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FHOD proteins in actin dynamics—a formin' class of its own

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Abbreviations: AML-1B, acute myeloid leukemia transcription factor; DAD, diaphanous auto-regulatory domain; DID, diaphanous inhibitory domain; DRF, Diaphanous-related formins; FH1, formin homology 1; FH2, formin homology 2; FH3, formin homology 3; GBD, GTPase-binding domain; Dia, Diaphanous related formin; FHOD, FH1/FH2 domain-containing protein; SRE, serum response element.

Eukaryotic cells have evolved a variety of actin-binding proteins to regulate the architecture and the dynamics of the actin cytoskeleton in time and space. The Diaphanous-related formins (DRF) represent a diverse group of Rho-GTPase-regulated actin regulators that control a range of actin structures composed of tightly-bundled, unbranched actin filaments as found in stress fibers and in filopodia. Under resting conditions, DRFs are auto-inhibited by an intramolecular interaction between the C-terminal and the N-terminal domains. The auto-inhibition is thought to be released by binding of an activated RhoGTPase to the N-terminal GTPase-binding domain (GBD). However, there is growing evidence for more sophisticated variations from this simplified linear activation model. In this review we focus on the formin homology domain-containing proteins (FHOD), an unconventional group of DRFs. Recent findings on the molecular control and cellular functions of FHOD proteins in vivo are discussed in the light of the phylogeny of FHOD proteins.

Introduction

Formins are highly conserved multidomain proteins that control the formation of diverse cytoskeletal structures required for many cellular functions such as cell shape, adhesion, cytokinesis and cell motility (For a review see refs.[1-5]). The common defining features for this protein family are the formin homology domains 1 and 2 (FH1 and FH2). The proline-rich FH1 domain delivers profilin-bound actin monomers to the growing barbed end and the homodimeric FH2 domain is thought to induce actin polymerization by stabilizing actin dimers. Hence the formins stay attached to the growing barbed end and thereby prevent binding of capping proteins during actin elongation. A subgroup of formins, the Diaphanous-related formins (DRF) contain 2 additional conserved domains: the N-terminal diaphanous inhibitory domain (DID, also referred to as FH3) and the C-terminal diaphanous auto-regulatory domain (DAD), which interact with one another in order to keep the protein in an inactive state. In some DRF proteins, the auto-inhibition can be released by RhoGTPase binding. However, there is growing evidence that points to distinct regulatory mechanisms rather than a simple linear activation pathway. The FH1/FH2 domain-containing FHOD proteins share many features in the domain architecture with other DRF family members, but their molecular activities and the mechanism of activation seem to be distinct from other DRFs.

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FHOD proteins evolved in unicellular relatives of metazoans

Most evolutionary analyses of formins focused either on the characterization of the formin complement of defined species[6] and taxons[7] or on the relationship of different formin families to each other.[8,9] Here, we trace the history of FHOD, i.e. one formin family, over its whole evolutionary life (**Fig. 1**). We calculated a phylogeny of orthologs identified in completely sequenced eukaryotes. The most basal species with an identifiable FHOD ortholog is the filasterean Capsaspora owczarzaki. Besides the choanoflagellates, where no FHOD was found, this species is the closest known unicellular relative of metazoans. Thus, the emergence of FHOD predates the evolution of metazoa and parallels the history of Diaphanous, another formin family.[10] Whereas most species contain only a single member of this protein family, at least 2 are found in vertebrates. Even in the most basal vertebrate, the lamprey Petromyzon marinus, clearly defined orthologs of FHOD1 and FHOD3 are present. The genome of the urochordate Ciona intestinalis also contains 2 homologs of FHOD, but these are fragmentary and therefore could not be placed with certainty in the phylogenetic tree. Thus, a duplication before the split of urochordates and chordates cannot be excluded. Following this duplication, FHOD1 evolved significantly faster than FHOD3 ($P < 0.001$), indicating either a relaxed selective pressure or the emergence of new functions. Finally, within teleost

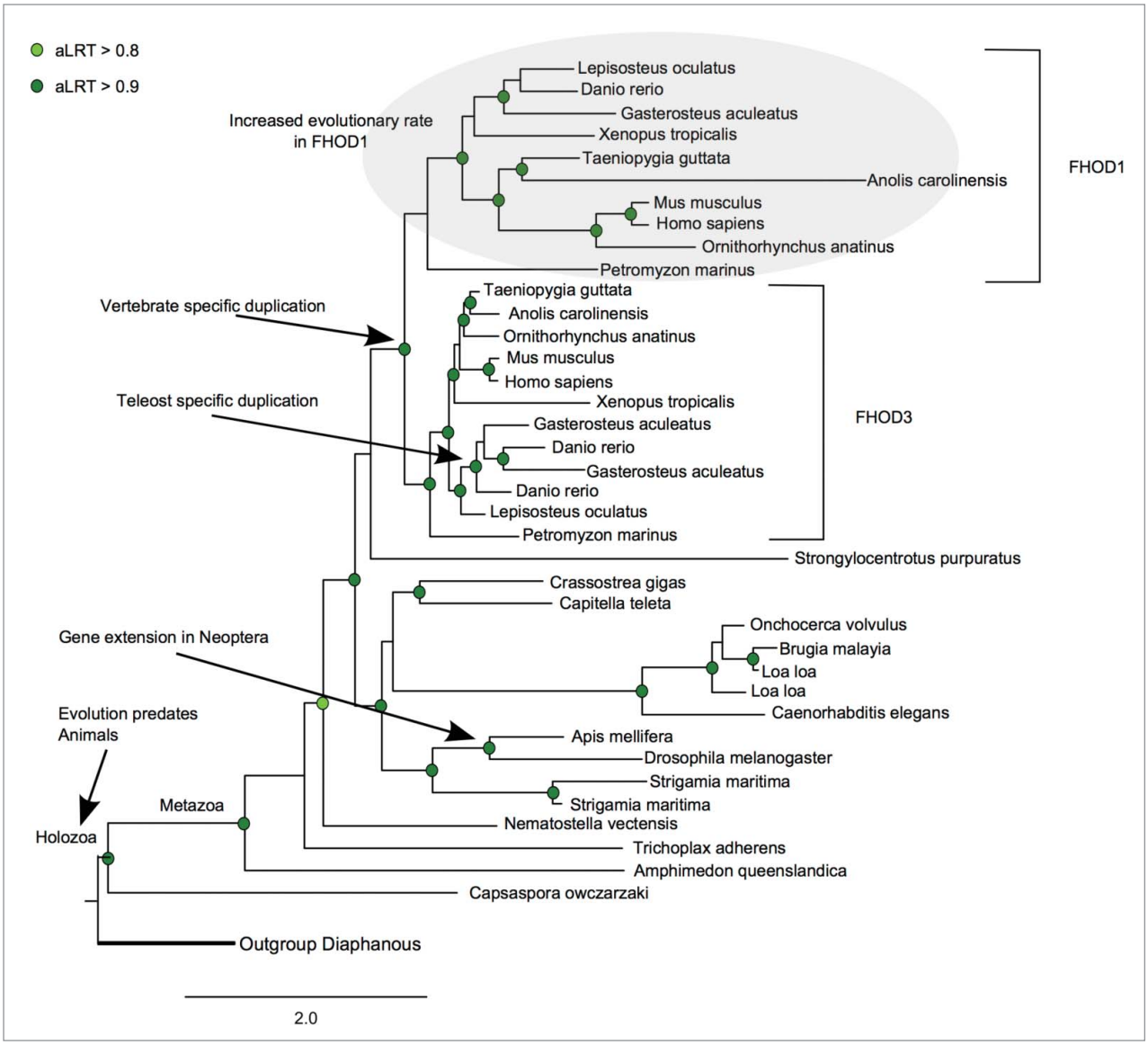

Figure 1. The evolutionary history of fhod gene. Candidate orthologues to D. melanogaster *knittrig* in selected species were extracted from ensembl.[35] As an outgroup to metazoans, the genomes of C. owczarzaki and Monosiga brevicollis were searched for candidate orthologues by reciprocal blastp. Protein sequences of the longest splice variant were aligned using muscle[36] and an evolutionary model was estimated with protTest.[37] The phylogenetic tree was calculated with phyml[38] using the approximate likelihood ratio as estimate for robustness. To compare the evolutionary rates of fhod1 and fhod2, a subset containing vertebrate sequences and the sea urchin Strongylocentrotus purpuratus as outgroup was aligned. Following a tree calculation with phyml, 2 models were tested using codeml from the PAML package.[39] In the first, a single evolutionary rate for the whole tree was assumed, whereas in the second the rate of the fhod1 clade was given as additional free parameter. According to a Chi2 test with one degree of freedom, the second model fit the data significantly better ($P < 0.001$).

fishes, the gene coding for FHOD3 duplicated again resulting in 3 FHOD genes in this clade (**Fig. 1**).

The Drosophila melanogaster ortholog of FHOD, *knittrig* distinguishes itself from the other FHODs by an N-terminal extension present in some splice variants. The function of this additional large exon is unclear. However, a short *knittrig* isoform lacking this extension substantially rescues the observed phenotypic traits of *knittrig* mutants including late-pupal lethality.[11] Analysis of further arthropod genomes revealed a similar extension in the pea aphid Acyrthosiphon pisum. Although no extension is annotated for other completely sequenced insects, inspection of adjacent genes revealed homologous sequences in the silk moth Bombyx mori, the bee Apis mellifera and the beetle Triboleum castaneum. Thus it can be assumed that within these species the FHOD genes are not correctly annotated and that they also encode an extended gene. As no traces of this extension was found in the body louse Pediculus humanus, this modification of the FHOD gene seems to be specific for Neoptera. The extension contains a repetitive structure with a conserved Trp-Trp repeat and its function is so far unknown. Still, an

evolutionary conservation for more than 370 million years implies its functional importance.

FHOD proteins differ from other DRFs in their activation mode

Initial studies by Westendorf and colleagues already revealed that FHOD1 is primarily auto-inhibited by an intramolecular interaction between N- and C-terminal residues.[12] However, unlike other DRFs, FHOD1 associates with the RhoGTPase Rac1 in a guanine nucleotide-independent manner in vitro. The N- and C-terminal regions of FHOD proteins differ significantly in overall composition and length of the particular domains (**Fig. 2A**). This suggests a different auto-inhibitory mechanism compared to so far known mDia1-RhoGTPase interactions. In vitro binding studies further confirmed that the N-terminal DAD-interacting FH3 domain does not overlap with the proposed Rac1-binding domain.[13] Thus, a competitive binding and subsequent activation by Rac1 seems unlikely. The auto-inhibitory interaction can be released by removal of the C-terminal DAD domain resulting in a stimulation of transcription from the serum response element (SRE) as well as in a strong induction of stress fibers.[12,14] Gasteier and colleagues provided first evidence that FHOD1-induced stress fiber formation depends on the activity of Rho-associated kinase Rock/Rok.[15] Further cell culture studies showed that Rock/Rok directly phosphorylates mammalian FHOD1 at highly conserved C-terminal residues, specifically at serine 1131, serine 1137 and threonin 1141.[16] Rock/Rok-induced phosphorylation seems to be a conserved activation mechanism in vivo. Serine phosphorylation of Knittrig, the only member of the FHOD family in Drosophila, is likewise enhanced upon Rok co-expression that promotes stress fiber formation in macrophages.[11] Thus, FHOD formins are likely downstream effectors of Rock/Rok signaling. While Rho-Rok function seems to be important for the activation, Rac1 signaling is thought to be required for the subcellular localization and recruitment of FHOD formins.

Structural analysis of the N-terminal region of mammalian FHOD1 provided further evidence for such a model of FHOD regulation.[17] The deletion of the Ras-like GBD domain does not result in an activation of FHOD1, but on the contrary, results in a complete loss of the biological activity.[17] In contrast, mutation of a central valine at position 228 within the FH3 domain results in a constitutive active mutant.[17] Thus, FHOD1 contains two distinct N-terminal regulatory modules. The GBD domain is required for the biological activity and the localization mediated by Rac whereas the adjacent FH3 domain provides the surface for the intramolecular interaction with the DAD domain, which

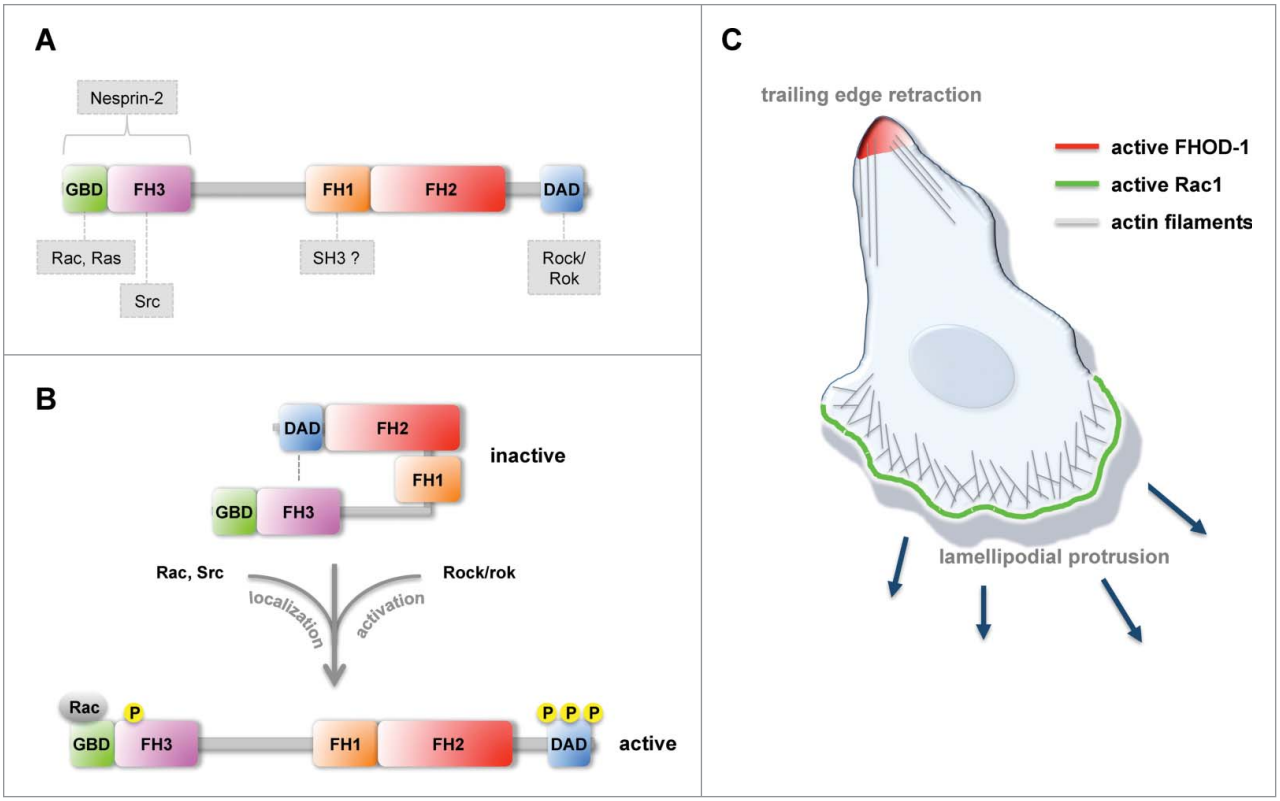

Figure 2. (**A**) Domain architecture of FHOD proteins and known signals/interactors linked to indicated FHOD domains. GBD: GTPase binding domain; FH3: formin homology 3 domain; FH1: formin homology 1 domain; FH2: formin homology 2 domain; DAD: Diaphanous autoregulation domain. (**B**) Proposed model of how different signaling pathways differentially control the localization/recruitment and the activation of FHOD proteins. Please compare text. (**C**) Schematic illustration of the two complementary activities at the trailing and leading edge of migrating macrophages as previously proposed.[11,40]

is controlled by Rho-Rok dependent phosphorylation. How opposing Rac- and Rho-dependent signaling on FHOD1 function are integrated in vivo still remains unknown (**Fig. 2B**). Given that the expression of active FHOD1 interfered with Rac1-induced lamellipodia formation, it has been proposed that Rho/Rock activity may be fine-tuned by simultaneous inhibition of Rac.[18] Such a scenario might be relevant in migrating cells. However, in vaccinia virus induced actin-based motility Rac1 suppression phenocopied effects of FHOD1 depletion suggesting a positive rather than an antagonistic relationship in vaccinia actin comet tail formation.[19]

The exact biochemical activity of FHOD proteins on actin filaments is also unclear. Recent in vitro actin polymerization assays with purified active FHOD1 lacking the DAD domain suggested that FHOD proteins might act as a combined actin capping and bundling factor rather than an actin nucleator promoting actin polymerization.[20] Thus, the induction of stress fibers might be the result of bundling or stabilizing pre-existing actin filaments. However, the deletion of the complete C-terminal DAD region may also affect an activity that is not related to auto-inhibition as recently observed for mDia1.[21] Supporting this notion, Schulze and colleagues found that the formation of stress fibers induced by the constitutively active V228E mutation is even stronger compared to a C-terminal truncation lacking the DAD region.[22] Future structural analysis of the C-terminus will certainly help to better understand the DAD-mediated auto-inhibition as well as the exact biochemical activity of FHOD1 on actin polymerization.

FHOD proteins play a major role in the formation of contractile actin bundles regulating cell migration and sarcomeric actin filament organization

Our current understanding of the cellular functions of FHOD proteins is largely based on cell culture studies although recent studies in Drosophila and C. elegans provided first insights into the physiological functions in vivo.

FHOD1, one of the 2 members of the mammalian FHOD family, was originally isolated as a yeast-2-hybrid binding partner of the acute myeloid leukemia transcription factor AML-1B from a B cell library.[23] The physiological relevance of this interaction has never been proven although FHOD1 has been described to be abundantly expressed in spleen, B cells and erythroid leukemia cells.[24] A recent quantitative real-time approach however revealed that FHOD1 is the most highly expressed human formin in haematopoietic as well as in non-haematopoietic cell types.[25] In contrast, the FHOD3 protein, which is most related to FHOD1, is the least expressed formin with a specific expression pattern in skeletal and cardiac muscle.[25]

FHOD proteins have been best characterized for their role in stimulating stress fiber formation upon over-expression of C-terminal truncated mutant proteins. A recent detailed cell culture study further demonstrated a more differential role of FHOD1 in regulating the formation and turn over of transverse arcs into mature ventral stress fibers.[22] This prominent function of FHOD1 on contractile actin bundles seems to be essential for proper cell spreading, cell adhesion and cell migration.[14,22] More recent RNAi-mediated knockdown experiments further revealed that FHOD1 assembles F-actin at integrin adhesions to drive cell spreading and migration by coordinating traction stress and adhesion maturation.[26] The ability of FHOD1 in forming actin bundles might also play an important physiological function in nuclear movement in migrating cells as previously observed in fibroblasts.[27]

The role of FHOD in cell spreading and cell migration seems to be conserved in Drosophila.[11] Loss of *knittrig* function in flies results in strong cell spreading defects of isolated primary macrophages. More importantly, mutant macrophages show strong defects in directional wound-induced cell migration in vivo.[11] Live imaging studies further revealed a remarkably dynamic accumulation of FHOD at the cell rear of migrating macrophages.[11] Consistent with this distinct localization, an active FHOD protein lacking the DAD region promotes the formation of actin bundles at the cell rear in a Rok-dependent manner (**Fig. 2C**). Based on these findings a model has been proposed in which FHOD acts downstream of Rok coordinating actin assembly and actomyosin-based retraction in the trailing edge of migrating macrophages.[11] However, it remains unclear how Drosophila FHOD is recruited to the cell rear in vivo while the same EGFP-tagged fusion localized throughout the cytoplasm in cultured macrophages, which might be an artifact due to the culturing of isolated cells. Targeting of human FHOD1 to adhesion sites depends on Src kinase phosphorylation on tyrosine Y99 that enables subsequent activation by Rock.[26] Drosophila FHOD contains several conserved tyrosine residues within its N-terminus, thus it might also be a downstream target of Src. Future genetic studies are required to rule out if the localization of FHOD is affected in src mutant macrophages. A positive activity on cell migration has recently been observed for human FHOD1, whose expression is up-regulated during epithelial-mesenchymal transition promoting cancer cell migration.[28] Consistently, overexpression of human FHOD1 in melanoma and breast cancer cells promotes cell migration and cell invasion.[29] The physiological function of mammalian FHOD1 in cell migration has not yet been addressed in a knockout model.

A knockout mouse model has recently been established to analyze fhod3 function in vivo.[30] The phenotypic analysis of mutant mice revealed an essential role in cardiogenesis by organizing myofibrillogenesis.[30] Fhod3 deficient mice are embryonic lethal with severe defects at early stages of heart development. These data confirmed previous findings obtained from RNAi-mediated depletion of FHOD3 in cultured cardiomyocytes that shows a reduction of filamentous actin and disruption of the sarcomeric organization.[31,32] Together, these studies document a differential requirement of FHOD1 and FHOD3, which is already reflected by their distinct expression pattern. A function of FHOD in Drosophila myogenesis has not yet been found. Loss of *knittrig* function affects trachea and wing morphology.[11] Analysis of the only fhod-1 ortholog in C. elegans revealed more functional similarities to mammalian FHOD3. Fhod-1 is present in sarcomeres of developing striated body wall muscles and worms lacking fhod-1 showed modest morphological and functional muscle defects.[33] A recent RNAi screen in C. elegans identified an additional essential function of fhod-1 in organizing

actin stress fibers in contractile lateral epidermal cells.[34] Unlike FHOD3 function in mammals it remains unclear if Fhod-1 acts downstream of Rho/Rok signaling. Future studies should also provide insights into a possible conserved function of C. elegans Fhod-1 in cell migration.

Taken together, recent studies advance substantially our understanding about the cellular functions of the FHOD proteins. Findings from different model systems underline a major role in the formation of contractile actin bundles that are required for cell migration and sarcomeric actin filament organization. The phylogeny of FHOD suggests that these 2 important cellular functions were first adopted by a single gene. During evolution both function were split between 2 genes in vertebrates (fhod1 and fhod3). Many questions about the exact mechanisms of how FHOD proteins promote actin bundles and how different signals control their in vivo activity in time and space remain important challenges for future research.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Chhabra ES, Higgs HN. The many faces of actin: matching assembly factors with cellular structures. Nat Cell Biol 2007; 9:1110-21; PMID:17909522; http://dx.doi.org/10.1038/ncb1007-1110
2. Faix J, Grosse R. Staying in shape with formins. Dev Cell 2006; 10:693-706; PMID:16740473; http://dx.doi.org/10.1016/j.devcel.2006.05.001
3. Liu R, Linardopoulou EV, Osborn GE, Parkhurst SM. Formins in development: orchestrating body plan origami. Biochim Biophys Acta 2010; 1803:207-25; PMID:18996154; http://dx.doi.org/10.1016/j.bbamcr.2008.09.016
4. Young KG, Copeland JW. Formins in cell signaling. Biochim Biophys Acta 2010; 1803:183-90; PMID:18977250; http://dx.doi.org/10.1016/j.bbamcr.2008.09.017
5. Chesarone MA, DuPage AG, Goode BL. Unleashing formins to remodel the actin and microtubule cytoskeletons. Nat Rev Mol Cell Biol 2010; 11:62-74; PMID:19997130; http://dx.doi.org/10.1038/nrm2816
6. Schonichen A, Geyer M. Fifteen formins for an actin filament: a molecular view on the regulation of human formins. Biochim Biophys Acta 2010; 1803:152-63; PMID:20102729; http://dx.doi.org/10.1016/j.bbamcr.2010.01.014
7. Grunt M, Zarsky V, Cvrckova F. Roots of angiosperm formins: the evolutionary history of plant FH2 domain-containing proteins. BMC Evol Biol 2008; 8:115; PMID:18430232; http://dx.doi.org/10.1186/1471-2148-8-115
8. Chalkia D, Nikolaidis N, Makalowski W, Klein J, Nei M. Origins and evolution of the formin multigene family that is involved in the formation of actin filaments. Mol Biol Evol 2008; 25:2717-33; PMID:18840602; http://dx.doi.org/10.1093/molbev/msn215
9. Rivero F, Muramoto T, Meyer AK, Urushihara H, Uyeda AQ, Kitayama C. A comparative sequence analysis reveals a common GBDFH3-FH1-FH2-DAD architecture in formins from Dictyostelium, fungi and metazoa. BMC Genomics 2005; 6:28; PMID:15740615; http://dx.doi.org/10.1186/1471-2164-6-28
10. Bogdan S, Schultz J, Grosshans J. Formin' cellular structures: Physiological roles of Diaphanous (Dia) in actin dynamics. Commun Integr Biol 2013; 6:e27634; PMID:24719676; http://dx.doi.org/10.4161/cib.27634
11. Lammel U, Bechtold M, Risse B, Berh D, Fleige A, Bunse I, Jiang X, Klambt C, Bogdan S. The Drosophila FHOD1-like formin Knittrig acts through Rok to promote stress fiber formation and directed macrophage migration during the cellular immune response. Development 2014; 141:1366-80; PMID:24553290; http://dx.doi.org/10.1242/dev.101352
12. Westendorf JJ. The formindiaphanous-related protein, FHOS, interacts with Rac1 and activates transcription from the serum response element. J Biol Chem 2001; 276:46453-9; PMID:11590143; http://dx.doi.org/10.1074/jbc.M105162200
13. Schonichen A, Alexander M, Gasteier JE, Cuesta FE, Fackler OT, Geyer M. Biochemical characterization of the diaphanous autoregulatory interaction in the formin homology protein FHOD1. J Biol Chem 2006; 281:5084-93; PMID:16361249; http://dx.doi.org/10.1074/jbc.M509226200
14. Koka S, Neudauer CL, Li X, Lewis RE, McCarthy JB, Westendorf JJ. The formin-homology-domain-containing protein FHOD1 enhances cell migration. J Cell Sci 2003; 116:1745-55; PMID:12665555; http://dx.doi.org/10.1242/jcs.00386
15. Gasteier JE, Madrid R, Krautkramer E, Schroder S, Muranyi W, Benichou S, Fackler OT. Activation of the Rac-binding partner FHOD1 induces actin stress fibers via a ROCK-dependent mechanism. J Biol Chem 2003; 278:38902-12; PMID:12857739; http://dx.doi.org/10.1074/jbc.M306229200
16. Takeya R, Taniguchi K, Narumiya S, Sumimoto H. The mammalian formin FHOD1 is activated through phosphorylation by ROCK and mediates thrombin-induced stress fibre formation in endothelial cells. EMBO J 2008; 27:618-28; PMID:18239683; http://dx.doi.org/10.1038/emboj.2008.7
17. Schulte A, Stolp B, Schonichen A, Pylypenko O, Rak A, Fackler OT, Geyer M. The human formin FHOD1 contains a bipartite structure of FH3 and GTPase-binding domains required for activation. Structure 2008; 16:1313-23; PMID:18786395; http://dx.doi.org/10.1016/j.str.2008.06.008
18. Baarlink C, Grosse R. A GBD uncovered: the FHOD1 N terminus is formin'. Structure 2008; 16:1287-8; PMID:18786389; http://dx.doi.org/10.1016/j.str.2008.08.002
19. Alvarez DE, Agaisse H. The formin FHOD1 and the small GTPase Rac1 promote vaccinia virus actin-based motility. J Cell Biol 2013; 202:1075-90; PMID:24062339; http://dx.doi.org/10.1083/jcb.201303055
20. Schonichen A, Mannherz HG, Behrmann E, Mazur AJ, Kuhn S, Silvan U, Schoenenberger CA, Fackler OT, Raunser S, Dehmelt L, et al. FHOD1 is a combined actin filament capping and bundling factor that selectively associates with actin arcs and stress fibers. J Cell Sci 2013; 126:1891-901; PMID:23444374; http://dx.doi.org/10.1242/jcs.126706
21. Gould CJ, Maiti S, Michelot A, Graziano BR, Blanchoin L, Goode BL. The formin DAD domain plays dual roles in autoinhibition and actin nucleation. Curr Biol 2011; 21:384-90; PMID:21333540; http://dx.doi.org/10.1016/j.cub.2011.01.047
22. Schulze N, Graessl M, Blancke Soares A, Geyer M, Dehmelt L, Nalbant P. FHOD1 regulates stress fiber organization by controlling the dynamics of transverse arcs and dorsal fibers. J Cell Sci 2014; 127:1379-93; PMID:24481812; http://dx.doi.org/10.1242/jcs.134627
23. Zeng C, van Wijnen AJ, Stein JL, Meyers S, Sun W, Shopland L, Lawrence JB, Penman S, Lian JB, Stein GS, et al. Identification of a nuclear matrix targeting signal in the leukemia and bone-related AMLCBF-alpha transcription factors. Proc Natl Acad Sci U S A 1997; 94:6746-51; PMID:9192636; http://dx.doi.org/10.1073/pnas.94.13.6746
24. Westendorf JJ, Mernaugh R, Hiebert SW. Identification and characterization of a protein containing formin homology (FH1FH2) domains. Gene 1999; 232:173-82; PMID:10352228; http://dx.doi.org/10.1016/S0378-1119(99)00127-4
25. Krainer EC, Ouderkirk JL, Miller EW, Miller MR, Mersich AT, Blystone SD. The multiplicity of human formins: Expression patterns in cells and tissues. Cytoskeleton (Hoboken) 2013; 70:424-38; PMID:23629878; http://dx.doi.org/10.1002/cm.21113
26. Iskratsch T, Yu CH, Mathur A, Liu S, Stevenin V, Dwyer J, Hone J, Ehler E, Sheetz M. FHOD1 is needed for directed forces and adhesion maturation during cell spreading and migration. Dev Cell 2014; 27:545-59; http://dx.doi.org/10.1016/j.devcel.2013.11.003
27. Kutscheidt S, Zhu R, Antoku S, Luxton GW, Stagljar I, Fackler OT, Gundersen GG. FHOD1 interaction with nesprin-2G mediates TAN line formation and nuclear movement. Nat Cell Biol 2014; 16:708-15; PMID:24880667
28. Gardberg M, Kaipio K, Lehtinen L, Mikkonen P, Heuser VD, Talvinen K, Iljin K, Kampf C, Uhlen M, Grenman R, et al. FHOD1, a formin upregulated in epithelial-mesenchymal transition, participates in cancer cell migration and invasion. PLoS One 2013; 8:e74923; PMID:24086398; http://dx.doi.org/10.1371/journal.pone.0074923
29. Jurmeister S, Baumann M, Balwierz A, Keklikoglou I, Ward A, Uhlmann S, Zhang JD, Wiemann S, Sahin O. MicroRNA-200c represses migration and invasion of breast cancer cells by targeting actin-regulatory proteins FHOD1 and PPM1F. Mol Cell Biol 2012; 32:633-51; PMID:22144583; http://dx.doi.org/10.1128/MCB.06212-11
30. Kan OM, Takeya R, Abe T, Kitajima N, Nishida M, Tominaga R, Kurose H, Sumimoto H. Mammalian formin Fhod3 plays an essential role in cardiogenesis by organizing myofibrillogenesis. Biol Open 2012; 1:889-96; PMID:23213483; http://dx.doi.org/10.1242/bio.20121370
31. Taniguchi K, Takeya R, Suetsugu S, Kan OM, Narusawa M, Shiose A, Tominaga R, Sumimoto H. Mammalian formin fhod3 regulates actin assembly and

sarcomere organization in striated muscles. J Biol Chem 2009; 284:29873-81; PMID:19706596; http://dx.doi.org/10.1074/jbc.M109.059303
32. Iskratsch T, Lange S, Dwyer J, Kho AL, dos Remedios C, Ehler E. Formin follows function: a muscle-specific isoform of FHOD3 is regulated by CK2 phosphorylation and promotes myofibril maintenance. J Cell Biol 2010; 191:1159-72; PMID:21149568; http://dx.doi.org/10.1083/jcb.201005060
33. Mi-Mi L, Votra S, Kemphues K, Bretscher A, Pruyne D. Z-line formins promote contractile lattice growth and maintenance in striated muscles of C. elegans. J Cell Biol 2012; 198:87-102; PMID:22753896; http://dx.doi.org/10.1083/jcb.201202053
34. Vanneste CA, Pruyne D, Mains PE. The role of the formin gene fhod-1 in C. elegans embryonic morphogenesis. Worm 2013; 2:e25040; PMID:24778933; http://dx.doi.org/10.4161/worm.25040
35. Flicek P, Amode MR, Barrell D, Beal K, Billis K, Brent S, Carvalho-Silva D, Clapham P, Coates G, Fitzgerald S, et al. Ensembl 2014. Nucleic Acids Res 2014; 42:D749-55; PMID:24316576; http://dx.doi.org/10.1093/nar/gkt1196
36. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004; 32:1792-7; PMID:15034147; http://dx.doi.org/10.1093/nar/gkh340
37. Darriba D, Taboada GL, Doallo R, Posada D. ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics 2011; 27:1164-5; PMID:21335321; http://dx.doi.org/10.1093/bioinformatics/btr088
38. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 2003; 52:696-704; PMID:14530136; http://dx.doi.org/10.1080/10635150390235520
39. Yang Z. PAML 4: phylogenetic analysis by maximum likelihood. Mol Biol Evol 2007; 24:1586-91; PMID:17483113; http://dx.doi.org/10.1093/molbev/msm088
40. Sander M, Squarr AJ, Risse B, Jiang X, Bogdan S. Drosophila pupal macrophages–A versatile tool for combined ex vivo and in vivo imaging of actin dynamics at high resolution. Eur J Cell Biol 2013; 92:349-54; PMID:24183239