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Formins



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disrupt Hem1 structure, leading to WRC degradation, except for the M371V mutation, which has been suggested to disrupt Arf1–WRC signaling.

## Outlook and future directions

Many important questions remain to be answered for a complete understanding of the function and regulation of the WRC, from both a biochemical/structural and cell biological perspective. A key remaining question is how the WRC interacts with or becomes activated by various ligands, including Rac1, Arf1, $PIP_3$ and many other molecules, both individually and cooperatively, and both *in vitro* and at the plasma membrane of cells. As an example, it is still unclear whether WRC activation can be separated from its recruitment to the membrane. Answering all of these questions will promote the development of inhibitors, activators, and chemical or optogenetic tools to control or track WRC functions in cells, which will be of both scientific and potential medical relevance. Such tools might also provide us with the ability to unravel additional, perhaps less canonical, functions to those summarized above. How different WRC variants containing distinct combinations of subunits are differentially regulated in cells is emerging as yet another exciting future topic. Additional questions that have remained almost entirely unanswered include the regulation of WRC assembly, recycling and degradation, as well as biochemical mechanisms of WRC regulation in plants. Last, but not least, it will be essential that a full understanding of WRC regulation and function establishes how its individual subunits also participate in other complexes, such as Sra1 with FMRP–eIF4E, and how cells balance all of these individual subunit activities in normal development and disease.

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Primer

# Formins

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Actin is one of the most abundant proteins in eukaryotes. Discovered in muscle and described as far back as 1887, actin was first purified in 1942. It plays myriad roles in essentially every eukaryotic cell. Actin is central to development, muscle contraction, and cell motility, and it also functions in the nucleus, to name a spectrum of examples. The flexibility of actin function stems from two factors: firstly, it is dynamic, transitioning between monomer and filament, and, secondly, there are hundreds of actin-binding proteins that build and organize specific actin-based structures. Of prime importance are actin nucleators — proteins that stimulate *de novo* formation of actin filaments. There are three known classes of actin nucleators: the Arp2/3 complex, formins, and tandem WASP homology 2 (WH2) nucleators. Each class nucleates by a distinct mechanism that contributes to the organization of the larger structure being built. Evidence shows that the Arp2/3 complex produces branched actin filaments, remaining bound at the branch point, while formins create linear actin filaments, remaining bound at the growing end. Here, we focus on the formin family of actin nucleators.

Formins are crucial proteins for a range of cellular processes, as demonstrated by their links to various pathologies, including cardiomyopathies, cancers, intellectual disabilities and other neuronal disorders, nonsyndromic deafness, and renal disorders. It follows that understanding how formins help to build actin-based structures is essential to our knowledge of normal physiology as well as many pathologies. In this Primer, we highlight the biochemical activities underlying actin assembly by formins and, where possible, we weave in links between biochemistry and biological roles. Finally, we discuss outstanding questions about formins.

## The importance of nucleators

Nucleators are essential for tightly regulated actin assembly. The initial step of actin filament

**Table 1. The metazoan formin superfamily.**

| Subtype (full name) | Human isoforms | Associated structures or processes | Associated diseases |
|---|---|---|---|
| **Dia** (Diaphanous) | Dia1, Dia2, Dia3 | Stress fibers, contractile ring, microtubule stabilization and cell migration | Deafness, seizures, cortical blindness, microcephaly syndrome |
| **Daam** (Disheveled-associated activators of morphogenesis) | Daam1, Daam2 | Planar cell polarity, cilia, sarcomeres | Unknown |
| **FMNL**/FRL (Formin-related proteins identified in leukocytes) | FMNL1, FMNL2, FMNL3 | Cell morphology, cortical actin | Unknown |
| **INF** ('inverted' formins) | INF1, INF2 | Mitochondrial fission | Focal segmental glomerulosclerosis, Charcot-Marie-Tooth disease |
| **FHOD** (Formin homology domain containing proteins) | Fhod1, Fhod3 | Sarcomeres, stress fibers | Hypertrophic cardiomyopathy and possibly other cardiac disorders |
| **GRID2IP** (Delphilin) | Delphilin | Purkinje cells | Unknown |
| **FMN** (the founding family of 'formins') | Fmn1, Fmn2 | Oogenesis, DNA damage response | Polydactyly, intellectual disability |
| **MWHF** (Multiple wing hairs formins) | N/A | Unknown | N/A |
| **PHCF** (PH-domain-containing formins) | N/A | Unknown | N/A |

formation — nucleation — is a kinetically unfavorable process. That is, actin alone spontaneously nucleates, but it does so at a much slower rate than that required for cellular actin dynamics. In addition, some actin-binding proteins (primarily profilin) bind to most available actin monomers in the cytoplasm, further limiting nucleation. Given the need to assemble structures at strictly controlled locations, at specific times, and in response to rapidly changing signals, the cell uses nucleators to 'turn on' actin assembly at will.

After the nucleation of a new actin filament, the filament grows or elongates. *In vitro*, one end of the filament, the so-called 'barbed' end, grows around ten times faster than the other end, the 'pointed' end. Growth at the barbed end is believed to be the dominant form of elongation in the cell. An important feature of formins, compared with other actin nucleators, is that they are elongation factors as well. That is, formins remain associated with the fast-growing barbed end, in some cases markedly accelerating growth and also competing with proteins that inhibit growth, such as capping protein. In this way, formins influence the length of actin filaments, in addition to their number.

## The formin superfamily

The first 'formin' was so named to reflect the defect in mouse limb formation that resulted from mutations in the *limb deformity* locus. In fact, the *limb deformity* phenotype is now attributed to two neighboring genes: Fmn1, of the FMN-subtype of formins, and gremlin, an unrelated gene. Shortly after Fmn1 was identified, a similar gene, *diaphanous*, was found to be essential for cytokinesis in *Drosophila*. When related yeast proteins were shown to nucleate actin assembly, the formin family of actin nucleators was established. Metazoans have nine subtypes of formins in the superfamily (Table 1), with humans having 15 different formin genes (and more isoforms due to alternative splicing) representing seven of the nine subtypes. Genomic evidence suggests that placental mammals lost the other two subtypes. The common yeast models, fission and budding yeast, have formins that are closely related to metazoan formins of the Dia subtype. Other fungi have formins that do not fall into the nine metazoan subtypes, although they likely arose from a Dia-like ancestor. More distant species show continued divergence. For example, plants have formins that fall into three distinct classes or subtypes. Phylogenetically, the core formin homology 2 (FH2) domain likely arose once during eukaryotic evolution and was connected to other structural domains through gene duplication events and divergence, resulting in varied subtypes.

Most cells contain a diverse complement of formins that is needed to build a collection of structures, including tightly organized stress fibers (require Dia, Fhod, Daam; metazoan formin subtypes, but not specific formin genes, are given here) and sarcomeres (Daam, Fhod), diffuse meshes that fill the oocyte (Fmn), long narrow filopodia (Dia, FMNL), transient structures on mitochondria (INF2), and the cytokinetic contractile ring (Dia), among many others (Figure 1 and

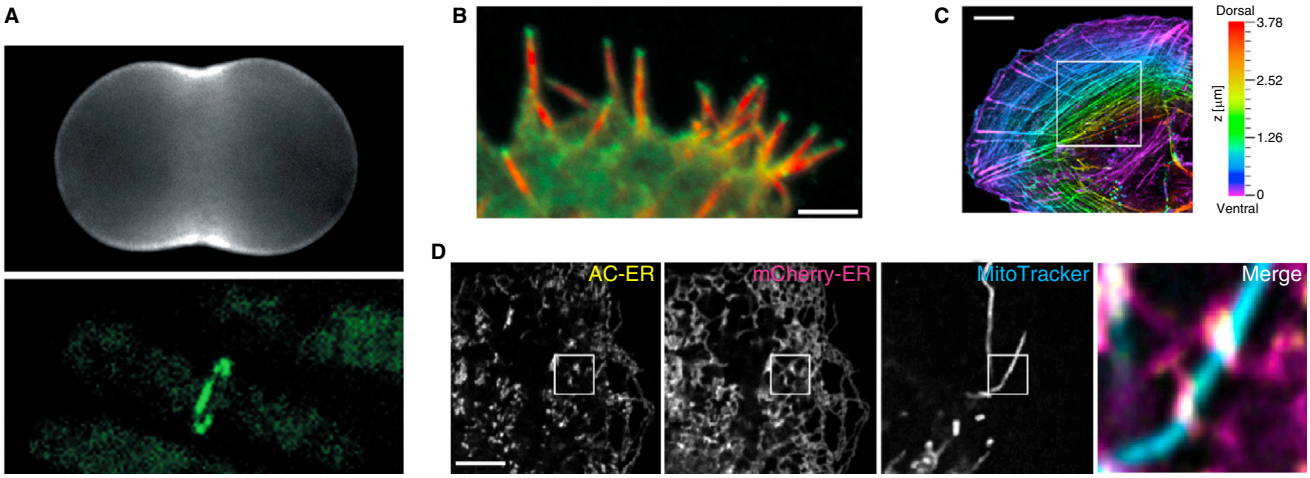


**Figure 1. Fluorescence images of formin-dependent actin structures.**
(A) Contractile rings in a large and a small cell. Sea urchin embryo (top) stained for actin filaments with rhodamine–phalloidin, and fission yeast cell (bottom) expressing a myosin light chain–GFP fusion protein. The fission yeast cell is approximately 14 times smaller than the sea urchin cell. (Reproduced from Chang and Burgess (2003) Curr. Biol. *13*, R692–R693.) (B) A *Dictyostelium* cell expressing GFP-tagged full-length dDia2 was fixed and labelled with anti-GFP antibodies to visualize dDia2 (green) and with TRITC–phalloidin to visualize F-actin (red). The formin is enriched at the tips of filopodia. Scale bar: 2 µm. (Image used with permission from Portland Press © Schirenbeck *et al*. (2005) Biochem. Soc. Trans. *33*, 1256–1259.) (C) Stress fibers in U2OS cells. Depicted is the maximum projection of a confocal Z-stack (F-actin), in which the z-coordinates are indicated by the color bar. Scale bar: 10 µm. (Reproduced from Schulze *et al*. (2014) J. Cell Sci. *127*, 1379–1393, with permission.) (D) Accumulation of actin, as indicated by an endoplasmic reticulum targeted actin nanobody (AC–ER), is observed at mitochondria–ER crossover points. Co-expression in U2OS cells of mCherry–ER and AC–ER, labeled with MitoTracker. Scale bar: 5 µm. (Reprinted by permission from Springer Nature, Schiavon *et al*. (2020) Nat. Methods *17*, 917–921 © 2020.)

Table 1). A clear example of the functional specificity of formins comes from fission yeast. This single-celled organism has four major actin-based structures built by four nucleators: the Arp2/3 complex and three different formins. The Arp2/3 complex nucleates actin in endocytic patches, and the formins For3, Cdc12, and Fus1 nucleate actin for cables, cytokinesis, and fusion during sexual reproduction, respectively. With so many formins, it is important to understand how their specificity of function arises.

## Formin structure and function

Each formin has characteristic nucleation and elongation activities, suggesting that precise actin assembly activity is central to the ability of the formin superfamily to build such a broad range of actin-based structures. Structurally, formins are defined by the eponymous formin homology (FH) domains 1 and 2 (Figure 2A). These domains are straddled by regulatory domains that modulate their activity (Figure 2B). Central to the actin nucleator role of formins is the FH2 domain, which forms a donut-shaped homodimer (Figure 2C). The FH2 domain is sufficient to nucleate and processively move with the growing barbed end of the actin filament (Figure 2D,E). The nucleation process remains poorly understood. Existing models suggest that the FH2 homodimer stabilizes otherwise short-lived actin dimers or trimers, thereby favoring filament formation (Figure 2C). Efficiencies as low as 0.02 filaments/nucleator and as high as 0.2 have been reported. The FH2 domain also influences the rate of filament elongation by a process termed gating, which is modeled as switching between two conformational states — 'open' and 'closed'. The fraction of time spent in the closed state determines the extent to which elongation is slowed by the FH2 domain. The gating fraction ranges from essentially 0 to 1 for different formins.

The rate of elongation is also controlled by the FH1 domain. This unstructured, proline-rich domain indirectly binds actin via the actin-monomer-binding protein profilin. The commonly accepted model for promotion of actin filament elongation by FH1 domains is the 'capture and delivery' model: the FH1 domain binds (captures) profilin-bound actin and rapidly delivers it to the growing end of the actin filament (Figure 2E). Under typical conditions used to study formin-mediated actin assembly *in vitro*, capture is the slowest step. Recent work has shown that profilin release from the actin filament can be rate-limiting at physiological concentrations of profilin–actin (~two orders of magnitude higher than common (and practical) concentrations used for *in vitro* studies). The composition of FH1 domains varies widely. Some FH1 domains have only one or two profilin-binding sites, while others have as many as ten. The number of profilin-binding sites, their distances from the FH2 domain and their affinities for profilin all influence the efficacy of a given FH1 domain. Consistent with the range of FH1 domains and FH2-mediated gating, the resulting effect on formin-mediated elongation rates varies widely, with FMNL1 slowing elongation by around fivefold and Dia1 accelerating elongation by around fivefold.

Given that formins set the elongation rate, the time that a formin remains associated with a filament is also important. This property is referred to as processivity. The product of the elongation rate and processivity (or off-rate) determines the characteristic run length of (or typical filament length built by) a given formin. Characteristic run lengths as short as 2 µm and as long as 200 µm have been reported.

To summarize, among the formins characterized to date, the range of nucleation activity is at least one order of magnitude, the range of gating is

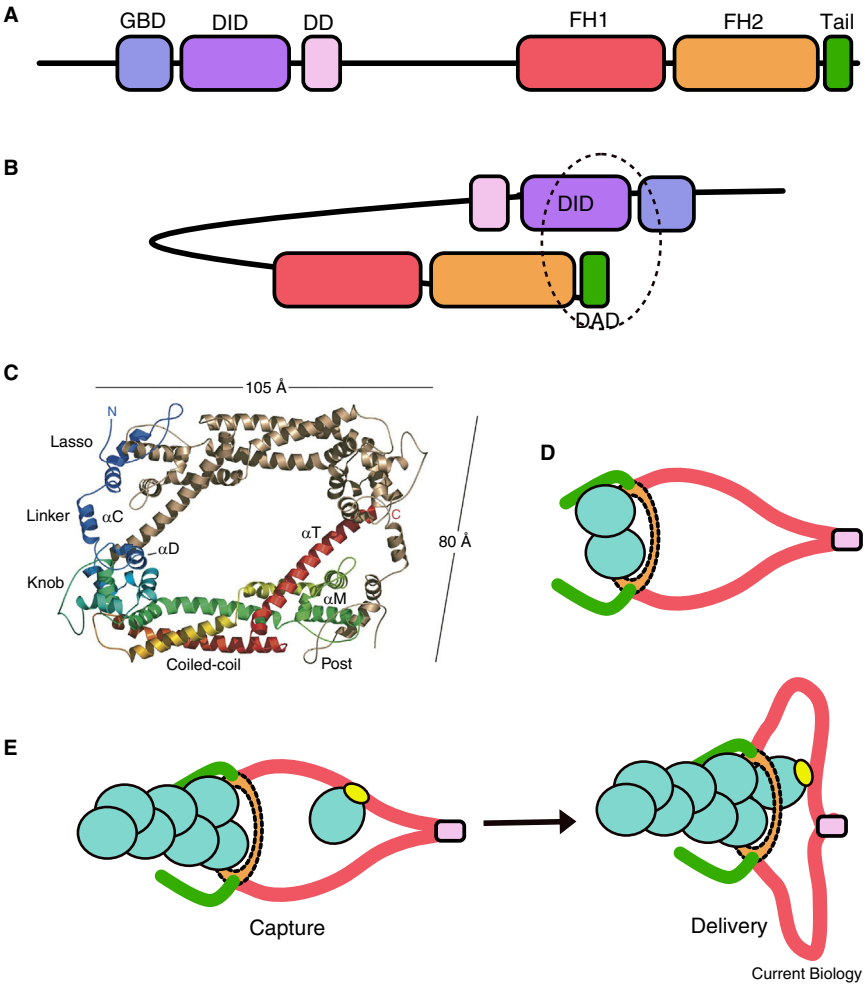


**Figure 2. Formin structures and models of assembly.**
(A) Domain structure of (Diaphanous-related) formins. GBD, GTPase-binding domain; DID, Diaphanous inhibitory domain; DD, dimerization domain; FH1, formin homology 1 domain; FH2, formin homology 2 domain; tail, includes the Diaphanous autoinhibitory domain (DAD). (B) Autoinhibitory interaction between the DID and DAD. (C) Crystal structure of an FH2 domain (Bni1p). Dimensions and subdomains are indicated. (Reproduced from Xu *et al*. (2004) Cell *116*, 711–723.) (D) Nucleation: the FH2 domain stabilizes an actin dimer with help from the tail. Domains are color matched to (A) and actin is light blue. (E) Elongation: the FH1 domain binds (captures) profilin-bound actin (profilin is yellow). The flexible FH1 domain then delivers actin to the barbed end of the growing filament.

two orders of magnitude, the range of elongation rates is at least one order of magnitude, and the range of processivities is at least two orders of magnitude. The combination of nucleation, elongation, and processivity can lead to a range of six orders of magnitude in overall activity, resulting in many short filaments, a few long filaments, and everything in between. Notably, this is the range of activities measured *in vitro*, without considering the proteins and post-translational modifications that could further modify any step of actin assembly mediated by formins.

## Regulation of formin activity

Controlling when and where new actin structures are built is critical. Thus, tight regulation of formin activity is a must. A third conserved domain, FH3, was found to be part of a larger regulatory domain in formins. First described in the formin Dia1, this domain is commonly referred to as the Diaphanous inhibitory domain or DID (Figure 2A). On either side of the DID is a GTPase-binding domain (GBD) and a dimerization domain. The DID makes an intramolecular interaction with a short motif at the carboxyl terminus, called the Diaphanous autoregulatory domain (DAD), which results in autoinhibition (Figure 2B). Thus, purified full-length formins are generally inactive. DIDs and DADs are present in four other subtypes of formins, in addition to the Diaphanous subtype. FMN-family formins are autoinhibited in an analogous manner, despite lacking recognizable DIDs and DADs. The three other subtypes — Delphilin, PHCF, and MWHF formins — appear to be regulated by distinct mechanisms. Only MWHF formins retain the DID and all three lack a recognizable carboxy-terminal DAD. While some formins might have a cryptic functional DAD, others do not have any carboxy-terminal sequence beyond the FH2 domains, necessitating an alternative mechanism to inhibit activity.

The first formin activator described was the small GTPase Rho, which binds to the GBD, adjacent to the DID of Dia1. However, Rho-mediated formin activation was incomplete when analyzed in biochemical assays. Subsequently, other small GTPases were linked to activation of specific formins. Mammals do not have fifteen small GTPases, suggesting that the specificity of activity is more complex. Recently, the contractile ring proteins anillin and IQGAP1 were proposed to mediate localization and co-activate specific formins in conjunction with Rho. Additional proteins and post-translational modifications are likely to modify formin activity. For example, purified full-length INF2 is active, despite the presence of DID and DAD domains. For this formin, an inhibitory complex of lysine-acetylated actin and cyclase-associated protein is required to stabilize or perhaps bridge the DID–DAD inhibitory interaction. A well-studied example of post-translational modification comes from the FHOD subtype of formins, including Fhod1 and Fhod3. Fhod3 is important for sarcomere formation in both skeletal and cardiac muscle, and two distinct phosphorylation events regulate this formin. One phosphorylation site is within an alternatively spliced exon and phosphorylation by casein kinase 2 drives the subcellular localization of Fhod3 to sarcomeres. Phosphorylation by the Rho-associated kinase ROCK of a consensus site within the DAD of both Fhod1 and Fhod3 weakens the intramolecular interaction between the DID and DAD and is sufficient to activate these formins in cells.

In addition to containing the DAD, the carboxy-terminal tail directly influences

actin assembly. Formin tails weakly interact with actin monomers and filaments, yet they can have a strong impact on nucleation and processivity. Data suggest that the ability of formin tails to bind actin monomers, holding them in proximity to the FH2 domain, contributes to nucleation (Figure 2C). A role for the tail in nucleation fits with the fact that the DAD is located within this region: the DID–DAD interaction would compete with actin monomer binding, thereby preventing or at least reducing nucleation. The tail also enhances processivity through electrostatic interactions with actin filaments. In one case, removing the tail decreased processivity by ~1.5 orders of magnitude. One can imagine that post-translational modifications of the tail, such as the phosphorylation reported for FHOD-family formins, could modulate nucleation and/or processivity in addition to the inhibitory state, though this is yet to be tested.

Actin filaments are commonly part of the cell's force-generating structures and/or modify physical conditions within the cell. Thus, formins might need to respond to external forces. An elegant demonstration of this concept comes from reconstituting components of the cytokinetic ring. In fission yeast, the formin Cdc12p contributes actin filaments to the ring. When myosin pulls on formin-bound actin filaments, Cdc12p-mediated actin assembly is inhibited. These observations were complemented by computational estimates consistent with force-induced formin inhibition being necessary for the formation of the cytokinetic ring. Quantitative measurements of formin mechanosensitivity have been made for Bni1, Dia1, and Dia2 using microfluidics and optical or magnetic traps. Both the FH1 and FH2 domains appear to be subject to force-mediated regulation, with growth slowing or accelerating depending on force amplitude (among other conditions) and the formin itself.

## Actin isoform specificity of formins

The six mammalian isoforms of actin have at least 93% sequence identity, with most of the small amount of variation occurring in the first 10 residues at the amino terminus. This high level of conservation makes it unclear exactly why we need so many actin genes and how they function differentially. Recent studies reported a role for formins as selectors of specific actin isoforms. β-actin is enriched in the contractile ring of dividing cells and γ-actin is distributed throughout the cortex. These isoforms differ by only four residues. The primary formin at the contractile ring is Dia2, and *in vitro* experiments indicate that it selectively nucleates and elongates β-actin, potentially establishing the biased localization of this actin isoform in this context. Delphilin, a formin specific to Purkinje cells, can also differentiate between actin isoforms. Delphilin selectively nucleates non-muscle actin (β and/or γ-actin) over muscle actin (α-actin). It is not immediately obvious why Delphilin has this specificity if it is predominantly expressed in neurons. However, removal of α-actin from neurons does result in a phenotype, suggesting that this actin isoform is expressed in and important for neurons. A more intuitive example is provided by Fhod1. This formin is ubiquitously expressed, including in cardiomyocytes. In the cardiomyocyte, Fhod3 is associated with sarcomeres, the contractile units of the muscle, while Fhod1 is linked to intercalated discs, structures that mediate communication for synchronized contraction of the heart. As in cell division, actin isoforms are differentially localized: muscle actin is in sarcomeres and non-muscle actin is in intercalated discs. While we do not know if it is causative or even necessary, we do know that Fhod1 preferentially nucleates non-muscle actin. Interestingly, Fhod3 nucleates both actin isoforms, with only a slight preference for muscle actin. In contrast to these examples, other formins that have been tested for isoform specificity are not selective: FMNL1 and the *Drosophila* FMN-family formin Cappuccino (Capu), in addition to Fhod3, potently nucleate both muscle and non-muscle actin.

The fact that some formins can select between actin isoforms suggests that they are sensitive to individual amino acids. Consistent with this, we now know that formins are sensitive to specific post-translational modifications of actin, and this is a new, exciting area of investigation.

## Formins in health and disease

Given the broad range of physiological effects, it should not come as a surprise that disruption of formin function has been associated with various diseases (Table 1). For example, the Dia formin subtype is linked to several cancers, as one might expect given its essential role in cell division. In addition, specific mutations in Dia1 and Dia3 have been linked with deafness and the developmental disorder termed seizures, cortical blindness, microcephaly syndrome (SCBMS), while Dia2 mutations are associated with premature ovarian failure.

INF2 mutations are linked to both renal (focal segmental glomerular syndrome, FSGS) and neural (Charcot-Marie Tooth) disorders. All of the mutations identified to date are in the amino-terminal regulatory region of INF2, which was interpreted as evidence that loss of INF2 regulation results in pathologies. Now evidence has revealed that some of these mutants are not properly transported to the foot processes of renal podocytes, which are essential for filtration. Specifically, an amino-terminal fragment of INF2 generated by proteolysis binds and inhibits Dia1 specifically in these processes. To date there have been few reports of interactions between formins. Cdc12p and For3p both play a role at the fission yeast contractile ring, but this appears to be an indirect interaction. Formin–formin interactions are an intriguing and complex issue, giving rise to many open questions, including what happens to the other half of INF2, which is presumably constitutively active, after proteolytic cleavage? And, why, if Dia1 can be autoinhibited, would it require *trans*-inhibition by another formin?

As noted above, Fhod3 plays a role in sarcomere formation and maintenance in both skeletal and cardiac muscle. Sequencing Fhod3 in a large cohort of patients with diagnosed hypertrophic cardiomyopathy (HCM) revealed 13 mutations that are likely to be causative. Similar to INF2, most of the mutations are located outside of the FH1 and FH2 domains, but their functional consequences remain unknown. Patients live for many years with HCM and FSGS. Presumably misregulation of a formin is less detrimental than loss of function, otherwise we might expect to find more mutations in the FH2 domain.

A contrasting example comes from the *Drosophila* formin Capu, a maternal effect gene, meaning that it is essential for egg development and female fertility, but not viability. Of the many

alleles that have been sequenced, most are nonsense mutations and the seven missense mutations identified were all in the FH2 domain, compromising actin assembly to different degrees. In this case, overactive Capu may be tolerated because the formin is autoinhibited and not essential, but loss of activity is detrimental to egg development. The mammalian homolog of Capu, Fmn2, is essential in the developing egg, with its loss leading to infertility in mice and likely in humans as well.

## Formin functions outside of actin assembly

The length of this section should not belie its importance. Formins can have additional activities aside from actin assembly. Many, but not all, formins bind to the sides of actin filaments and most of these bundle filaments, creating larger assemblies. At least one formin, INF2, severs actin filaments in addition to building them. Interestingly, several formins, either alone or in conjunction with microtubule-binding proteins, stabilize microtubules. They may crosslink and align actin filaments with microtubules, making formins central to overall cytoskeletal organization in some cases. Finally, some formins have additional conserved domains, such as PDZ, pleckstrin homology (PH), and WH2 domains, which likely contribute to specificity of function by determining localization or additional binding proteins. This variety of capabilities is part of how formins contribute to a broad array of structures and functions.

## Remaining questions

There is more to learn. On the biochemical front, nucleation is still poorly understood. We have a firmer grasp on elongation but there is plenty to learn about the elements that control gating, processivity, and potential crosstalk between the FH1 and FH2 domains. For example, how does a formin remain bound to a continuously growing filament end? On the cell biological front, roles for many formins are yet to be determined. In some cases, this may be complicated by crosstalk and/or compensation. For example, researchers have long sought the mechanism of actin filament formation and regulation within the sarcomere. Many proteins have been identified, including at least three formins. With advances on these two fronts we will begin to understand the link between the specific activity of a formin and its function. Finally, many proteins are known to have strong effects on different elements of actin assembly, but clear common themes have yet to be identified: there are certainly more formin-interacting proteins to be found and studied. In conclusion, all nine metazoan subfamilies of formins share common activities in terms of nucleation and elongation, but are also distinguished by activity level, localization, and cell-type-specific expression, which are probably among many other aspects that remain to be discovered!

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Primer

# Intermediate filaments

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Cell morphology, architecture and dynamics primarily rely on intracellular cytoskeletal networks, which in metazoans are mainly composed of actin microfilaments, microtubules and intermediate filaments (IFs). The diameter size of 10 nm — intermediate between the diameters of actin microfilaments and microtubules — initially gave IFs their name. However, the structure, dynamics, mechanical properties and functions of IFs are not intermediate but set them apart from actin and microtubules. Because of their nucleotide-independent assembly, the lack of intrinsic polarity, their relative stability and their complex composition, IFs had long been overlooked by cell biologists. Now, the numerous human diseases identified to be associated with IF gene mutations and the accumulating evidence of IF functions in cell and tissue integrity explain the growing attention that is being given to the structural characteristics, dynamics and functions of these filaments. In this Primer, we highlight the growing evidence that has revealed a role for IFs as a key element of the cytoskeleton, providing versatile, tunable, cell-type-specific filamentous networks with unique cytoplasmic and nuclear functions.

## Elucidating IF physiological functions

IFs are generated by a wide variety of proteins encoded by 73 genes in humans. With the exception of nuclear lamins, which are found in all cells of multicellular organisms, the expression of IF proteins, such as keratins, desmin or vimentin, is cell-type specific and also varies during cell differentiation (Figure 1). IF-related diseases affect particular tissues or organs that mostly correspond to the sites of expression of the particular IF proteins. More than 90 pathologies, including the so-called laminopathies, keratinopathies and desminopathies, have been associated with mutations in IF proteins. Mutation of desmin, which is specifically expressed