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Review

The role of formins in human disease

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ABSTRACT

Formins are a conserved family of proteins that play key roles in cytoskeletal remodeling. They nucleate and processively elongate non-branched actin filaments and also modulate microtubule dynamics. Despite their significant contributions to cell biology and development, few studies have directly implicated formins in disease pathogenesis. This review highlights the roles of formins in cell division, migration, immunity, and microvesicle formation in the context of human disease. In addition, we discuss the importance of controlling formin activity and protein expression to maintain cell homeostasis.

1. Introduction

Formin family proteins—so-called because of conserved formin-homology-1 and -2 (FH1 and FH2) domains—have emerged as key regulators of actin and microtubule cytoskeletal dynamics during cell division and migration. The FH1 and FH2 domains were identified by Castrillon and Wasserman in the initial characterization of the *Diaphanous* gene in *Drosophila* [1], and both domains participate in the control of cytoskeletal remodeling. The proline-rich FH1 domains have been shown to bind to numerous WW- and SH3-domain containing proteins in addition to profilin-actin, which contributes to the ability of formins to produce non-branched actin filaments. FH2 domains dimerize, then nucleate and processively elongate linear actin filaments by associating with their growing barbed (+) ends. These FH2 dimers create an environment that favors actin monomer addition to generate actin filaments.

While formins are important for actin remodeling events, formins can also modulate microtubule dynamics [2] in at least two different ways. Diaphanous-related formins (DRFs), the family of formins most closely related to the canonical formin Diaphanous, bind directly to microtubules to promote their stabilization [3]. In addition, mammalian DRF (mDia) proteins have been shown to associate with microtubule-end binding proteins EB1 and APC [4]. APC is the product of the adenomatous polyposis coli (*APC*) familial colon cancer tumor suppressor gene, and its role in disease progression may be mediated in part by mDia family proteins. Emerging evidence suggests that the formin mDia1 possesses tumor suppressor activity [5], again pointing to a role for formins in cancer formation.

Defects in cytoskeletal remodeling proteins have previously been implicated in malignancy [6] and the aforementioned association between mDia proteins and tumor suppression may point to specific roles for formins in cancer and other diseases. However, despite the significant roles formins play in cell biology and development [7], there have been relatively few studies that directly link formins with disease pathogenesis. This review highlights the existing body of

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knowledge that suggests defects in formin gene and gene product function contributes to disease.

2. Cytoskeletal remodeling and the cell cycle

Inappropriate cell cycle regulation and cell division are often responsible for the cellular changes that lead to human disease. Changes in cell morphology, chromosome segregation, and vesicular trafficking are all fundamental events that occur during cell division. Each of these events are governed by cytoskeletal remodeling, and it is not surprising then that formins are centrally involved in many aspects of cell division. In fact, one of the best-characterized roles for formins is a necessary function during cytokinesis [1,8,9].

Cytokinesis occurs in the last stage of cell division to physically separate the mother cell into two daughter cells. This process requires the formation of an actin-rich contractile ring that constricts to induce plasma membrane invagination. Completion of cytokinesis is marked by abscission of the daughter cells. Importantly, disruption of formin function by mutation or genetic deletion often results in cytokinesis failure (Fig. 1). This initial discovery was made in *Drosophila* after loss of the *Dia* allele led to aneuploidy in germ cells [1]. Subsequent work in other species has shown that numerous formins play a critical role in cytokinesis [7].

Failure to divide the daughter cells after karyokinesis results in a tetraploid cell. Surviving tetraploid cells are prone to genomic instability, widely thought to contribute to cancer initiation and progression [10]. Therefore, inappropriate control of formin function or expression in humans may be a critical event in cancer development. However, there is no evidence that directly links cytokinesis failure with cancer as a consequence of defective formin function, despite the conserved role for formins in cytokinesis.

As mentioned previously, formins can also modulate microtubule dynamics. How formins stabilize microtubules is reviewed in detail by Bartolini and Gundersen [2]. In the context of cell division, microtubule stabilization is required to facilitate chromosome segregation and midbody formation [11]. mDia1 has been shown to localize to spindle microtubules in dividing HeLa cells [12]. However, the contribution of mDia function toward spindle assembly and dynamics remains unclear. mDia1 and mDia2 have also been shown to decorate the midbodies of dividing cells [13]. The midbody, a dense region of stable microtubules at the site of abscission, helps coordinate vesicle trafficking to promote the membrane remodeling required for cell separation. It is likely that formin-mediated microtubule stabilization contributes to the trafficking events during cell division, especially considering that formins control vesicle trafficking in other cellular contexts as well [14–16].

In summary, formin activity is critical for proper cell division and thus for the maintenance of genomic integrity during cell division. Future studies are likely to link formins with cancer initiation or other diseases directly, given the fundamental role of formins during cytokinesis.

3. Formins in cancer cell migration and invasion

Mammalian cells display remarkable capacities for migration, invasion, and morphological plasticity, and these attributes make possible numerous biological processes of central interest in the understanding of development, homeostasis and disease. Cells of the immune system, in particular, are capable of precisely targeted homing and invasion of tissues; cells in metastatic cancer are similarly capable of migration and invasion. These processes are known to depend on dynamic modulation of the cytoskeleton. A thorough understanding of these processes is essential for progress in diagnosis

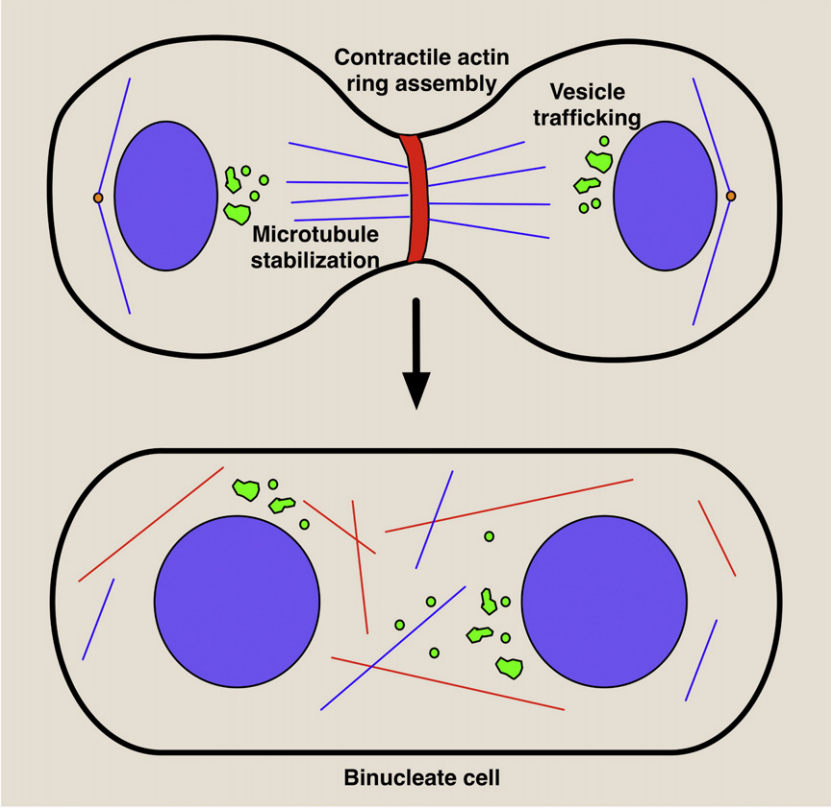

Fig. 1. Formins in cytokinesis. Formins are required for cytokinesis through the assembly of the contractile actin ring. The ability of formins to stabilize microtubules and control vesicle trafficking may also be a required formin function during cytokinesis. Loss of formin activity or deregulation of formin activity has been shown to interfere with cytokinesis and lead to binucleate cells, which can result in chromosome instability.

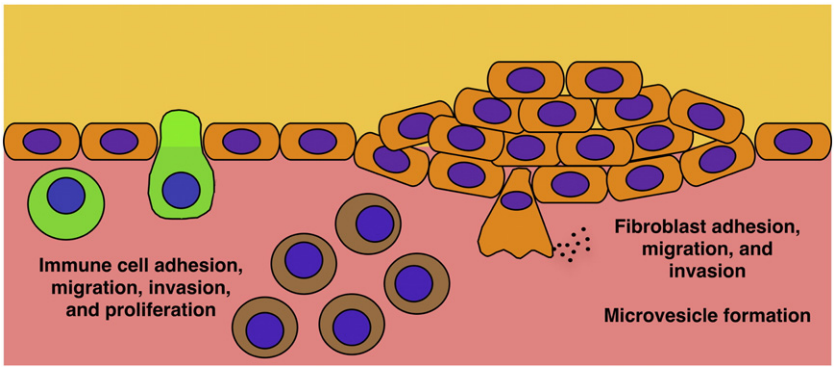

Fig. 2. Formins and cell migration. Formins control adherent cancer cell migration and invasion by assembling actin-rich protrusive structures and stabilizing microtubules. Formins perform similar functions in immune cells, and studies from mDia1 knockout mice show that they also play a role in immune cell proliferation. In prostate cancer cells, the formin mDia2 was shown to control microvesicle formation, which could lead to oncogenic signal transmission to nearby cells.

and therapy of malignancy and other disease states in which cell migration or invasion is centrally involved.

The reorganization of the actin cytoskeleton drives morphological changes during directed cell migration and invasion in normal and malignant cells. Much attention has focused upon the Rho subfamily of small GTPases, and their enhanced expression and/or activation in human cancers, proteins fundamental to actin remodeling; yet, historically, little is known about the role of key downstream effector proteins propagating Rho signaling in migrating/invading cancer cells. In the past several years, significant progress in the understanding of cytoskeletal dynamics has led to the construction of detailed and useful models of these pathways. In particular, an emerging understanding of the functions and roles of formins has generated a model of formin-based actin polymerization and microtubule stabilization, usually in the context of Rho GTPase signaling. Collectively, this emerging evidence (briefly discussed below) suggests a role for the formin family of proteins in modulating both actin- and microtubule-based cytoskeletal networks to promote cancer cell adhesion, migration and ultimately invasion (Fig. 2).

3.1. mDia1

mDia1 has been implicated in a variety of distinct processes driving normal and cancer cell polarity and migration. mDia1 has been demonstrated to bind, stabilize and polarize microtubules from the cell center to the periphery in migrating cells [4,17–21]. mDia1-dependent microtubule polarization and reorientation of both the microtubule organizing center (MTOC) and the Golgi toward the direction of cellular migration is fundamental in driving migration and is dependent upon members of the Rho family of GTPases, including Cdc42 and RhoA (reviewed in [22,23]).

Acting as a Rho GTPase effector protein, mDia1 also has a critical role in promoting the formation of actin-rich protrusions by building F-actin filaments and directing signaling networks at the leading edge of migrating cells. For instance, in the context of N-WASp-depleted adenocarcinoma cells, mDia1 drives actin-enriched cellular protrusions and stress fiber formation in a RhoA-dependent manner [24]. Furthermore, through interaction with the cytoskeletal scaffolding protein IQGAP, mDia1 was localized to the leading edge of migrating fibroblasts [25]. mDia1 has been shown to control the formation of stable actin filaments and promote the formation and turnover of focal adhesions [13,17,19,20]. Recently, it was shown that mDia1 knockdown inhibited formation of focal contacts, decreased lamellipodial thickness and impeded leading edge dynamics in migrating cells [20], while another study showed that mDia1 depletion inhibited Src accumulation into focal adhesions in migrating glioma cells, impairing focal adhesion function and stability, as well as cellular migration [19]. Furthermore, through direct interaction with the cell-surface receptor for advanced glycation end products (RAGE), mDia1 was shown to promote RAGE-ligand-stimulated cellular migration in a manner dependent upon activated Rac and Cdc42 [26]. Thus, the role of mDia1 in cellular migration appears to be complex, involving multiple platforms (actin and microtubule cytoskeletons) and affecting cell polarity and the assembly of cytoskeletal structures supporting leading edge dynamics.

3.2. mDia2

Like mDia1, the related formin mDia2 has also very recently been implicated in cellular migration in both normal and cancer cells. For instance, the zebrafish homologue of mDia2, zDia2 (sharing approximately 50% and 80% sequence homology to human and mouse mDia2, respectively), was shown to be involved in cell motility observed during gastrulation [27]. A morpholino-based zDia2 knockdown strategy uncovered a role for zDia2 in the formation of actin-rich protrusions observed during gastrulation and revealed that profilin I was coordinately involved downstream of zDia2 signaling to control cellular migration during gastrulation. Interestingly, the authors showed that zDia2 expression directed the formation of membrane blebs in the front row of marginal deep cells in zebrafish at the germ-ring stage of gastrulation. As membrane blebbing is an initial indicator of cellular motility accompanying the transformation of non-motile blastomeres into motile blastula cells, these results suggest a role for mDia2 homologues in cellular migration *in vivo*. Moreover, these results confirm *in vitro* evidence that mDia formins control cortical actin contractility and that their disruption promotes the plasma membrane blebbing that is a hallmark of the amoeboid type of cellular motility demonstrated in cervical and prostate cancer cells [28,29]. In a recent study by Di Vizio et al. (discussed further below), mDia2 depletion in DU145 and LNCaP prostate cancer cells enhanced blebbing upon EGF addition and a concomitant increase in motility and invasion was observed. Furthermore, invasion of MDA-MB-231 breast cancer cells in Matrigel was dependent upon mDia formins, and mDia2 was localized with Src to invadopodia; mDia2-depleted MDA-MB-231 cells had few invadopodia, pointing toward mDia2 as an important component in breast cancer cell invasion [30].

Finally, in addition to affecting the actin cytoskeleton during cellular migration and invasion, it is very likely that, like mDia1, mDia2 also influences the microtubule cytoskeleton in migrating cells. Indeed, it was recently shown that mDia2 stabilized microtubules independent of its ability to modulate actin filament assembly [3]; this study illustrated the importance of understanding whether the microtubule stabilizing activity of mDia2 formin is also fundamental to cellular processes in which this formin previously

had been implicated, including cellular adhesion and focal adhesion stability [31].

3.3. Other formin family members (FHOD/FHOS, Formin 1, dDia2, FRL)

In addition to the mDia family, other formins have been shown to play roles in generating actin-rich protrusions that promote cellular migration in a variety of cells and organisms. The Formin-homology-2-domain-containing protein (FHOD1) was demonstrated to enhance cellular migration upon overexpression in human melanoma cells, as well as in NIH 3T3 cells, without significantly affecting integrin expression/activation or cellular adhesion as a whole [32].

Other formins with only moderate homology to mDia formins were also shown to function in cellular migration, including Formin 1 isoform IV (Fmn1-IV), *D. discoideum* Dia2 (dDia2), and formin-related gene in leukocytes (FRL). For instance, Fmn1-IV is a formin protein with some sequence similarity to dishevelled-associated activator of morphogenesis-1 (DAAM1); Dettenhoffer et al. generated Fmn1-IV knock-in mice and demonstrated in primary kidney epithelial cells a role for Fmn1-IV in cell spreading and focal adhesion formation [33]. Conversely, mouse embryonic fibroblasts (MEFs) derived from Fmn1-IV knockout mice, which show weakly penetrent kidney aplasia [34], had altered protrusive behavior at the leading edge of the cells and had defective cell spreading and focal adhesion formation [33]. Overexpression in *D. discoideum* of dDia2, which has some sequence similarity to mammalian DAAM1 (~42%) and to a lesser extent hDia2, led to the formation of more persistent, larger adhesive contacts between filopodia and substrate, suggesting a role for dDia2 in controlling filopodia dynamics and the formation of adhesive structures important for cell motility [35]. Finally, Formin-related gene in leukocytes (FRL) was shown to influence cell adhesion, cell spreading, lamellipodia formation and chemotactic migration [36]. In those studies, overexpression in macrophages of the FH3 domain (referred to as the DID domain in recent studies [37]), containing the Rac-binding domain, was sufficient to inhibit cell spreading as well as lamellipodia formation upon stimulation with the chemokine SDF1α (CXCL12); expression of the FH3 domain was also sufficient to ablate cell adhesion to fibronectin and inhibit chemotactic migration toward SDF1α, indicating a role for this understudied formin in cell motility.

4. Formins in microvesicles

In addition to soluble proteins and other hormones, cells shed membrane vesicles into the extracellular environment. These extruded vesicles have been shown to contain various signaling proteins and microRNAs, and their contents can be transferred to neighboring cells, resulting in changes in proliferative and/or migratory capacity [29,38]. These structures range in size from ~50 nm to several microns in diameter and have been characterized in various guises as exosomes, ectosomes, argosomes, microparticles, and oncosomes [39]. We will refer to them as microvesicles here.

Microvesicles are believed to arise by budding directly from the plasma membrane, but they appear to originate from multiple sources, which include organelles from the endocytotic and endosome processing/trafficking apparatus. Several lines of evidence suggest that formins are involved in both the formation and the emission of microvesicles (Fig. 2). Early work on mDia1 and mDia2 found both proteins on endosomal structures along with Src non-receptor tyrosine kinases [13]. Subsequent studies demonstrated that all mammalian Diaphanous-related formins (mDia1-3) have roles in endosome trafficking [15,40]. Moreover, formin inhibition leads to an induction of non-apoptotic membrane blebbing in numerous cell types [28].

Whether any of the formin-associated vesicles gives rise to microvesicles has yet to be demonstrated. However, recent work by Di Vizio et al. [29] has provided solid evidence that formins directly participate in microvesicle production and thereby function to encourage tumor growth and metastasis. Their studies focused on EGF-induced blebbing of prostate tumor cells, which results in the shedding of microvesicles termed “oncosomes.” These microvesicles contain various signaling proteins and alter signaling in other cells. Notably, Akt phosphorylation is induced in cells exposed to microvesicles shed by prostate cancer cells. Similar results obtained in glioblastoma cells [38] suggest that such phenomena are occasionally associated with metastatic cancer cells and/or amoeboid cell migration. Di Vizio et al. also tested whether mDia2 knockdown in cells produced microvesicles. They incubated recipient cells with vesicles shed from mDia2 knockdown cells and saw measurable increases in proliferation and migration in *in vitro* assays.

These intriguing findings do not specifically implicate mDia2 or other formins in cancer progression. However, they raise interesting questions about such roles. Expression of dominant interfering mDia1 (truncated mDia1 FH2 domain), the same variant known to induce blebbing, inhibits both mDia2 and mDia1 signaling to SRF [41]. Moreover, expression of the interfering mDia1 significantly enhances the tumor-forming capacity of Ras-transformed mouse embryo fibroblasts [42]. Whether the boost in tumor-forming ability is due to increased microvesicle production (perhaps secondary to enhanced non-apoptotic membrane blebbing), or is due to diminished signaling to the apoptotic machinery through SRF signaling to Egr1 [43], or to other effects is unknown. Clearly, the role of mDia2 in such signaling systems needs to be addressed in more detail.

5. Formins in immunity

Cytoskeletal remodeling is required to generate an effective immune response. While formins are critical for cellular migration and invasion (discussed in the previous section), they also mediate polarity, adhesion, and activation of immune cells. Because of their critical roles in T cells, neutrophils, and other hematopoietic cells, formins may contribute to disease when their activity is disrupted in immune cells.

5.1. T cell responses

Recent work by our group, and independently confirmed by others, discovered a central role for the formin mDia1 for *in vivo* dynamic cytoskeletal remodeling driving T cell responses [28,44]. Focusing upon mice with targeted deletion of the gene encoding mDia1, these studies collectively revealed the involvement of mDia1 in T cell development, proliferation and emigration into peripheral lymphoid organs, including spleen and lymph node. These data are consistent with considerable genetic evidence that Rho GTPases (e.g., RhoA), their regulatory exchange factors (e.g., Vav), and their effectors (e.g., WASp) participate in normal T cell function. For example, while both $Vav^{-/-}$ and $WASp^{-/-}$ animals are defective in dynamic remodeling of actin following T cell receptor (TCR) ligation [45,46], it is possible that the RhoGEF activity of Vav (specifically toward RhoA) directly affects cytoskeletal remodeling upon TCR stimulation. Indeed the small GTPase RhoA regulates integrin-mediated T cell adhesion and migration [47,48]; while mDia1 is a known RhoA effector, it is plausible that the defects in adhesion and chemotactic migration may be due to disruption of RhoA-mediated mDia1 activation and dysregulation of formin-mediated actin dynamics. Indeed, constitutively activated mDia1 (lacking the GTPase binding and a portion of the Dia-inhibitory domain) dramatically enhanced actin accumulation in Jurkat T cells *in vitro* while decreasing chemotactic migration upon TCR engagement [49]; these results suggest that arresting the dynamic actin and microtubule

cytoskeletons controlled by mDia1 [4,50,51] inhibits normal T cell function, consistent with our results utilizing T cells derived from the *Drf1* knockout mouse. However, a recent study suggested that upon depletion of mDia1, actin dynamics at the immune synapse were not affected upon TCR stimulation [52]. mDia1 depletion was incomplete in those studies (90% depletion as estimated by Chhabra and Higgs [53]), and, along with the modest effects observed in F-actin accumulation and cellular adhesion/migration, these results suggest that a lower level of mDia1 protein is sufficient to mediate actin dynamics in stimulated T cells.

5.2. Neutrophil responses

Following the discovery of a role for mDia1 in regulating actin dynamics critical for T cell migration, two recent studies also demonstrated a role for mDia1 in neutrophil migration and activation [54,55]. Using neutrophils isolated from mDia1 [56], WASp [46] or mDia1/WASp double knockouts, these studies revealed an association between mDia1 and WASp that was important for mediating neutrophil polarization and chemotactic responses toward the chemokine MIP-2 or the fMLP formyl peptide. These studies further demonstrated that both Src family kinases and LARG/RhoA/ROCK signaling contributed to the mDia1-mediated signaling axis promoting neutrophil chemotaxis.

5.3. Other hematopoietic cells

The assembly of F-actin filaments into a contractile actin ring (CAR) was previously shown to be fundamental to enucleation [57,58], a process fundamental to erythyrocyte maturation in which the pycnotic nucleus is extruded from the mature erythroid cell. Until recently, the identity of actin-assembly factors mediating CAR formation and enucleation remained elusive. However, Ji et al. recently demonstrated a specific requirement for the formin mDia2, through an association with Rac1 and Rac2, for CAR formation and subsequent enucleation [59]. In those studies, Rac inhibition ablated enucleation, while constitutively activated mDia2 was shown to rescue the defect. As it has been postulated that enucleation is a specialized form of cytokinesis, these results are consistent with previous findings revealing a role for mDia2 in contractile ring formation and in the completion of cytokinesis [13,60,61].

Finally, platelets exposed to thrombin are rapidly activated and undergo dramatic RhoA-dependent reorganization of their actin cytoskeleton [62,63]; yet until recently, it was unclear what Rho effector proteins are required for propagating Rho-dependent signaling in platelets. Two studies demonstrated a role for the formins mDia1 and DAAM1 in promoting actin assembly/remodeling and cell spreading in activated platelets in response to RhoA signaling [64,65]. Collectively these findings, and those obtained in the experiments on hematopoietic cells described above, suggest an essential role for formin-mediated actin assembly in maintaining proper homeostasis during immune function. Additional *in vivo* analysis of mice deficient in formin protein expression and validation in human samples will be an important avenue for future studies.

6. Mouse models linking formins with disease

Multiple formin family members have been genetically manipulated in mice to determine their *in vivo* functions. These studies have revealed important roles for formins in development, immunity, and disease. In some instances, the subtle phenotypes suggest that there is functional redundancy between formins. Based on the current mouse models, several diseases have been associated with impaired formin function.

6.1. Models of Fmn gene function

Leder and colleagues identified the initial *Limb Deformity* (*Ld*) loci genes as having key roles in development programs that guide limb formation [66,67]. Different transcripts with potential truncations were identified at those loci and hybridization experiments pointed to their expression in limb buds. It was then hypothesized that defects in their gene function affected limb formation [7]. Continued analysis of the *Ld* locus showed that while the *Formin* (*Fmn*) gene was near the *Ld* locus, the protein encoded by *Fmn* was not affected in the original knockout mice. Instead, defects in the *Gremlin* gene, which has important functions in limb formation, were shown to account for defects in *Ld* mice [7].

Recently, *Fmn1* or Formin 1 knockout mice were shown to have a limb deformity phenotype by reduction of digit number to four [68]. Importantly, there were no effects on Gremlin expression or function as a result of *Fmn1* knockout. These data suggest that Formin 1 function is indeed required for normal limb development in certain contexts. Nevertheless, a role for Formin 1 in human development or disease remains to be described.

Fmn2 knockout mice identified a role for Fmn2 function in the meiotic cell divisions of mouse oocytes [69]. Knockout mice had reduced fertility as a result of defects in spindle positioning and polyploidy in oocytes. Since defects in oocyte maturation often result in birth defects and pregnancy loss in humans, it is interesting to speculate that problems with formin activity or expression may contribute to these errors [69]. However, neither Fmn2 nor any other formin family member has been specifically implicated in human fertility defects to date.

6.2. Models of Drf gene function

Human myelodysplastic syndrome (MDS) is a hematopoietic disorder due to defects in the control or differentiation of hematopoietic stem or progenitor cells [70]. Clinically, MDS patients present with various cytopenias, hypercellular marrow, dysplastic erythrocytes, and an increased risk to develop AML [71]. One subset of MDS is 5q- syndrome, which involves deletion of all or part of chromosome 5. Interestingly, *DIAPH1*, which encodes mDia1, is located at or near commonly deleted sites in 5q- patients. In fact, gene expression analysis of patients with 5q- MDS shows decreased *DIAPH1* expression [43]. This led to the hypothesis that mDia1 may have suppressor functions in the maintenance of hematopoietic stem or progenitor cell proliferation. This idea was recently supported through genetic deletion of the *Drf1* gene in mice. Knockout mice developed an age-dependent myeloproliferative disorder, including splenomegaly, hypercellular bone marrow, and expansion of the myeloid and erythroid compartments in the spleen and bone marrow [5]. The specific mechanism by which loss of mDia1 contributes to this phenotype remains to be determined, but it is suggested that inappropriate signaling through the transcription factor SRF plays a critical role [7,43,72].

Moreover, additional loss of RhoB enhanced the myeloproliferative phenotype observed in mDia1 knockout mice alone. Compared to mDia1 knockout mice, mice lacking both RhoB and mDia1 developed a more severe myelodysplasia [73]. This is intriguing, given that RhoB has been proposed to have tumor suppressor activity [74], and low expression often correlates with late stage malignancy [75,76]. It will be important to examine the role of formins and RhoB in the context of stem or progenitor cell function, since myelodysplasia is considered a disease of the stem cell compartment.

7. Alterations in formin expression or function associated with disease

Aberrant formin function and expression have been directly implicated in maladies as diverse as deafness [77], fertility defects

Table 1
Formin chromosome locations and human disease association.

Formin subfamily	Name	Gene ID	Chromosome	Disease relevancy
Dia—Diaphanous	mDia1/*DIAPH1*	1729	5q31	5q- Myelodysplastic syndrome [5,43], DFNA1 non-syndromic deafness [77]
	mDia2/*DIAPH3*	1730	13q21.2	Chromosome deletion in metastatic prostate cancer [29]
	mDia3/*DIAPH2*	81624	Xq21.33	Premature ovarian failure [78]
FRL—Formin-Related gene in Leukocytes	FMNL1	752	17q21	Increased expression in lymphoid malignancies and peripheral blood leukocytes from CLL patients [80]
	FMNL2	114793	2q23.3	Increased expression in colorectal cancer [79]
	FMNL3	91010	12q13.12	
DAAM—Dishevelled-Associated Activator of Morphogenesis	DAAM1	23002	14q23.1	
	DAAM2	23500	6p21.2	
Delphilin	GRID2IP	392862	7p22.1	
INF—Inverted Formin	FHDC1	85462	4q31.3	
	INF2	64423	14q32.33	
FHOD—Formin Homology-Domain-containing protein	FHOD1/FHOS	29109	16q22	
	FHOD3/FHOS2	80206	18q12	
FMN—Formin	FMN1	342184	15q13.3	
	FMN2	56776	1q43	

[78], and cancer [79] (Table 1). However, the mechanisms demonstrating a causal role for formins in the formation of these diseases have not been rigorously tested. Nevertheless, these studies point to the importance of controlling formin activity for proper cell function.

7.1. DFNA1—autosomal dominant non-syndromic deafness

A progressive deafness disorder was discovered among a kindred in Costa Rica that had descended from the Conquistadore invasions. This disorder was named *DFNA1* and was subsequently mapped to the *DIAPH1* gene [77]. The mutation in *DIAPH1* is a four base pair (TTAA) insertion that generates a frameshift mutation in the coding sequence that is predicted to generate an inappropriate stop codon, truncating 32 amino acids (Fig. 3). However, the contribution of this mutation to formin function has never been tested. Given the location of the truncation, it is possible that the mutation may induce a gain-of-function, by disrupting the autoinhibitory mechanism of mDia1.

7.2. Premature ovarian failure (POF)

Previous work has shown that a critical region on the X chromosome is interrupted by a breakpoint associated with a familial case of POF [78]. Mapping of the gene responsible for the ovarian defect revealed that *DIAPH2* (mDia3) may be the gene responsible due to a breakpoint in the last intron. These findings suggest a possible role for mDia3 in ovarian development. The cellular consequences of the breakpoint for mDia3 function are unknown. However, a role for formins in ovarian development is consistent with the spermatogenesis and oogenesis defects observed in mutant versions of the *Drosophila dia* gene [1].

7.3. Expression data associated with disease

Formin-like 2 (FMNL2) expression was shown to be elevated in colorectal metastatic cancer cell lines compared to normal colorectal cancer cell lines [79]. In addition, FMNL2 expression was higher in primary colorectal cancer and lymph node metastases, with the highest expression in the metastatic-derived cell lines. However, a correlation between FMNL2 expression and clinical diagnosis was not performed. Also unknown is whether increased FMNL2 expression contributes to colorectal cancer formation, or if it is simply a passenger effect due to other required genetic abnormalities.

The human leukocyte formin (FMNL1) is highly expressed in the thymus, spleen, and peripheral blood leukocytes. It was reported

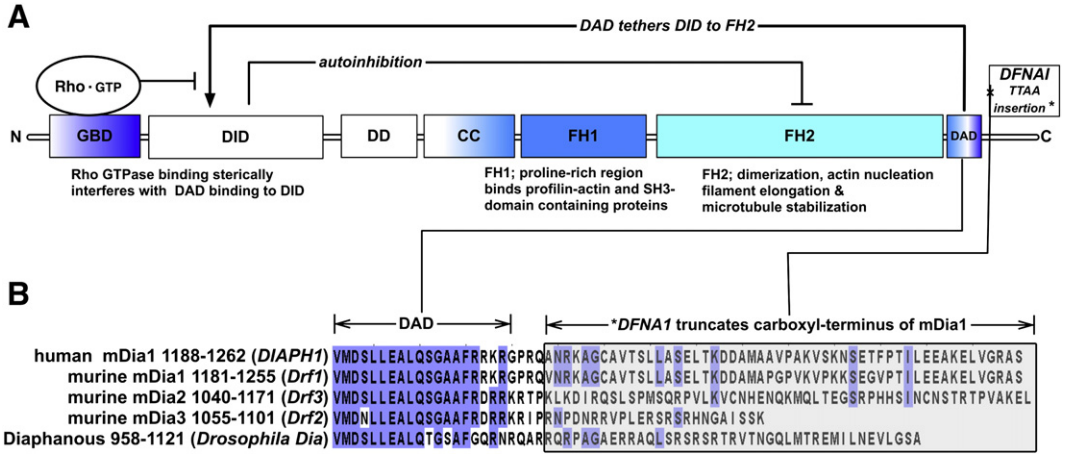

Fig. 3. Mutation in *DIAPH1* leads to an autosomal dominant non-syndromic deafness disorder. (A) Schematic diagram of the *DIAPH1* encoded mDia1 protein. GTP-bound Rho binds the GTPase binding domain of autoregulated formins (e.g. mDia1) to sterically interfere with Dia inhibitory domain interaction with the Dia autoregulatory domain (DAD). Release of autoregulation promotes formin-homology-2-domain-mediated actin polymerization. The DFNA1 insertion is located at the C-terminal end of the DAD domain. (B) ClustalW amino acid sequence alignment of human mDia1, murine mDia1-3, and *Drosophila* diaphanous. The DFNA1 insertion generates a truncated version of mDia1 that may disrupt autoregulation given the proximity of the truncation to the DAD domain. However, the truncation is on the C-terminal end of the most conserved DAD region, so the DFNA1 insertion may result in defective protein function not related to autoregulation.

that high expression of FMNL1 is present in several lymphoid cancer cell lines [80]. However, whether FMNL1 expression correlates with lymphoid cancers from patient samples has not been tested.

Experiments that implicate mDia2 in the negative regulation of non-apoptotic blebbing and microvesicle formation suggest that mDia2 (and perhaps other formins) exerts control over amoeboid cell migration and/or other aspects of cancer progression [28,29]. For instance, genomic analysis of prostate cancer samples, comparing primary tumors to metastases, suggests that deletions at the *DIAPH3* locus are significantly more common in metastatic disease [29]. *DIAPH3* is located in a region of the q arm of chromosome 13 (13q21.2), and while this region has been long thought to harbor tumor suppressor genes [81], no tumor suppressor genes have been specifically identified there. Further work will clarify the extent to which mDia2 is functioning in that role, but the findings to date indicate that such hypotheses are worthy of careful examination.

8. Concluding remarks

Formins govern both actin and microtubule cytoskeletal dynamics and play important roles in cell division, cell migration, immunity, and development. While much of the knowledge about formin function has been established using model organisms and *in vitro* systems, there have been relatively few studies that directly link formins with disease pathogenesis. The generation of new mouse models and a more extensive characterization of the current models will be useful to provide insights regarding the function of formin family members *in vivo*. Future studies should address specific hypotheses related to aberrant formin expression and activity in disease. Are there disease-causing mutations in formins that affect their activity? How does increased or decreased formin expression facilitate disease, if at all? At this point, there are many more questions than answers regarding the role of formins in disease. Given the numerous cellular functions mediated by formins, determining answers to these questions should prove to be a rewarding endeavor.

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