriction of the contractile ring during cytokinesis.[14] At the plasma membrane, both mDia1 and mDia2 have been shown to form lamellipodia[8,15] and filopodia.[7,16] A role for formins in lamellipodial protrusion is not surprising—once thought to be structures comprised of dendritic networks of branched microfilaments, lamellipodia have recently been reported to contain linear actin filaments as well.[17] We have found mDia3 to be capable of inducing filopodia in N1E115 neuroblastoma cells, despite not being able to detect the presence of endogenous mDia3 protein in this particular cell line.[16] All three mDia isoforms have also been linked to invadopodia protrusion,[18] while mDia2 alone plays a role in forming the filopodial precursors of dendritic spines.[19] Actin dynamics leading to the formation of the phagocytic cup in macrophages are believed to involve mDia1 and mDia2 too.[20]
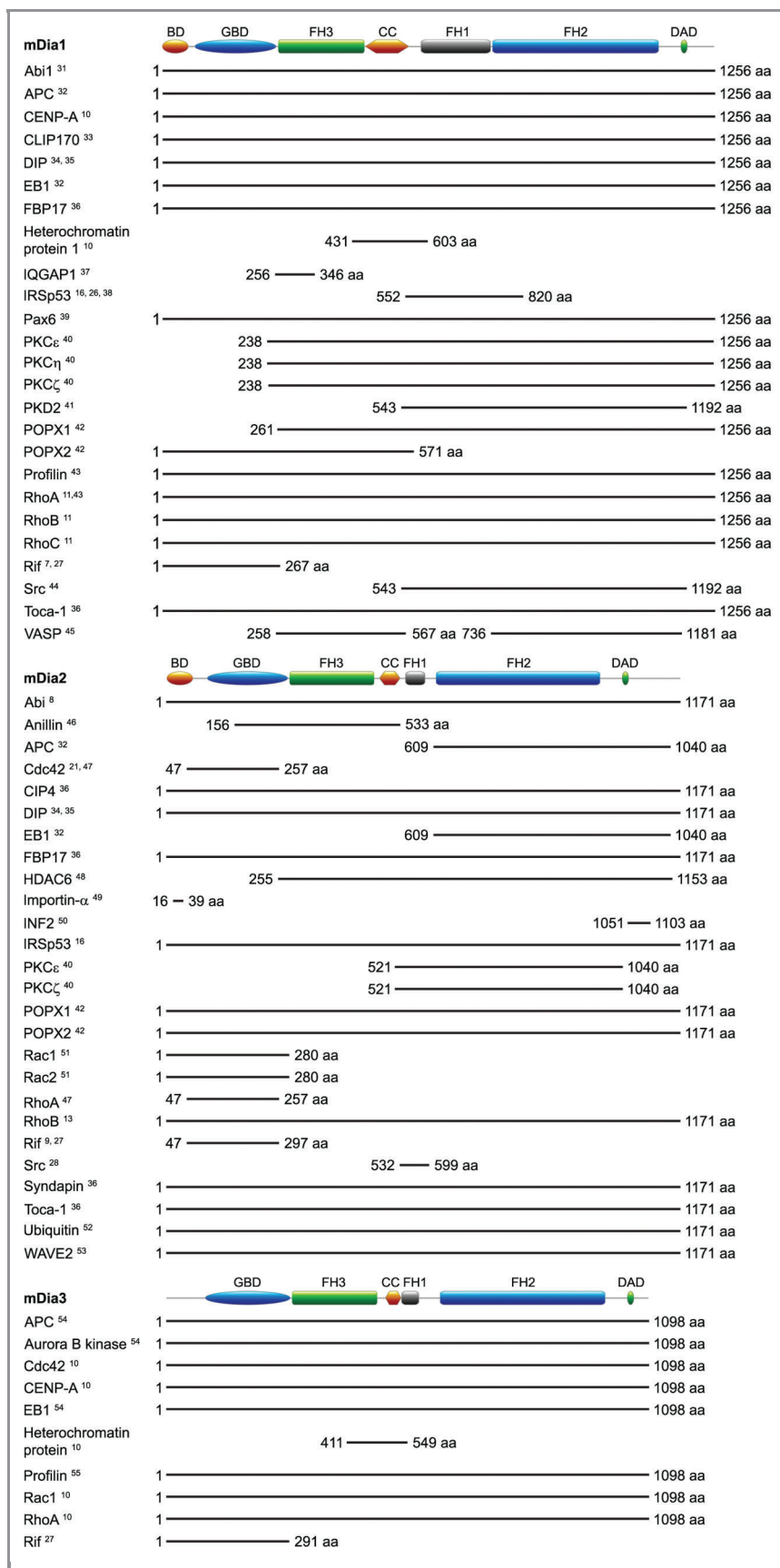
Figure 1. Domain organization and interacting partners of mDia1-3. The shortest fragment(s) known to bind the respective mDia isoforms is shown for each interacting protein. Excluded from this diagram are YWK-II, which binds a 223 aa fragment of hDia1 that shares 96% aa sequence identity with mDia1 (903-1125 aa),[56] and INF2, which binds to aa 1181-1262 and aa 1051-1193 of what might be longer splice variants of mDia1 and mDia3 respectively.[50] Domain architecture diagrams were created using MyDomains Image Creator (prosite.expasy.org/cgi-bin/prosite/mydomains/).

mDia Proteins and Filopodium Formation in Mammalian Cells

Previous studies have pointed to a role for mDia2 in mammalian filopodia. A decrease in filopodial protrusion was seen in mouse fibroblasts overexpressing constitutively active Cdc42 and microinjected with anti-mDia2 antibodies, as well as cells cotransfected with activated Cdc42 and a non-functional mDia2 mutant.[21] In NIH3T3 fibroblasts, mDia2 localized to the tips of filopodia in cells over-expressing constitutively active Rif.[9] Constitutively active mDia2, when overexpressed alone in B16F1 melanoma cells, also accumulated at filopodial tips.[22] Furthermore, knockdown of mDia2 protein in mouse hippocampal neurons reduced the formation of the filopodial precursors of dendritic spines.[19] In more recent work, mDia1-3 have been shown to induce filopodia in neuronal cells when overexpressed on their own.[7,16] However, only mDia1 was seen within filopodia, as observed by time lapse imaging of live cells.[7,16] The lack of mDia2 and mDia3 in neuronal filopodia implies that these Drfs might be involved in the initiation of filopodium formation but not the elongation of the structures. One possibility is that mDia2 and mDia3 generate short microfilaments that are subsequently elongated by mDia1 to form mature filopodia. This would tie in with the suggestion that mDia2 is a relatively strong nucleator but poor elongator of microfilaments,[23] based on observations that it elongates microfilaments at a slower rate than mDia1.[24] The filopodia induced by full-length wild-type mDia2 appeared cylindrical and of even thickness along their length,[7,16] and so did the filopodia formed by FH1FH2-mDia2, a fragment of mDia2 that consists of only the FH1 and FH2 domains.[8] This is unlike the club-shaped filopodia obtained by transfecting

B16F1 cells with constitutively active mDia2,[8,22] where the structures are packed with shorter microfilaments at their distal ends but contain relatively few long microfilaments that extend toward the base of the protrusions.[22] The high overexpression of constitutively active mDia2 could have resulted in endogenous mDia1 protein becoming a limiting factor on the process of filopodial microfilament elongation, giving rise to the club-shaped morphology of the filopodia. In addition, both full-length mDia2 and FH1FH2-mDia2 localized mostly to the cytoplasm[7,8]—in these experiments it is likely that less of the protein was present in filopodia, and there was enough endogenous mDia1 to elongate the smaller number of short microfilaments generated, thus resulting in filopodia of even thickness along their shafts. These interpretations of the findings would further implicate mDia1 as the key mDia isoform that elongates microfilaments to form mature filopodia. It would be interesting to see if knocking out mDia1 affects mDia2-driven filopodial protrusion—will the resulting mDia2-induced filopodia be shorter in length?

mDia1 was seen throughout the shafts of filopodia when overexpressed alone or together with IRSp53 or constitutively active Rif in neuronal cells.[7,16] This is in contrast to the 'tip nucleation' model of filopodium formation,[1] where formins are expected to be found only at the tips of the protrusions. One possible explanation is that mDia1 dimers play a dual role in filopodium formation and are involved in not just polymerising the actin filaments but bundling them together as well. Another possibility is that filopodia consist of short, discontinuous actin filaments that do not span the entire length of the filopodial shaft. This has been shown by cryo-electron tomography studies to be the nature of the actin filaments that constitute Dictyostelium filopodia.[25] Superresolution microscopy studies would be able to reveal detail at the nanometre scale and help elucidate the specific locations of mDia1 and mDia2, as well as other proteins associated with filopodial protrusion, within the structures with much greater accuracy. This would help in establishing a better understanding of the roles of these proteins in the various stages of filopodium formation.

mDia Proteins in Filopodia Induced by Cdc42 and Rif

The Rho GTPases Cdc42 and Rif regulate distinct pathways to filopodium formation. Cdc42 works through IRSp53, which recruits to the plasma membrane the following proteins that modulate actin dynamics: N-WASP, Mena, WAVE2 and Eps8.[26] The Rif pathway to filopodia does not require IRSp53, N-WASP, Mena or WAVE2.[7] As for the mDia proteins, mDia1 appears to be the only isoform common to both pathways. In the filopodia of neuronal cells overexpressing IRSp53, mDia1 but not mDia2 was

present, and was observed to interact with IRSp53 within the structures.[16] While both mDia1 and mDia2 were present in Rif filopodia, only mDia1 interacted with the RhoGTPase.[7] In addition, knockdown of either of these two isoforms resulted in a decrease in Rif-driven filopodium formation,[7] while IRSp53 filopodia were affected only by the silencing of mDia1 expression.[16] Taken together, it appears that mDia2 is not required for IRSp53 to form filopodia, and we have found that coexpressing mDia2 with IRSp53 leads to a loss of filopodia instead.[16] It remains to be seen as to why cells need two or even more-yet-undiscovered pathways to form filopodia, and why IRSp53 requires only mDia1 when Rif appears to require both mDia1 and mDia2. Also, Rif has been shown to bind the GBD of mDia3, however the significance of this interaction has yet to be investigated.[27]

mDia1[11,27] and possibly mDia2[28] are involved in stress fiber formation in addition to filopodial protrusion. These two types of actin-based cellular structures appear to be linked—in fish fibroblasts, microfilaments in filopodia can become incorporated into stress fibers,[29] and it has been suggested that the reverse might occur in rat embryonic fibroblasts, with the actin freed up by the dissolution of stress fibers facilitating the protrusion of filopodia.[30] Rif interacts with both mDia1 and mDia2[27] and is able to trigger the formation of both filopodia[7,9] and stress fibers.[27] The dual role of these three proteins might point to a major role for them in controlling the balance between these two types of actin structures in cell migration.

Conclusions

It is clear that mDia proteins play an important role in mammalian filopodium formation. mDia2 appears to be specific for the Rif-mediated pathway whereas mDia1 is required for the pathways controlled by Cdc42 and Rif. It remains to be seen how exactly Rif utilizes two different formins, mDia1 and mDia2, to form filopodia. How Rif couples membrane deformation with actin dynamics to give rise to these structures has also yet to be resolved. Apart from mDia1 and mDia2, are there other proteins specific to the Rif pathway to filopodium formation? What potential roles do IRSp53 family proteins (IRTKS, MIM, ABBA and PinkBar) play in filopodial protrusion? These are some of the important questions to address in future studies on mammalian filopodia.

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