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Review

Fifteen formins for an actin filament: A molecular view on the regulation of human formins

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ABSTRACT

The regulation of the actin cytoskeleton is a key process for the stability and motility of eukaryotic cells. Besides the Arp2/3 complex and its nucleation promoting factors, WH2 domain-containing proteins and a diverse family of formin proteins have recently been recognized as actin nucleators and potent polymerization factors of actin filaments. Formins are defined by the presence of a catalytic formin homology 2 (FH2) domain, yet, the modular domain architecture appears significantly different for the eight formin families identified in humans. A diverse picture of protein localization, interaction partners and cell specific regulation emerged, suggesting various functions of formins in the building and maintenance of actin filaments. This review focuses on the domain architecture of human formins, the regulation mechanisms of their activation and the diversity in formin cellular functions.

1. Introduction

The actin cytoskeleton is formed by the non-covalent assembly of globular actin proteins into polymeric, helical actin filaments [1,2]. These filaments can be further arranged in a broad variety of higher order structures, such as filopodia, lamellipodia or actin stress fibers. Actin is abundant in all eukaryotic cells that are characterized by a membrane enveloped nucleus, but also bacteria express polymerizing actin orthologues [3,4]. Actin filaments are major determinants of cell morphology and adhesion site formation in multicellular organisms. In addition, the assembly of actin filaments and their association with motor proteins of the myosin superfamily can generate forces, which mediate cellular processes such as migration, cell division, endocytosis, exocytosis, organelle morphology, transport during gene transcription and muscle contraction [5,6].

Actin nucleation from actin monomers does not occur spontaneously but requires factors which help to overcome the kinetic barrier of nucleation [5]. These actin nucleation factors can be classified into three groups, the Arp2/3 complex and its nucleation promoting factors, formins and the WH2-containing nucleators [7,8]. In addition, leiomodin was discovered only recently as a nucleation factor of muscle actin that stabilizes tropomyosin-decorated filaments [9]. All these factors employed different mechanisms to accomplish the nucleation and elongation of actin filaments. The Arp2/3 complex binds to the sides of pre-existing actin filaments and is thought to generate branched actin networks. Spir, which contains four WH2 domains, nucleates the assembly of straight actin filaments and remains bound to the pointed end of the nucleated filament. In contrast, formins nucleate linear actin filaments from the barbed end and remain associated with the barbed end during filament elongation. This review focuses on the eight formin families found in man, their cellular functions, as far as established in human or other species, and the modular architecture of these multi-domain proteins.

2. Formin families

The term 'formin' was introduced in 1990 to describe protein products of the *limb deformity* gene in mice that were suggested to play a role in the formation of several organ systems [10,11]. Four years later, the product of the *Drosophila* gene *diaphanous*, which was shown to be required for cytokinesis, was found to be homologous to the formin protein [12]. Together with the protein Bni1 from *S. cerevisiae*, two regions of sequence homology were identified, which gave rise to the definition of a proline-rich FH1 domain followed by an FH2 domain, whose region of highest homology was initially described to comprise 130 residues [12]. As an increasing number of proteins from different species with similar domain composition emerged, a third homologous domain (FH3), albeit of higher sequence variability, was described at the N-terminus of the *S. pombe* protein Fus1 [13]. While the FH2 domain became the defining element of all formins, it was soon recognized that

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the flanking regions vary considerably between different formins, likely resulting in different cellular functions and regulation mechanisms. Although the *limb deformity* phenotype was later ascribed to the *gremlin* gene located on the same chromosomal locus [14], Formin and Diaphanous became the founding members of the formin families, now designated as FMN and Dia, respectively.

The effect of formins on actin polymerization was first recognized in the yeast homologue Bni1 [15] and at around the same time GTPases of the Rho family were identified as activators of formin function [16,17]. An autoregulatory domain at the C-terminus of the mouse Diaphanous-like formins was identified, which gave rise to the classification of Diaphanous-related formins (DRFs) [17,18]. Phylogenetic analyses of formins from different organisms were performed on the FH2 domain [19], the FH1/FH2 and GBD/FH3 domain assemblies [20] or formins from all organisms [21]. Here we focus on the fifteen formins found in man that cluster into eight different families (Figs. 1 and 2). In the following we give a short overview on the cellular and mechanistic functions of these formin families.

2.1. Dia

Diaphanous 1 (Dia1) and its mouse homologue mDia1 are the best analyzed formins both on a cellular and mechanistic level to date. mDia1 was described first as an effector of the small GTPase RhoA that activates the formin by disruption of an intramolecular interaction to induce the formation of thin actin stress fibers [16,17]. This process is catalyzed in addition by the Rho-associated kinase ROCK [17,22,23]. The activation of mDia1 is achieved by binding of a guanosine triphosphate loaded Rho GTPase that relieves an autoinhibited state between the N- and C-termini of the protein [17,18]. Truncation of the C-terminal autoregulatory domain, in contrast, leads to a constitutive active formin variant that exhibits a strong phenotype of actin cytoskeleton remodeling also in the absence of a Rho family GTPase. Of note, in the original report the prefix 'm' in the name p140mDia was indented to denote "mammalian" to emphasize that this protein is the homologue of the *Drosophila* gene product Diaphanous [16]. While mDia is to date often understood as mouse Diaphanous, Dia might be the most unbiased designation.

mDia1 was also recognized to mediate transcriptional activation of specific promoters such as the serum response factor (SRF) [22,24]. Complex morphological and migratory behavior as observed during embryogenesis, or during inflammatory and invasive processes is governed by cytoskeletal rearrangements that involve the reprogramming of gene expression. SRF relays RhoA/Dia1 induced actin assembly to transcription by its actin binding cofactor MAL (megakaryocytic acute leukemia). To amplify this signal Dia1 was shown to act upstream of RhoA by binding to the leukemia-associated RhoGEF (LARG) [25]. The FH2 domain was shown to stimulate the guanine nucleotide exchange activity of LARG *in vitro*, leading to a positive feedback loop that regulates cell morphology and invasion [25]. The nuclear protein SCAI was recently identified as cofactor between Dia1 and SRF for the transcriptional control of β_1-integrin and cell invasion [26]. Moreover, the cellular localization of Dia1 was linked to the cytoskeletal scaffold protein IQGAP1 and the microtubule tracking protein CLIP170, whose interaction is required for phagocytosis and recruitment to phagocytic cups [27,28]. The fast directional movement of actin filaments in living cells and *in vitro* was shown for an mDia1 FH1–FH2 construct that associated persistently with the growing barbed end showed actin elongation rates [29].

A construct encompassing the FH1–FH2 domains of mDia2 induced stable microtubules independently of its dimerization and actin nucleation properties [30]. This observation raised the possibility that formins not only act as actin assembly factors but also as microtubule-binding proteins to cross-link the two cytoskeletal components [23,30]. A microtubule polarization event is found in cell migration toward the direction of migration. In addition, the RhoGAP protein DLC1 was recently shown to control cell migration through a Dia1-dependent signaling pathway. Silencing of DLC1 in breast cancer cells led to stabilization of stress fibers and focal adhesions and enhanced cell motility, displaying a migratory cancer cell phenotype [31,32]. Likewise, membrane-derived microvesicles secreted from prostate cancer cells were shown to depend on Dia2 expression, whose knockdown enhanced the oncosome formation [33]. For a

protein name	*short name*	*alternative names*	*accession number*	*gene locus*	*length*	*no. of isoforms*	*homology relations*
protein Diaphanous homolog 1 (Diaphanous-related formin-1)	**Dia1**	(DRF1)	**O60610**	5q31.3	1272 aa	2	90.3% sequence identity to mouse mDia1 (O08808)
Diaphanous-related formin-2	**Dia2**	(DRF2)	**O60879**	Xq21.33	1101 aa	3	49.0% ident. to Dia1
Diaphanous-related formin-3	**Dia3**	(DRF3)	**Q9NSV4**	13q21.2	1193 aa	7	43.4% ident. to Dia1; 53.5% ident. to Dia2
Disheveled-associated activator of morphogenesis 1	**Daam1**		**Q9Y4D1**	14q23.1	1078 aa	3	26.9% ident. to Dia1
Disheveled-associated activator of morphogenesis 2	**Daam2**		**Q86T65**	6p21.2	1068 aa	1	67.7% ident. to Daam1; 25.3% ident. to Dia1
Formin-like protein 1 (Leukocyte formin)	**FMNL1**	(FRL1)	**O95466**	17q21.31	1100 aa	2	23.6% ident. to Dia1
Formin-like protein 2	**FMNL2**	(FRL3, FHOD2)	**Q96PY5**	2q23.3	1086 aa	2	59.4% ident. to FMNL1; 22.8% ident. to Dia1
Formin-like protein 3	**FMNL3**	(FRL2, FHOD3)	**Q8IVF7**	12q13.12	1028 aa	3	56.5% ident. to FMNL1; 69.2% id. to FMNL2; 22.4% id. to Dia1
WH2 domain-containing formin 1	**WHIF1**	(INF2)	**Q27J81**	14q32.33	1249 aa	2	23.8% ident. to Dia1
Inverted formin 1	**INF1**	(FHDC1)	**Q9C0D6**	4q31.3	1143 aa	1	16.9% ident. to Dia1, 19.2% ident. to INF2
FH1/FH2 domain-containing protein 1	**FHOD1**	(FHOS)	**Q9Y613**	16q22.1	1164 aa	1	20.2% ident. to Dia1; 20.3% ident. to FMNL2
FH1/FH2 domain-containing protein 3	**FHOD3**		**Q2V2M9**	18q12.2	1422 aa	2	43.4% ident. to FHOD1; 19.9% ident. to Dia1
Delphilin	**Delphilin**	(GRID2IP)	**A4D2P6**	7p22.1	1211 aa	1	21.3% ident. to Dia1
Formin-1 (Limb deformity protein homolog)	**FMN1**		**Q68DA7**	15q13.3	1419 aa	4	21.9% ident. to Dia1
Formin-2	**FMN2**		**Q9NZ56**	1q43	1722 aa	1	28.5% ident. to FMN1; 22.8% ident. to Dia1

Fig. 1. Summary of the 15 formin proteins identified in man. The protein length and the degree of homology relation are given each for the most prominent isoform. The number of isoforms and the definition of the most prominent isoform were derived from the UniProt databases (www.uniprot.org). FMNL2 is also described as FHOD2 and FMNL1 as FHOD4. Delphilin is also known as "Glutamate receptor, ionotropic, delta 2-interacting protein 1" or GRID2IP.

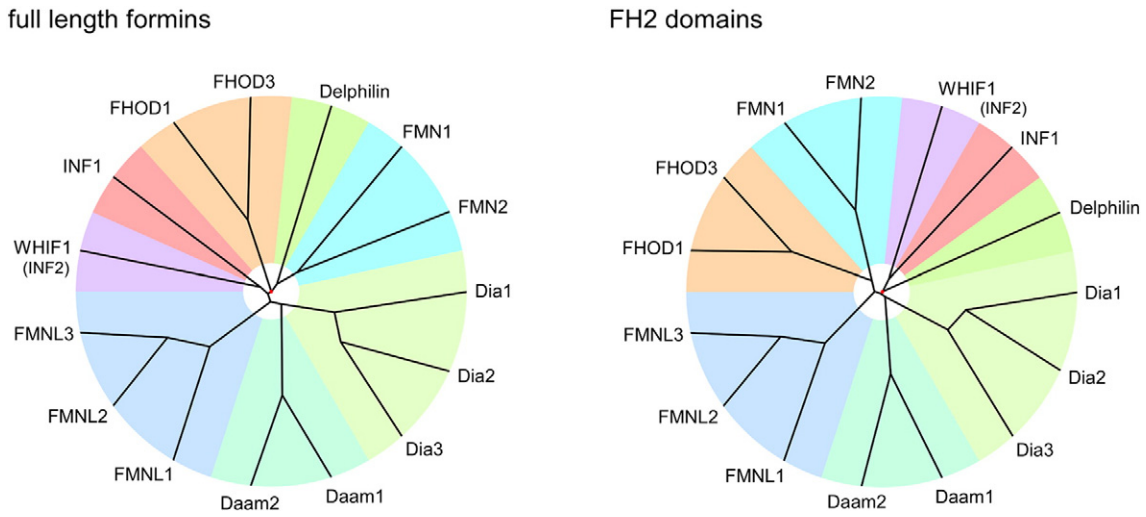

Fig. 2. Phylogenetic analysis of human formins. (left panel) Full length sequences of the most prominent isoform of each formin orthologue were used to derive the degree of relationship. Starting from the origin, three main branches can be identified that include Dia, Daam, FMNL, WHIF and INF, followed by FHOD and finally Delphilin and FMN. The three diaphanous-related formin families Dia, Daam and FMNL are particularly close in sequence homology and proposed domain composition. (right panel) Phylogenetic analysis of the FH2 domains only of human formins. Now the formin relationships cluster differently with FHOD and FMN being closest, followed by FMNL and Dia/Daam. WHIF/INF and Delphilin diverge from the origin directly into two branches while the other five families constitute a separate line of homology relation. Note the large divergence of WHIF and INF FH2 domains, underlining their affiliation into two different families. On average, the FH2 domains of all 15 formins share a sequence identity of 24.8% as determined from a multiple sequence alignment, while the identity within the individual families is on average 61.4%. The phylogenetic tree was determined using the program ClustalW2 and displayed with EPOS [126]. The central dot indicates the origin of the phylogeny.

topical overview on loss of Dia function in cancer metastasis and invasion we like to refer to the reviews by Narumiya et al., Eisenmann et al. and Deward et al. [34–36].

2.2. Daam

Daam formins were identified as binding proteins of the cytoplasmic phosphoprotein Dishevelled (Dvl) and therefore referred to as Dishevelled associated activators of morphogenesis [37]. Dishevelled is a component of a non-canonical Wnt signaling pathway, also termed the planar cell polarity (PCP) pathway, which regulates cell movements through modifications of the actin cytoskeleton. The establishment of cell polarity is a fundamental process critical for cell division, migration, and convergent extension cell movements during development. This pathway requires activated RhoA although RhoA does not significantly activate Daam1. Instead, the latter is activated through interaction of its DAD with the PDZ domain of Dishevelled, releasing the autoinhibited state [38]. Since active Daam1 was reported to lead to RhoA activation, a positive feedback loop that amplifies the levels of active GTPase has been proposed [37,38]. It has been speculated that either a RhoGEF is recruited to active Daam1 to increase the pool of GTP-loaded RhoA. Alternatively, a RhoGAP might be silenced by Daam1 such that less RhoA-GTP is hydrolyzed and the pool of active RhoA therefore increased. A mechanistic explanation how this function might be achieved on the molecular level, however, is not yet clear and awaits further clarification.

Daam1 is essential for PCP signaling during *Xenopus* gastrulation [37]. This complex is supposed to be mediated by β-arrestin 2 [39]. In addition, activated Daam1 and Dvl2 form a complex with EphB, a receptor tyrosine kinase important for cell migration and adhesion in development, and the formin is required for dynamin-dependent endocytosis of this receptor in the development of the notochord in zebrafish [40]. Furthermore, Daam1 proteins are expressed during development of neuronal tissues [41] and play a critical role in axonal morphogenesis [42]. Daam1 has been demonstrated to bind Profilin 1 and 2 via its FH1 domain [43,44] and to utilize each profilin homologue differently for distinct actin assembly events during gastrulation [44]. The two Daam orthologues Daam1 and Daam2 are highly identical and may fulfill similar functions.

2.3. FMNL

"Formin-like" proteins FMNL1, FMNL2 and FMNL3 constitute a third family of Diaphanous-related formins in mammals. The founding member of this family was described as formin-related gene in leukocytes (FRL) [45], but this term can be mistaken in the databases with activators of the fibroblast growth factor receptor. FMNL1 is autoinhibited by N- to C-terminal interactions and becomes activated by the GTPase Cdc42 [46], while in FMNL2 the DAD–FH3 interaction is described to not inhibit its activity [47]. FMNL formins were shown to bind and bundle actin filaments by their FH2 domain [47–49]. In T-cells, FMNL1 has been identified as essential regulator of centrosome polarity, which connects the microtubule organizing center with F-actin filament outgrowth to engulf the antigen presenting cell [50]. Depletion of FMNL1 or Dia1 in cytotoxic lymphocytes abrogated cell-mediated killing indicating a crucial function of these formins in the dramatic morphological changes that occur upon T cell activation. Furthermore, two recent studies implicated FMNL2 in colorectal cancer and mental retardation. First, immunohistochemical analysis showed FMNL2 expression to be considerably higher in colorectal tumors and corresponding lymph nodes than in normal colorectal mucosa, suggesting thus a role in metastasis [51]. Secondly, a sporadic 3.9 Mb deletion in gene locus 2q23.3 was found in a 2.5 year old child that showed severe mental retardation, onset of puberty, short stature and hand anomalies [52]. The authors speculated that changes in the morphology of dendritic spines caused by defects in the actin cytoskeleton may cause the observed mental retardation.

2.4. WHIF1 (also referred to as INF2)

The open reading frame of WHIF1 (or INF2) was previously misaligned, starting at positions 733 or 534 (accession numbers NM_001031714.1 and NM_001031714.2, respectively) directly at the beginning of the FH2 domain. This suggested the presence of an inverted domain composition in the formin, which led to the designation INF2. Later, the coding sequence was realigned unraveling now to the presence of the regulatory FH3 and FH1 domains at the N-terminus of INF2, as found in most other formin families (Fig. 3). Since also the FH2 domains of INF1 and INF2 share only 28% identity,

while on average human FH2 domains are typically 61% identical within distinct formin families (Fig. 2), we suggest that INF2 and INF1 should be separated into two different formin families. We therefore propose to name this formin 'WHIF1' for WH2 domain-containing formin 1.

An obvious difference of WHIF1 (INF2) as compared to Diaphanous-related formins is the presence of a WH2 domain that replaces the DAD at their ancestral location C-terminal to the FH2 domain. Interestingly, the WH2 domain seems to adopt functional features of the DAD, most notably by binding to the FH3 domain [53], which might suggest an evolutionary relation between both sequence motifs. The WH2 domain binds to actin monomers and its presence causes WHIF1 (INF2) not only to accelerate polymerization of actin but also to accelerate depolymerization of actin filaments under certain conditions [53]. The WH2–FH3 interaction does not inhibit WHIF1's (INF2's) polymerization but depolymerization activity of FH2 domain-containing fragments [54]. WHIF1 (INF2) contains a CaaX motif at its C-terminus, which is a signal for post-translational prenylation and membrane targeting. WHIF1 (INF2) is modified by a farnesyl group, which is essential for its localization to the endoplasmic reticulum (ER). However, the function of WHIF1 (INF2) at the ER is not well understood, since the ER in cells not expressing WHIF1 appears to have no defect in ER structure [54].

2.5. INF1

The inverted formin 1 (INF1) or FH2 domain-containing protein 1 (FHDC1) is the "real" inverted formin as being the only formin that contains an FH1–FH2 domain module at the N-terminus, whereas the C-terminal half consists of a unique polypeptide sequence (Fig. 3). INF1 has been found to associate with microtubules by a C-terminal microtubule-binding domain (MTBD) composed of two well conserved regions [55]. Expression of INF1 in fibroblasts induces actin stress fiber formation, coalignment of microtubules with actin filaments, and the formation of bundled, acetylated microtubules, while knockdown of INF1 results in decreased levels of acetylated microtubules. INF1 might thus act in microtubule modification and potentially in the coordination of F-actin and microtubule structure.

In a phylogenetic analysis of the full length sequences, INF1 and WHIF1 (INF2) appear more distantly related than *e.g.* the Dia and Daam family formins (Fig. 2). This result is different from previous phylogenetic analyses when the open reading frame of WHIF1 (INF2) did not include its N-terminal FH1 and FH3 domains. The domain architecture is thus significantly different in both formins. Therefore, these two proteins represent distinct formin families that are found throughout various species [21]. As a consequence of these differences in function and domain composition, we propose to keep the designation of INF1 (which is intuitive compared to FHDC1) and to change the name of INF2 to WHIF1.

2.6. FHOD

The FH1/FH2 domain-containing proteins FHOD1 and FHOD3 are grouped into the Diaphanous-related formins, although they appear more distant in sequence than Dia, Daam and FMNL family formins (Figs. 2 and 3). FHOD1 shares typical features with other DRF family members, such as overall domain organization, multimerization and autoinhibition [56–59], but its GTPase interaction and modes of activation are not fully understood. FHOD1 was originally identified in spleen as a downstream effector of Rac1 [60,61], but while active Rac1 recruits FHOD1 to the plasma membrane it seems insufficient for its activation [62,63]. More recently, FHOD1 was shown to become phosphorylated at three specific sites within the C-terminal DAD by the Rho effector kinase ROCK [64,65]. This modification disrupts the autoinhibitory state of FHOD1 and leads to F-actin stress fiber formation [64]. In addition, FHOD1 and ROCK1 were shown to induce non-apoptotic plasma membrane blebs that required F-actin integrity, the Rho pathway and Src activity [65]. The most prominent phenotype

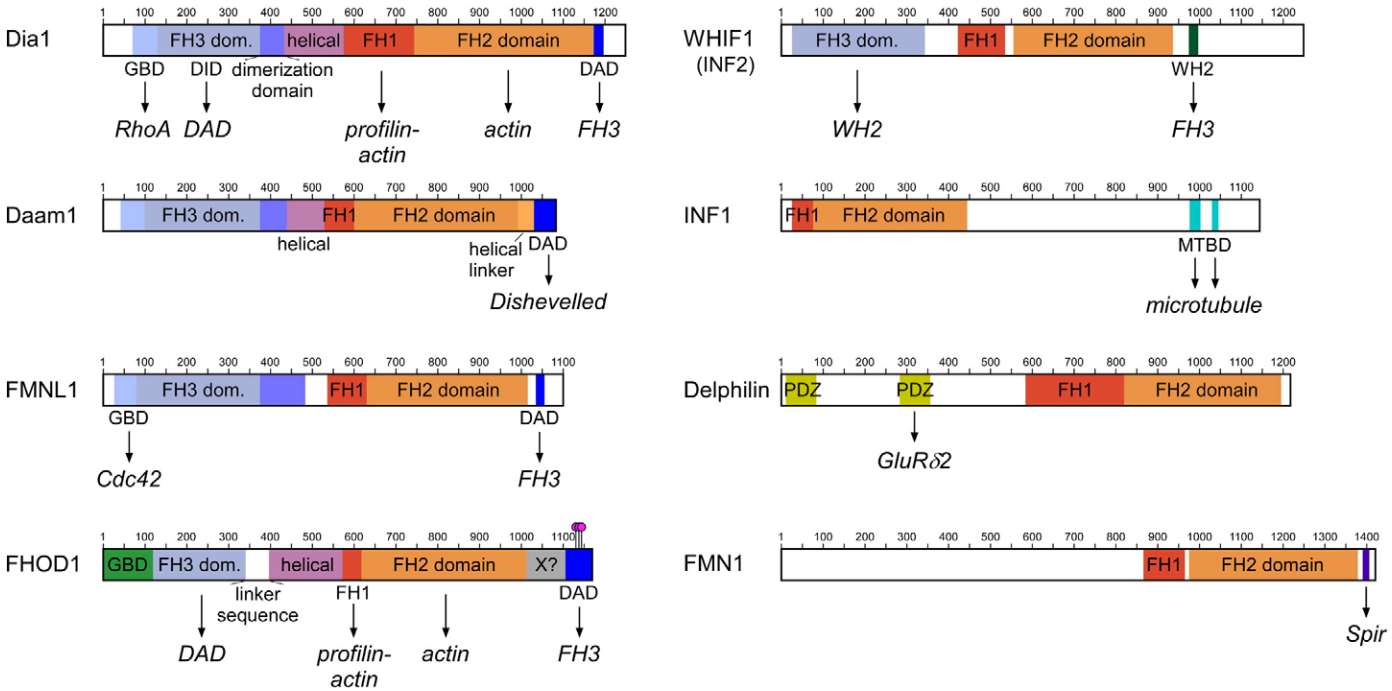

Fig. 3. Modular domain architecture of human formins. A schematic view on the domain composition of one member each for all eight formin families is shown as derived from structural analysis and computational prediction. The most prominent isoform of the first family orthologue is always shown. The domain nomenclature denotes the formin homology 1 (FH1), 2 (FH2) and 3 (FH3) domains, the GTPase-binding domain (GBD), the Diaphanous inhibitory domain (DID), a dimerization domain (DD) followed by a helical region, and the C-terminal Diaphanous autoregulatory domain (DAD). In addition some formins contain a WH2 actin binding motif and PDZ domains. Interaction partners as far as they are known from cellular and biochemical studies are indicated by arrows below the bar diagrams. The intramolecular interaction between the DAD and FH3 domain leads to autoinhibition of the formins. The balloons at the C-terminus of FHOD1 indicate serine and threonine phosphorylation sites in the polybasic region of the DAD, while the preceding region of ~90 residues (denoted X?) is unique to Diaphanous-related formins.

of FHOD1, however, is the formation of F-actin stress fibers, which is achieved by C-terminal truncation of the autoregulatory DAD domain.

2.7. Delphilin

This unique formin was first described as an interacting protein of the glutamate receptor δ2 (GluRδ2) subunit in neuronal cells of the cerebellum [66], and therefore named "glutamate receptor, ionotropic, delta 2-interacting protein 1" or GRID2IP. The membrane protein GluRδ2 is predominantly expressed at parallel fiber-Purkinje cell postsynapses and plays crucial roles in synaptogenesis and synaptic plasticity, *e.g.* by controlling the endocytosis of AMPA receptors. The originally described splice variant of Delphilin contained only one N-terminal PDZ domain required for GluRδ2 binding and localized at the dendritic spines of hippocampal neurons, while the full length isoform identified later encodes an additional PDZ domain, and is clustered in soma and dendritic shafts [66,67]. Delphilin ablation in mice exerted little effect on the synaptic localization of GluRδ2 and no abnormalities in Purkinje cell synapse formation was detected [68]. However, induction of long-term depression was facilitated in Delphilin mutant mice, indicating a role in motor learning and cerebellar wiring [68]. The proposed link of Delphilin to the actin cytoskeleton via its FH2 domain has not been addressed yet, and remains elusive at a molecular level.

2.8. FMN

The mouse *formin* gene, formerly known as *limb deformity* gene, is the founding member of the formin families [10]. With 158 and 180 kDa its two orthologues are the largest in size but on a molecular level the least well understood. Functionally, FMN1 has been implicated in interphase microtubule binding [69], and its contribution to protrusions of the cell's leading edge and to focal adhesion formation of primary cell is considered relevant to wound healing and cell spreading [70]. Moreover, genetic variants in the chromosome 15q13.3 locus that harbours FMN1 and GREM1 (*gremlin* gene) were hypothesized to influence colorectal cancer risk [71]. In contrast, FMN2 is associated with the asymmetric spindle positioning in meiotic oocytes [72–76]. Maturation of an oocyte to a competent egg requires extrusion of the chromosomes into a small polar body that is relocated from the center of the oocyte to the cortex to gain sufficient storage material for the development of the embryo after fertilization. While spindle relocation requires F-actin, it was shown in mice oocytes that FMN2 concentrates around chromosomes through its N-terminal region [75] and coordinates actin network nucleation [74–76]. Oocytes from female mice without the formin gene *fmn2* cannot correctly position the metaphase I DNA-spindle. This produces daughter cells with an abnormal number of chromosomes, the leading cause of female infertility, birth defects, and embryo loss. These observations connect formin function with infertility [72]. A conserved sequence motif in the far C-terminus of FMN1 and FMN2 was recently shown to interact with Spir, which is a WH2 containing actin nucleator [77]. FMN2 is the human homologue to the *Drosophila* protein Cappuccino which has been shown to be required for the polarity of the egg and embryo [78]. A detailed discussion on the functions and phenotypes of FMN formins has been recently presented in a review by Liu et al. [79]. Together, these studies point toward a diverse range of actin nucleation activities and broad cellular functions of human formins.

2.9. Yeast, plants and other organisms

An even larger diversity of formin architecture and function is found in plants as well as in fungi and lower organisms as *Dictyostelium discoideum* or protists as the malaria *Plasmodium berghei* [80], *Plasmodium falciparum* [81] or the related parasite *Toxoplasma gondii*. Three different classes of plant formins were described that contain *e.g.* an N-terminal transmembrane region, a PTEN-like domain that exhibits phosphatase activity or a RhoGAP domain that stimulates GTPase activity. *Arabidopsis thaliana* contains *e.g.* 21 different formins, while budding yeast contains only two formins (Bni1 and Bnr1) and fission yeast three (Cdc12, Fus1 and For3). For further information, we like to refer to some recent, excellent reviews [21,79,82].

3. Modular domain architecture of eukaryotic formins

A subset of formins is classified as Diaphanous-related formins (DRFs) based on the presence of a C-terminal autoregulation domain (DAD) that was first identified in mDia1 [83], the mammalian homologue to the *Drosophila* Diaphanous protein. This class encompasses the families Dia, Daam, FMNL and FHOD, which share similar domain architecture. In the following we will describe functional and structural aspects of the domain modules that constitute human formins. Further information is given in two recent, excellent reviews [84,85]. A schematic view that correlates domain architecture with structural elements of DRFs is shown in Fig. 4.

3.1. FH2 domain

All formins contain an FH2 domain, which is the defining element of this protein superfamily. The FH2 domain binds G- and F-actin directly and has been shown for many formins to nucleate actin filaments [86–90]. The FH2 domain remains bound to the barbed end of the nascent actin filament leading to unbranched actin filaments [29,91–93]. FH2 domains are only functional in a dimeric state [90,94]. In addition, some formins (Bnr1p, AFH1, FMNL1 and mDia2) contain an FH2 domain with F-actin bundling activity [48,95,96].

FH2 domains have a length of about 400 amino acids and share on average 24.8% sequence identity within the human species. To date structural information of three different FH2 domains exists. The FH2 domain from yeast Bni1 was determined in its free state (PDB accession code 1UX5, [97]) and in complex with TMR-labeled actin (1Y64, [98]). The structure of the FH2 core domain from murine mDia1 was determined (1V9D, [99]) and recently two structures of the human Daam1 FH2 domain were reported (2J1D, [100]; 2Z6E, [101]). The FH2 domain showed an almost completely α-helical fold: Viewed from the "top", the FH2 domain looks like a parallelogram with a hole in the middle (Fig. 5A). This geometry is generated by two arch-formed FH2 domains, which dimerize in a head to tail orientation. Two sides of a FH2 domain are essential for the dimerization: at the N-terminus each FH2 domain contains the "lasso" subdomain (which is named after its circular shape) that envelops the compatible region of the second molecule at the "post" subdomain. This central region contains the highly conserved GN(Y/F)MN motif that was recognized as the FH2 domain defining element [12]. The region between the lasso and post subdomain is formed by an extended "linker" and spherical "knob" subdomain as well as a "coiled coil" region. The lasso, linker and knob subdomains are composed of the N-terminal ~120 residues whereas the coiled coil and post region together contain the central and C-terminal part of the FH2 domain. Because the knob, coiled coil and post subdomains of one polypeptide form a hydrophobic inner surface with the lasso subunit of the opposite FH2 polypeptide, this subdomain composition is referred to as a structural "hemidimer".

The linker regions of both FH2 domains connect the two hemidimers and provide flexibility for the nucleation and processive capping activity. Each hemidimer has two patches with highly conserved residues that mediate the interaction with actin. The first patch is in the knob region that contains a solvent exposed isoleucin (I1431 in Bni1, I698 in Daam1), the second patch is located in the lasso-post dimerization interface and possesses a lysine (K1601 in Bni1, K847 in Daam1) that is important for the actin interaction. Mutation of either of these two residues completely eliminates actin nucleation and capping activities. In contrast, a monomeric wild-type FH2 domain

missing the lasso-linker subdomains caps actin filaments [99]. The circular structure of the FH2 domain dimer ("doughnut" or *torus* form) with a diameter of approximately 11 nm is already suggestive to accommodate actin molecules in its center, which have a diameter of approximately 8 nm in the filamentous form. The linker region could provide the required flexibility for processively stepping on the barbed end as the filament elongates.

Valuable insights into the actin-FH2 domain interaction were derived from a structure of the Bni1 FH2 domain in complex with a polymerization defective tetramethylrhodamine- (TMR-) labeled actin [98]. In the crystal, the actin monomers showed an arrangement almost like F-actin: Two adjacent actin molecules have a translation of 180° and 28 Å in the FH2 actin complex in contrast to 166° and 27.5 Å essential for the introduction of the twist in helical F-actin [1]. The FH2 domain is not dimeric in this case but rather a spiral polymer that wraps around the counterfeit F-actin. This is possible because the lasso subunit of each molecule interacts with the post subunit of the next polypeptide through a domain swap during crystallization. In the generated model of the actin FH2 structure with the dimeric FH2 domain two actin molecules are bridged by one hemidimer and thus, the FH2 dimer has four possible binding sites, which correspond to the above-mentioned patches at the knob and post subunits. This arrangement is the basis of the current model for FH2 domain mediated nucleation and elongation of F-actin.

The formation of an actin filament nucleus comprising an actin dimer or trimer is kinetically unfavorable [5]. The dimeric FH2 domain could stabilize two or three actin molecules and defeat the kinetic hurdle. In the crystal, one actin molecule is hold by the post side of one hemidimer and the knob side of the other hemidimer, whereas the adjacent actin molecule contacts the knob of one hemidimer. However, the flexibility of the FH2 dimer and the length of the linker subdomain would allow filament growth at the barbed end with an intact ring structure. In this model one hemidimer has to dissociate – while the opposite one remains bound – from two actin molecules at the barbed end and moves to a later incorporated actin molecule. The incorporation of a new actin molecule is possible at the free post site and leads to the initial setup but with the filament elongated by one actin monomer. This barbed end growth in presence of the intact FH2 dimer is termed "processive capping". As mentioned above actin monomers bound to the FH2 domain have a different orientation to each other than those in F-actin. This might also be true for the conformation of actin monomers in the FH2 complex and those in filamentous actin, where they were recently shown to be flattened [2]. Furthermore, it has been shown that the FH2 domain changes the conformation of the actin monomers in F-actin leading to a more flexible filament [102]. Thus, after nucleation and/or incorporation of new actin molecule, the "strained" conformation of one actin molecule could relax upon dissociation of the hemidimer, which would allow the stable incorporation of this actin molecule into the actin filament.

Sequence analysis of mammalian and ascomycota FH2 domains showed that the biggest differences besides the linker length reside in the knob region [19]. While mDia1 and Daam1 contain a short loop in the knob domain with a length of 8 amino acids, Bni1 has a 25 residue long loop. As a consequence, the orientation of the knob subdomain with respect to the rest of the domain differs from that in Daam1 and mDia1, respectively. However, both crystal structures of Daam1 contain two short antiparallel β-strands near the inter-hemidimer interface that is formed by the N- and C-terminal residues of the linker region [100,101]. These residues are conserved among Daam1 proteins, but are not present in other Diaphanous-related formins [100]. Disruption of the β-strands by mutation of residues involved in this inter-hemidimer interface leads to an increased activity of the Daam1 FH2 domain in actin assembly [100]. These observations implicate that the β-strands lock the Daam1 FH2 domain through occlusion of the actin binding interfaces in a state of reduced activity and suggest a role of these secondary structure elements for the specific Daam1 regulation. It is conceivable that binding of regulators like Dishevelled [37] and/or modifications like phosphorylation in this region might trigger the nucleation and processive capping activity of Daam1.

Another interesting aspect of FH2 domain activity addresses the role of the linker length that connects the lasso with the knob subdomains (Fig. 5B). Intrinsic nucleation and elongation activities of FH2 domains differ between several members of the formin family [103]. It was thus suggested that the length of the linker could correspond to the individual formin nucleation activity. One important hint in this direction came from the observation that mDia1 contains a long linker and is most potent in actin assembly, while Bni1 and mDia2 have shorter linkers and showed reduced activity. Cdc12, in turn, has the shortest linker and does not elongate actin filaments, at least in the absence of profilin. The FH2 domain structures of free and actin bound Bni1 showed indeed that the α-helical linker became expanded to accommodate two actin molecules (Fig. 5B) [97,98]. It has been shown that deletion of 23 out of 30 amino acids in the linker region led to loss of actin assembly activity, while a 20 amino acid deletion mutant has activities in the range of the wild-type protein [101,104]. The length of an extended 22 amino acid long linker is about 5.9 nm that fits well with the difference between the short edge in the parallelogram of the free FH2 domain and the extended edge in the almost quadratic assembly of actin bound Bni1 [98]. In a recent study, wild-type Bni1 and Bni1 chimeras with FH2 linkers from Cdc12, mDia1 and mDia2 were investigated [105]. Unexpectedly, no correlation between the

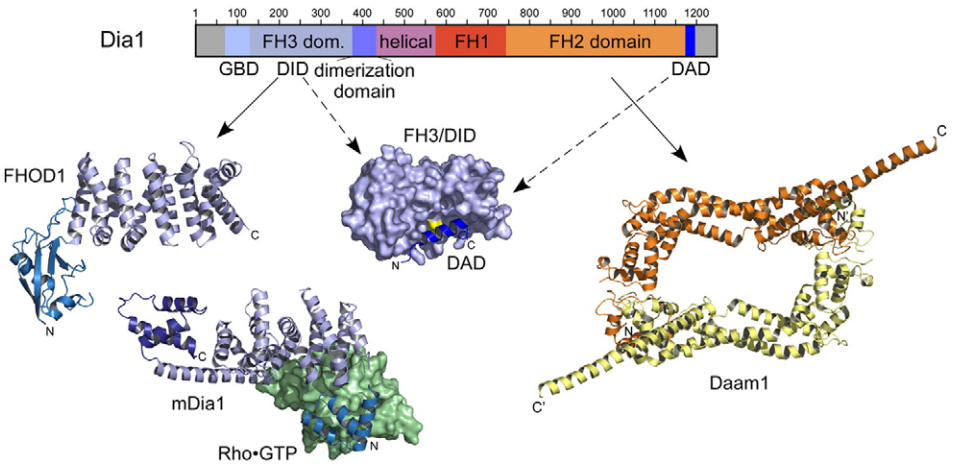

Fig. 4. Overview of the domain architecture and the structure assemblies of Diaphanous-related formins as known to date. The bar diagram corresponds to human Dia1, while structure elements are shown from mDia1, Daam and FHOD1.

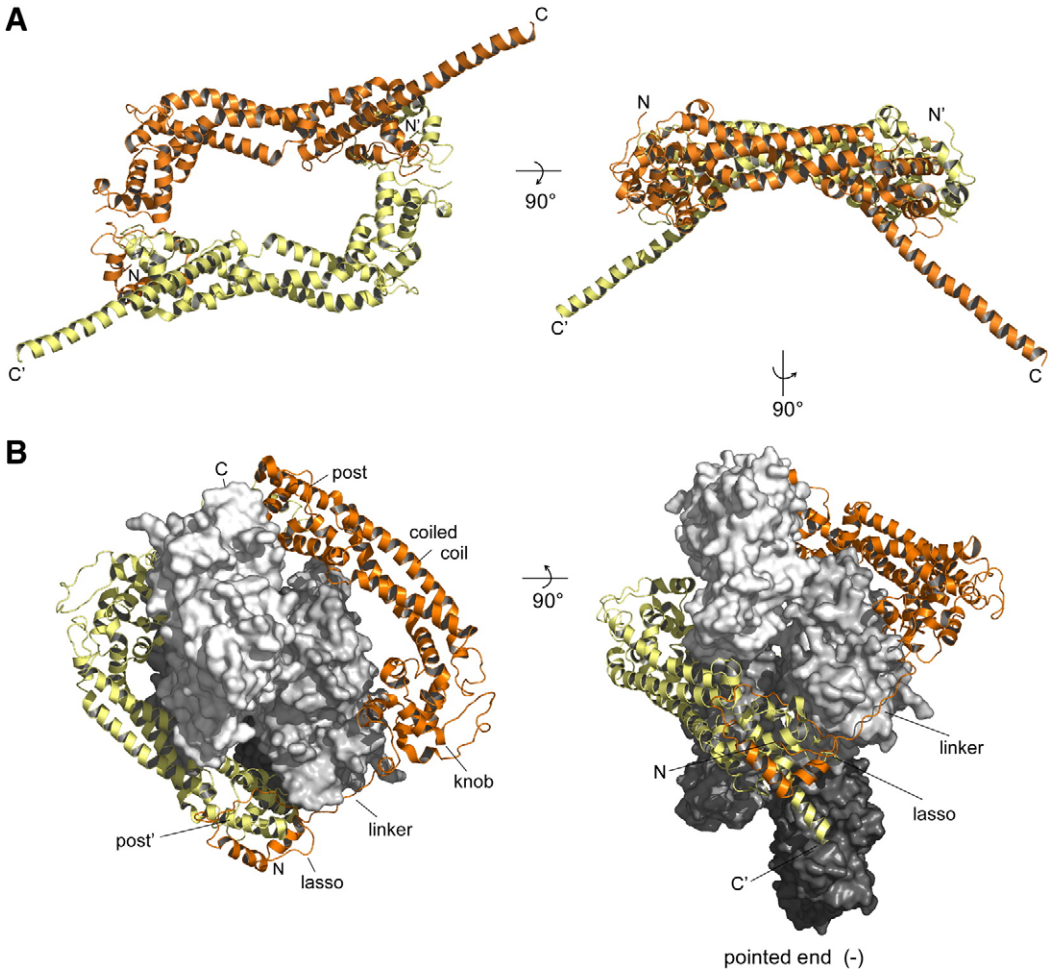

Fig. 5. Structures of the FH2 domain. (A) Overall assembly of the head-to-tail dimeric structure of the human Daam1 protein (2J1D, [100]). The N-terminal lasso subdomain interacts with the post domain of the opposing chain. In the actin free form, the linker region is laced up, leading to a lasso-knob interaction. The proposed DAD sequence motif FDDLVSAL is only 10 residues downstream of the last helix turn (right panel). (B) Structure of the FH2 domain dimer from Bni1 bound to actin (1Y64, [98]). Now the linker segment is expanded to wrap around the shifted actin molecules in the nascent filament. The flexibility of this segment is thought to contribute to the stair-stepping FH2 domain mechanism of filament elongation.

linker length and the nucleation activity was found. Instead, the activities of the Bni1 chimeras were in the range of those from wild-type Bni1, while the chimeras tended to dissociate easier from barbed ends [105]. These results suggest that the linker length is optimized for the distinct FH2 domains, and that specific determinants for the nucleation activity are probably located in the hemidimer.

FH2 domains not only nucleate and elongate actin filaments, but were also shown to bundle filamentous actin. So far, FH2 domains from yeast Bnr1 [96], murine FMNL1 [94] and mDia2 [48] as well as the plant formin AFH1 [95] were shown to bind to the sides of filaments and to bundle those. In contrast to the specific FH2 domain binding sites that mediate the actin elongation activity, no conserved motif or binding patch for bundling activity was identified. Instead, bundling activity severely depended on the salt concentration, which suggested a rather unspecific adherence of positively charged FH2 domains to the negatively charged F-actin surface [48]. The mechanism of how FH2 domains bind to the sides of filaments is thus not understood and requires further investigation. Furthermore, the cellular function of these binding and bundling activities as well as the mechanism of regulation in the cellular context remains an open question.

3.2. FH1 domain

The FH1 domain is preceding the FH2 domain and composed of proline-rich stretches that vary in number and length for the individual formins [91,92,95,106]. For some formins, binding of the FH1 domain to the small cytosolic protein profilin was shown [16,107,108]. Profilins form a stoichiometic complex with actin and regulate the pool of free G-actin inside the cell. They bind to the barbed end of actin leaving the nucleotide binding site accessible and decrease the nucleotide affinity of actin for higher nucleotide exchange rate. In addition, proline-rich stretches can bind to SH3 and WW domains, but such interaction might be promiscuous and a cellular meaning for formin function has not been shown yet. The only known formins lacking an FH1 domain are the *D. discoideum* formin ForC and the malaria parasite formin MISFIT [80,109].

It is a matter of debate whether the FH2 domain needs the FH1 domain for processive capping [92], or whether the FH1 domain simply increases the elongation rate of the FH2 domains [106]. Many experiments that were performed to elucidate the mechanism of FH1–FH2 domain cooperation on actin polymerization rely on specific assay conditions and protein constructs and are thus hardly comparable. Here we summarize two general effects of the FH1 domain on FH2 domain mediated actin assembly.

Profilin influences both FH2 mediated nucleation of new filaments and elongation at the barbed end. First, in the presence of profilin nucleation by the FH2 domain is completely inhibited while FH1–FH2 domain nucleation is reduced but not abolished [92,94,110]. Second, individual FH1–FH2 constructs increase the elongation rate of actin filaments to rates faster than free barbed ends in presence of profilin,

whereas FH2 constructs do not [29,91,106,110]. Profilin recognizes proline-rich regions and the number of proline-rich stretches within the FH1 domains correlates with increased elongation rates [110]. The FH1 domain might thus bind profilin–actin complexes in order to recruit actin close to the FH2 domain at the barbed end of the growing filament. A recruited profilin–actin pool near the FH2 domain would ensure an increased elongation rate not dependent on diffusion of free actin monomers to the FH2 domain. In addition, the FH1 domain could provide profilin–actin complexes in a pre-oriented conformation, which would likewise increase the elongation rate. The elongation activities of the FH2 domain from individual formins have always to be considered in context of the respective FH1 domain. As already mentioned, the number of proline-rich regions varies strongly among different formin proteins. A comparison of FH1 domains in human formins reveals *e.g.* 2–3 putative profilin binding sites in FHOD1 compared to about 33 sites in FMNL2, which could influence the elongation rates or serve as storage pool for actin.

3.3. FH3 domain (DID and DD)

The FH3 domain is the least conserved module in the canonical composition of mammalian formins. The domain is located N-terminal to the catalytic FH1–FH2 elements and involved in the regulation of formin activity. The FH3 domain can be functionally most clearly described as the DAD recognition domain, although its decomposition into various subdomains is viable. The Dia, Daam, FMNL and FHOD formin families contain an FH3 domain, whose appearance seems to be coupled to the presence of the C-terminal DAD autoregulation motif. WHIF1 (INF2) is the only formin that is supposed to contain an FH3 domain while the DAD is replaced by an actin binding WH2 motif. To date, structures of the FH3 domain, either alone or in complex with the DAD, are available for two different formin families, mDia1 and FHOD1 (Fig. 6) [59,111–115]. Both formin structures exhibit an all helical armadillo repeat fold consisting of five repeats, although the two domains share only 19.2% sequence identity (Fig. 6A). The concave site of the armadillo repeat structure forms the recognition surface for the C-terminal DAD motif, which is stretched out axial over the interacting helices (Fig. 6B). A single point mutation of an aliphatic residue in the central repeat, A256D in mDia1 and V228E in FHOD1, led to loss of binding to the DAD [59,113], and hence activation of the formin [59]. This phenotype, which is similar to the truncation of the C-terminal DAD, has been described in cells only for FHOD1, but is expected to manifest similarly in all Diaphanous-related formins.

The flanking regions of the FH3 domains vary significantly in different formins and a distinction into subdomains or stable entities cannot be generalized unambiguously for all formin families. At the N-terminus, the GTPase-binding domain is linked to the FH3 domain, while the C-terminus is leading into a dimerization subdomain in mDia1. This dimerization domain (DD) encompasses approximately 60 residues in mDia1 [111,112]. The DD is formed by three α-helices, two of which from each monomer form a tightly intertwined four helical bundle (Fig. 6C). In contrast, in FHOD1 the region following the armadillo repeat structure forms a flexible linker sequence that is rich in basic, acidic and small residues as glycine, serine and alanine. An indication for dimerization in the N-terminal domains of FHOD1 could indeed not be observed [59,116]. Due to the low sequence identity and the repetitive nature of the armadillo repeat fold, the individual helices and the exact boundaries of the FH3 domain are difficult to predict by homology comparisons and computational methods.

The FH3 domain was first described by Petersen et al., who identified a region of repetitive character in the fission yeast formin Fus1 that was required for localization of the protein to the projection tip during conjugation [13]. Later, this region was functionally described as Diaphanous inhibitory domain (DID) in mDia1 based on *in vitro* actin polymerization experiments that showed inhibition of a catalytic FH2–DAD protein fragment by addition of a fragment spanning residues 129–369 of mDia1 [117]. The inhibitory effect of the DID showed an IC_{50} value of 200 nM at a concentration of 2.5 nM FH2–DAD [117]. Despite the low efficacy the presence of such inhibitory effect might be unique to mDia1 and warrants further confirmation *in vivo*. In context of the full length protein one might expect that expression of the DAD recognition domain would lead to activation rather than inhibition of the formin, similarly as the Dishevelled PDZ domain releases the autoinhibited state in Daam1 by competition binding to the DAD.

3.4. DAD

The C-terminal Diaphanous autoregulatory domain (DAD) is essentially composed of two signal sequences, a highly conserved MDxLL motif followed by a polybasic region of different length and composition [17,18]. The identification of this domain gave rise to the

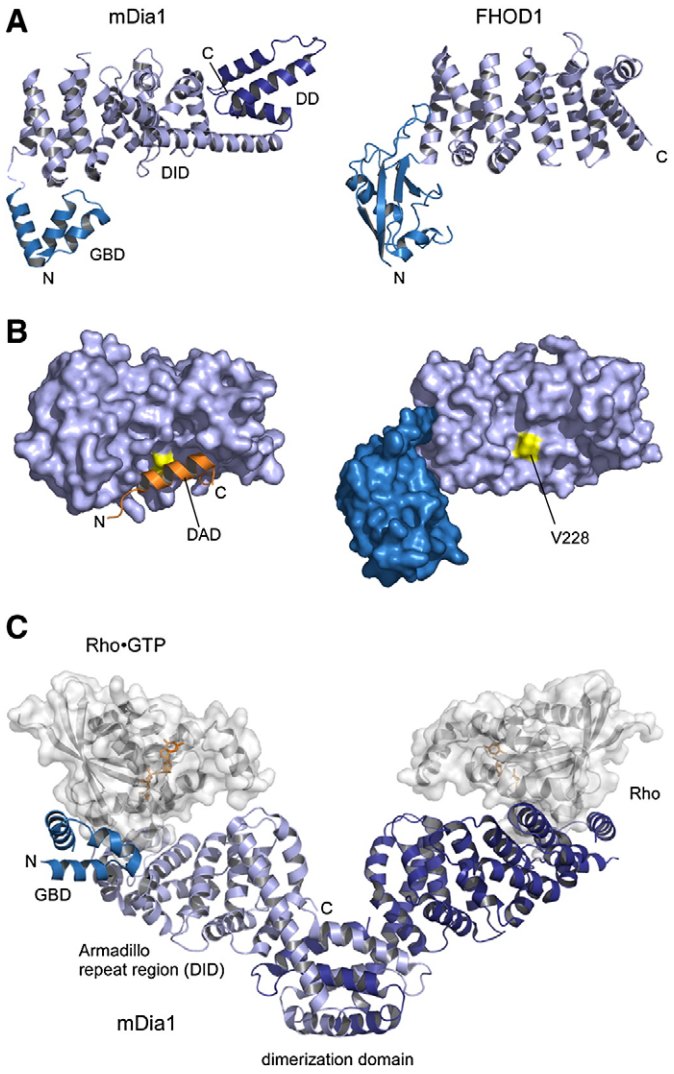

Fig. 6. Structures of the N-terminal regulation domains of mDia1 and FHOD1 formins. (A) Assembly of the GBD–FH3 and dimerization domain (DD) structures in mDia1 (1Z2C, [112] left panel). The three-helical bundles of the N- and C-terminal GBD and DD appear as subdomains within the entire domain assembly rather than as stable entities. In FHOD1 the GBD is composed of an ubiquitin superfold that is tightly linked to the FH3 domain (3DAD, [59] right panel). (B) Binding of the DAD to its recognition domain. The concave site of the armadillo repeat fold constitutes a surface that interacts with the MDxLLxxL motif of the DAD in mDia1 (2F31, [115]). The position of a central residue in the binding interface, A256 in mDia1 and V228 in FHOD1, whose mutation to an acidic residue abolishes DAD binding, is indicated. (C) Interaction of guanosine triphosphate loaded RhoC with mDia1 (83–443) in the dimer conformation (1Z2C, [112]).

classification of a subset of formins as Diaphanous-related formins (DRFs) that encompass Dia, Daam, FMNL and FHOD family formins [18]. In cellular transfection experiments, truncation of the DAD leads to activation of the formin, which constitutes the most stable and reproducible phenotype of Diaphanous-related formins examined. The DAD is recognized by the FH3 domain, leading to an autoinhibition of the formin. While on average DAD and FH3 domains are about 800 residues apart, it is not clear yet if the DAD binds to an FH3 domain of the same polypeptide chain (*cis*) or if the interaction occurs with the mutually opposing chain of the head-to-tail FH2 domain dimer assembly (*trans*). Even higher order assemblies of dimers with dimers by cross-interacting FH3–DAD formations could be envisioned, leading *e.g.* to homologous formin networks for the formation of filopodia at specific subcellular sites. An advantage of such networks would be that they could be triggered synchronously. Mechanistically, one may at least suspect that an electrostatic attraction between the polybasic region of the DAD and a negatively charged surface patch on the FH3 domain [59,111,112] will help to bring the two sequentially distant sites together in either conformation of the autoinhibited state.

The MDxLL motif forms a helix that folds into the concave site of the armadillo repeat structure of the FH3 domain and interacts with helices of the second, third and fourth repeat. In fact, the motif extends to MDxLLxxL for Diaphanous-related formins with the polybasic region five to eight residues further downstream. In the absence of its recognition domain the DAD does not adopt a stable fold but rather contains some helical secondary structure elements as shown for FHOD1 by NMR [58]. Single point mutations of the consensus motifs led to partial activation of the formin [18,58,118]. Binding analyses by isothermal titration calorimetry and fluorescence polarization experiments revealed dissociation constants between the DAD and the GBD–FH3 domain of approximately 110 nM for mDia1 and 1.0 μM for FHOD1 [58,114], while the affinity of mDia1 to GTP-bound RhoA is about 10-fold higher [114]. In FHOD1, the polybasic region is interspersed with serine and threonine residues and cellular studies showed that the threonine kinase ROCK1 can activate FHOD1 by phosphorylation of S1131, S1137 and T1141 [64].

The sequence composition as well as the helical structure of the C-terminal DAD seems reminiscent to the WH2 domain sequence, that directly interacts with actin and stimulates filament polymerization. The WH2 domain again exhibits similarities to β-Thymosin and RPEL sequences, which all bind to a hydrophobic cleft between subdomain 1 and 3 in G-actin [119–121]. These motifs seem to display a variation on a theme [119–121]. Indeed, a WH2 domain is found in the formin WHIF1 (INF2) that replaces the DAD while the N-terminal FH3 domain is maintained. These variations may indicate an evolutionary development of a WH2 domain into a DAD, which may correlate with the appearance of its recognition domain, FH3, and a regulating GBD. A supporting function of the DAD in actin recruitment or filament formation, however, has not been described to date.

3.5. GBD

The GTPase-binding domain (GBD) is a component of the Diaphanous-related formin families Dia, Daam, FMNL and FHOD and directly precedes the FH3 domain at the N-terminus of the protein. The domain was first identified in mDia1 to interact with GTPases of the Rho family [16,17]. Small GTPases of the Rho, Rac and Cdc42 type are known to regulate the actin cytoskeleton and to induce filamentous structures such as actin stress fibers, lamellipodia and filopodia, respectively [122,123].

In mDia1, the GBD is composed of three helices (residues 84–121) that appear as subdomain within the N-terminal domain assembly rather than as discrete domain entity [112,113]. Rho binds via switch I and part of switch II to a hydrophobic patch on the GBD and to the inner helix of the first armadillo repeat of the FH3 domain, which therefore senses the active state of the GTPase (Fig. 6C). The specificity of formins for particular GTPases, however, does not arise from interaction with the switch II region, which is completely conserved between Rho, Rac and Cdc42, and only to a minor degree involves the switch I region (the so-called “effector loop” [124]). Instead, aromatic residues in a helix distal to the nucleotide binding site of the GTPase (residues H105, F106 in RhoA) interact with a successive motif of three residues at the C-terminal turn of the first armadillo repeat helix (N164, N165, N166 in mDia1). A second structural epitope that contributes to the recognition specificity is an insert helix that represents a unique feature of Rho family GTPases. This helix loosely interacts with the last armadillo repeat of the curved DAD recognition surface and might therefore as well contribute to the activation of DRFs [114].

Sequential homology suggests that the GBD domain structure of Daam and FMNL formins is similar to that in Dia. In contrast, structural analysis revealed a fully different domain fold in FHOD1. Surprisingly, a ubiquitin superfold was discovered for the N-terminal domain that is tightly connected to the succeeding FH3 domain by a long loop between the first two β-strands which folds into a hydrophobic patch of the first armadillo repeat [59]. The ubiquitin superfold is indeed known as a bona fide GTPase interaction domain from c-Raf1, PI3-kinase or RalGDS and forms a stable domain structure on its own [124]. However, biochemical and *in vivo* co-localization studies failed so far to identify a GTPase that interacts with FHOD1 and directly leads to its activation. Furthermore, deletion of the GBD domain led to inactivation of the formin even in conjunction with a truncation of the DAD [59]. While this points to a pivotal function of this domain in FHOD, it is currently not clear if this region should at all be designated as GTPase-binding domain or if any other function will be assigned to it.

3.6. The helical region (between FH3 and FH1)

The central part in between the FH3 and FH1 domain is the most capacious region within diaphanous-related formins, whose function and structure is still unknown. This segment is sometimes referred to as coiled coil region, due to the typical disposition of hydrophobic and polar residues, but may be seen more generally as helical domain. The region encompassing 100 to 180 residues may function as a bridging or scaffolding section that connects the N-terminal regulation domains with the catalytic head-to-tail dimer of the FH2 domain. This points to a yet unresolved enigma in the overall architecture of Dia, Daam and FMNL formins as how the dimeric N-terminus is descended into the dimeric C-terminus with the far C-terminal DAD interacting again with the N-terminus. The helical region and the FH1 domain might thus cross-bridge in between the opposing chains and provide the conformational flexibility required for the stair-stepping actin polymerization mechanism.

3.7. Other regions

While most functional and structural information is achieved today from Diaphanous-related formins, the composition of WHIF1 (INF2), INF1, Delphilin and FMN family formins is diverse, suggesting various functions and modes of regulation. Delphilin *e.g.* contains two N-terminal PDZ domains that are often found to be involved in the clustering of signaling molecules and play important roles in organizing protein networks on membranes. PDZ domains typically interact with short signature motifs occurring in flexible regions of their target proteins, which is again reminiscent to the GBD/FH3–DAD autoregulation interaction. Dia and to a lesser extent Daam and FMNL but not FHOD formins contain an N-terminal region of up to 70 residues whose structure and function is not known yet. First insights were recently reported for mDia2 that contains a bipartite nuclear localization signal (NLS) for the Ran-Importin mediated nuclear transport [125]. Although this motif is specific for mDia2, but not mDia1 or mDia3, and it exhibits some similarity with the polybasic region of the

C-terminal DAD, a function of this region in protein localization could support the specific recruitment of formins by activated GTPases.

4. Activation of formins

While the mechanism of FH2-mediated actin filament elongation might be uniform for all formins, the regulation of formin activation appears significantly different for every formin family and potentially even for individual orthologues within a specific family. For the autoinhibited formins – those that carry a DAD motif – varying mechanisms of regulation were reported that can be distinguished into three different modes: (*i*) GTPase mediated activation, (*ii*) DAD competition binding or (*iii*) DAD modification. All three mechanisms facilitate the release of the DAD from its recognition site on the FH3 domain, which is likely to relieve a tension in the mutual lasso to post interaction of the FH2 domains. The conformational change induced by the DAD release is thought to provide the flexibility in the FH2 dimer assembly that allows actin binding in the torus center and the stair-stepping actin polymerization mechanism. Since the three reported activation schemes are not mutually exclusive, they might also act synergistically for some kind of "initiation" and "full" activation state of the formin.

The high affinity binding of an activated Rho GTPase to the GBD/FH3 domain assembly was shown to actively displace the DAD from the N-terminal region in mDia1 [114]. However, the two binding sites only partly overlap for the MDxLL motif, while the polybasic region of the DAD may not be directly affected by the GTPase-FH3 domain interaction. The displacement of the DAD from the FH3 domain is due to a competition binding with the GTPase that binds with an about 10-fold higher affinity to the GBD–FH3 domain assembly [58,59,112,114]. A different mechanism is suggested to activate Daam1 where the PDZ domain of the Dishevelled (Dvl) protein binds to the DAD of the formin and thereby releases autoinhibition [38]. Instead of a displacement mechanism there seems to be an active competition for binding to the MDxLL motif of the DAD (which happens to be FDDLVSAL in both Daam1 and Daam2 orthologues), which is recruited to the Dvl protein. A simplified and potentially more ancestral version of such regulation mechanism is a substrate based activation mechanism that we propose for the WHIF1 (INF2) formin. Actin binds to the WH2 domain of WHIF1, which therefore competes for the intramolecular FH3–WH2 domain autoregulation interaction and might release the autoinhibited state. Such mechanism would be self-regulated by the concentration of actin as the competitor binder for the WH2 domain and as substrate for actin filament elongation. Finally, two serines and one threonine residue within the polybasic region of the DAD of FHOD1 were shown to be phosphorylated by the Rho kinase ROCK [64]. This phosphorylation event leads to destabilization of the electrostatic interaction and therefore potentially to the release of the DAD from its recognition surface on the FH3 domain. While ROCK might be recruited to FHOD1 via active Rho, which again involves a GTPase in the activation mechanism, this modification might be unique to FHOD formins since other polybasic regions, as *e.g.* those of mDia1, are not similarly interspersed with serine and threonine residues. In addition, it has been suggested that some formins might be regulated by phosphorylation or association of individual factors at the FH2 domain. However, no such effect was observed for Bni1, whose FH2 domain can become phosphorylated [96].

Where does the DAD go after release from the GBD–FH3 heterodomain? For formins that are recruited to cellular membrane compartments by a lipidated GTPase, the presence of a negatively charged membrane may offer an alternative interaction site in close proximity to the FH3 domain that will attract the polybasic region of the DAD. Such interaction could stabilize the anchoring of the formin at the membrane, while strong deformations take place due to the filament growth. Likewise, G- and F-actin are negatively charged molecules that could be recruited by the DAD, similarly as WH2 domains interact with actin. The localization of the DAD relative to the formin core structure, the actin filament, the GTPase and potentially the surrounding membranes implies also if the GTPase forms a stoichiometric complex with one formin or if one GTPase could potentially activate several formins. The latter mechanism scheme might at least facilitate the synchronization of activation for multiple formins, *e.g.* in cases when several actin filaments are required to form a filopodium. So far, all mechanistic analyses on the regulation of formins were derived either by over expression in cells or by using purified domains *in vitro* that encompass the FH2 or the FH1–FH2–DAD domain assembly only. For a more comprehensive analysis on formin regulation it will be desirable to use full length proteins to probe the activation mechanisms by GTPases in the presence of actin, profilin or other potential co-factors and preferentially also in the presence of liposomes.

5. Concluding remarks

Formins have been recognized as actin nucleation factors for twelve years, and their importance in cytoskeleton regulation is becoming considerably evident. A diverse picture of cellular functions emerged that is augmented by biochemical and structural insights into the mechanism of actin polymerization and formin regulation. Since formins are implicated in dynamic but also static cytoskeleton remodelling processes, major determinants of formin function are the expression levels and localization properties of endogenous protein. On a molecular level it is not yet understood why the FH2 domain of some formins catalyzes actin filament elongation while other FH2 domains bundle filaments. These different functions should be reflected also by expression levels of the respective formin in various tissues. In addition, the targeting of formins to cellular membranes is considered a major determinant of function. While GTPases of the Rho family that activate DRFs are localized at specific cellular compartments, it remains unclear if formins support membrane binding. Such a task could be achieved *e.g.* by the polybasic region of the DAD that is released from its autoregulatory interaction with the FH3 domain upon activation by the GTPase. Likewise, the overall assembly of the multi-domain protein in either the stalled dimer formation, or the catalytically active state, or the actin bundling conformation is of major interest. The combination of kinase domains, transmembrane regions, phosphatase activity or RhoGAP function with FH2 domains is even more diverse in formins from plants, fungi or parasites and awaits further functional description. Since formin mediated cytoskeleton regulation is implicated in affecting pathogenesis and diseases ranging from cancer, neuronal disorders, infertility to pathogens as malaria, the mechanistic analyses of the function of this protein family remains an important task.

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