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BACHELOR'S THESIS

NON-TRADITIONAL ROLES OF FORMINS BESIDES ACTIN NUCLEATION

Netradiční funkce forminů mimo nukleaci aktinu

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I hereby declare that the work presented here is, to my best knowledge and belief, original and the result of my own investigations and consultations with my tutor, except as acknowledged, and has not been submitted, either in part or whole, for a degree at this or any other University.

Formulations and ideas taken from other sources are cited as such. This work has not been published.

In Prague, 27 August 2012 Jáchym Metlička

LIST OF ABBREVIATIONS:

APC	adenomatous polyposis coli
Bni1p	bud neck involved protein
Bnr1p	Bni1p related protein
Capu	Cappucino
Cdc12p	cell division cycle 12 protein
DAAM	Disheveled-associated activator of morphogenesis
DAD	Diaphanous autoregulatory domain
Dia	Diaphanous
DID	Diaphanous inhibitory domain
DRF	Diaphanous related formins
EB1	end-binding protein 1
ER	endoplasmic reticulum
F-actin	filamentous actin
FH	formin homology
FHOD	formin homology domain-containing protein
FMN	formin
FMNL	formin - like protein
For3p	formin-3 protein
FRL	formin related protein
Fus1p	fusion-1 protein
G-actin	globular actin
GAP	GTPase-activating protein
GBD	GTPase binding domain
GEF	guanine nucleotide exchange factor
GFP	green fluorescent protein
INF1	inverted formin-1
INF2	inverted formin-2
KIND	kinase noncatalytic C-lobe domain
ld	limb deformity
MT	microtubule
MTBD	mictotubule binding domain
NETO	new end take off
ORF	open reading frame
SRE	serum response element
SRF	serum response factor
TMR	tetramethyl-rhodamine
WH2	Wiskott-Aldrich syndrome homology region 2
WASP	Wiskott-Aldrich syndrome protein
wt	wild-type

CONTENTS:

ABSTRACT

Formin homology 2 (FH2) domain-containing proteins (formins) have, since their discovery in 1990, been observed in all analyzed species of eukaryotic kingdoms. Our knowledge of structure and function of the defining FH2 domain has greatly increased over the last couple of years. Its function in nucleation, polymerization and processive capping of actin filaments designates formin protein family an important cytoskeleton-remodelling factor. But FH2 domain is just one part of the puzzle – additional optional conserved peptide structures surrounding it, as well as concrete variation of the FH2 domain itself, greatly influence the functional properties and cellular localization of the resultant formin protein. Formins have been implicated in variety of cellular processes, which often (but not always) involve the cytoskeleton – e.g. F-actin network management, crosstalk of F-actin filaments and microtubules or plasma membrane. They also partake in processes integral to cell division, function in conserved signalling pathways and much more. This thesis explains the structure and function of FH2 and FH1 domains, outlines the main formin phylogenetic clades in multicellular eukaryotes and reviews various roles that formins fulfill or are thought to fulfill. Such goal, however, is very bold and (considering the spatio-temporal constraints of this thesis) unattainable in the extent, which topic such as this needs.

Keywords:

formin, FH2 domain, actin, cross-talk, profilin, SH3 domain, polyproline helix, barbed end, cytoskeleton, GBD domain, auto-inhibition

ABSTRAKT

Od objevu v roce 1990 byly proteiny s Formin homology 2 (FH2) doménou (forminy) pozorovány ve všech analyzovaných druzích spadajících pod eukaryota. Znalost struktury a funkce FH2 domény se za posledních několik let výrazně zlepšila. Její schopnosti nukleace, polymerace a procesivního cappingu aktinových filament činí z proteinů forminové rodiny významné faktory ovlivňující podobu cytoskeletu. Ale FH2 doména tvoří pouze dílek skládanky – další volitelné konzervované peptidické struktury, které ji obklopují, stejně jako konkrétní podoba samotné FH2 domény, výrazně ovlivňují konečné vlastnosti forminu a jeho umístění v buňce. Forminy se podílejí na řadě buněčných aktivit, často (ale ne vždy) souvisejících s cytoskeletem. Spravují například aktinovou složku cytoskeletu, propojují aktinová vlákna s mikrotubuly či plazmatickou membránou. Dále se účastní buněčného dělení a fungují jako složky tradičních signalizačních drah atd. Tato práce popisuje strukturu a funkci FH2 a FH1 domén, poskytuje přehled fylogenetických větví forminů u mnohobuněčných eukaryot a shrnuje rozličné role, kterých se forminy v buňkách (pravděpodobně) účastní. Není to malý cíl a (vzhledem k časovým a prostorovým omezením této práce) je nemožné ho splnit v míře, jakou si toto téma žádá.

Klíčová slova:

formin, FH2 doména, aktin, cross-talk, profilin, SH3 doména, polyprolinová šroubovice, +konec, cytoskelet, GBD doména, autoinhibice

1. INTRODUCTION

Formins are a large and diverse family of proteins characterized by presence of a Formin homology 2 (FH2) domain. They are multi-domain polypeptides that readily form homodimers by interactions of the FH2 domain and as such then mediate actin filaments assembly and other functions related to cytoskeleton remodeling.

The name formin was first introduced in 1990 to describe a product of a gene, whose mutation or loss was thought to disrupt the proper course of murine embryonic development. The mice exhibited incorrect limb bone anatomy – limb deformity (*ld*) phenotype[1]. The evidence suggesting the *formin* gene as the culprit was that in 2 of 4 independently isolated mutant alleles responsible for *ld* phenotype, mRNA transcripts of regions coding for formin were absent (Woychik et al., 1990). Later research into the cause of *ld* phenotype revealed that expression of *formin* is not directly responsible. Two new mutant alleles resulting in limb deformity were discovered, but the *formin* gene was intact and its mRNA could be detected. In wild-type (wt) mice, there is another gene called *gremlin* located ~40kb downstream from *formin*. Deletion of the entire *gremlin* ORF and substitution mutation in *gremlin* gene (leading to incorrect pre-mRNA splicing) were found as a cause for the mutant phenotype in these 2 cases. Experiments were conducted to further explain the role of *formin* gene – deletion of a single exon from *formin* sequence (which introduced framing error) did not induce the *ld* phenotype. However, when larger region of *formin* gene was deleted, the abnormalities were observed. In both experiments, *formin* expression was disrupted, but only in the second case had this any influence on phenotype. It was discovered that part of the genetic sequence of *formin* exerts cis-effect on *gremlin* expression. When mutated or deleted, this results in non-expression of *gremlin* and subsequent *ld* phenotype (Zuniga et al., 2004).

Even though formins are no longer thought of as linked to the mice *ld* phenotype, the name is quite fitting, considering the wide range of actin structures that this protein family helps to assemble and maintain.

[1] Reduction and fusion of the distal bones and digits of all limbs and abnormalities in kidney

2. CONSERVED FORMIN DOMAINS

2.1. FORMIN HOMOLOGY 2 DOMAIN

Formin homology (FH2) domain is the main feature common to all formin proteins. It has the ability to nucleate and progressively elongate actin filaments from their barbed ends. The domain is very ancient – no FH2 homologue has been detected in prokaryotic species, but proteins containing well-conserved FH2 domain have been observed in all sampled species across all eukaryotic kingdoms. This suggests that FH2 domain was with great probability already present in the "toolbox" of the last common ancestor of all eukaryotes (Rivero and Cvrčková, 2007).

2.1.1. STRUCTURE OF FH2 DOMAIN

In vitro, full-length FH2 domain readily forms dimers. FH2 monomer is ~400 – 500 amino acid residues long (Paul and Pollard, 2008) and is mostly of α-helical character. It is usually located near the C-terminus of formin polypeptide chain. It is quite well conserved across species and comprises 5 different sub-domains (listed here in order from N- to C-terminus): *lasso*, *flexible linker*, *globular knob*, *coiled-coil* and *post* (Fig. 1a).

Knob together with *post* and *coiled coil* form an elongated part of the FH2 domain. N-terminal to *knob* lies *linker* of variable length that connects with *lasso*. *Lasso* sub-domain tethers to its respective partner (*post*) on the second FH2 subunit and vice versa (Fig. 1b) (Lu et al., 2007; Xu et al., 2004).

Interactions between *Lasso* and *Post* of two formin subunits are crucial for successful assembly of formin dimer – the *lasso* region of one subunit winds around the *post* sub-domain on the other subunit. Studies of crystal structures of yeast Bni1 FH2 domain show that first 28 residues of *lasso* do not form a traditional secondary structure but they contain 2 specifically localized tryptophan residues. Those residues insert into hydrophobic pockets located on the *post* of the other subunit of the formin dimer (Xu et al., 2004). The tryptophan residues located on *lasso* and glycine residues flanking pockets of *post* are relatively well conserved in FH2 domains – similar method of interface stabilization was observed in human Daam1 FH2 domain (Lu et al., 2007) and 7 out of 10 known *Dictyostelium* formins also contain stabilizing Trp residues. The other 3 formins substitute Trp with phenylalanine, which has similar stabilizing properties (Rivero et al., 2005).

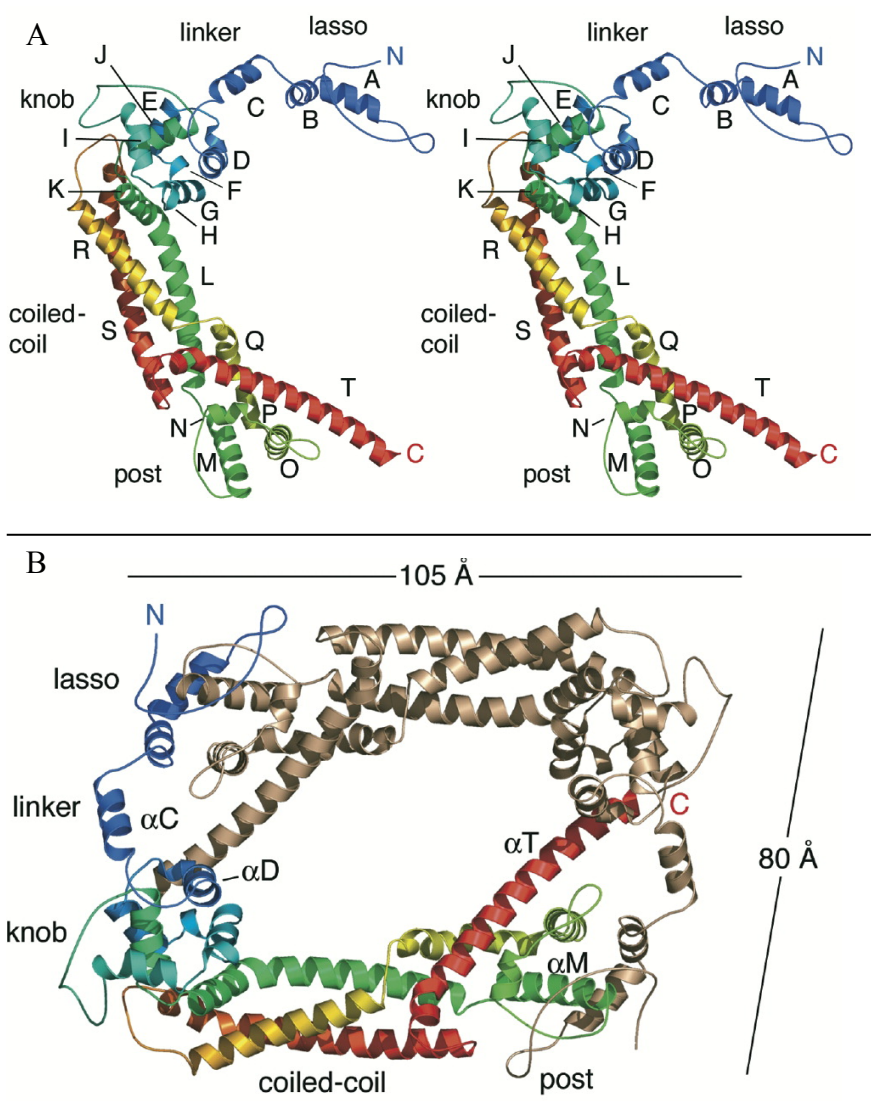

Figure 1

(A) FH2 domain subunit of Bni1p formin of budding yeast

'Knob', 'coiled-coil' and 'post' sub-domains form a rod-like structure. 'Linker' provides flexible connection to 'lasso', which in vitro attaches to 'post' subdomain on the other subunit.

(B) FH2 dimer - unstructured part of 'lasso' subdomain is 'post' and FH2 subdomains form a parallellogram-shaped dimer. Resulting structure is quite rigid except for the 'linker' region, which provides the flexibility necessary for the nucleation and elongation of actin.

modified from (Xu et al., 2004)

2.1.2. DOMAIN VARIABILITY

All FH2 domains share the same sub-domain arrangement, but structure of the sub-domains has been shown to differ to a certain degree between formins - e.g. number of α-helices that compose individual subdomains varies among different clades. When comparing structure of FH2 in human formin Daam1 with FH2 in yeast Bni1p, arrangement in *lasso-post* interaction is quite similar in a general sense. The difference is αA and αB helices, which are both 1 turn shorter than the corresponding helices in Bni1p. Higher lever of variability is present in the *globular knob* - Bni1p *knob* contains 8 helices (αA, - αH), whereas Daam1 *knob* consists of only 6 (αA - αE, αG). αD and αG helices of Daam1 are 1 turn longer than in Bni1p and a *knob loop* connecting helices αD and αG is a lot shorter. In Bni1p, the *knob loop* is in contact with *coiled-coil* sub-domain and thus limits overall flexibility of the FH2 structure and influences the respective positions of the sub-domains. The lack of contact between knob loop

and coiled-coil in Daam1 suggests higher level of flexibility between domains (Lu et al., 2007). Variability in the domain explains different elongation rates and activation conditions among formins.

2.1.3 FUNCTION OF FH2 DOMAIN

Main function of the FH2 domain is nucleation of actin monomers and subsequent elongation of an actin filament from its barbed end. FH2 domain prevents barbed-end capping proteins from binding the filaments and therefore makes creation of longer linear actin filaments possible (Rivero et al., 2005). However, some formins such as e.g. the fission yeast Cdc12p can act also as microfilament-capping proteins without apparent nucleation activity (Kovar et al., 2003)

FH2 dimer has in total 4 actin binding patches – 2 on each subunit. One is located on *post* sub-domain and is in-fact composed of residues from both *post* and *lasso*. The other one is on *knob* sub-domain. Amino acid residues involved in actin binding are generally well conserved and their deletion or substitution leads to loss of function (Lu et al., 2007; Xu et al., 2004).

2.2. FORMIN HOMOLOGY 1 DOMAIN

FH1 domain is present in almost all known formins. It is located just N-terminal to FH2 (reviewed in Higgs, 2005). Length of the domain varies significantly among formin subfamilies. Polypeptide chains 10 to 500 residues long have been observed (Rivero et al., 2005).

The only unifying feature among FH1 domains is a high content of proline residues. Relative abundance varies between 35 and 100 percent. The proline residues are not spread equally in the domain, instead they form a variable number of separate polyproline strips. These strips of 5 or more residues form stiff type-II polyproline helices that are known as capable binders of a variety of proteins (Paul and Pollard, 2008). Profilin, WW domain-containing proteins or SH3 domain-equiped proteins are some of the potential binding partners. Of these, profilin deserves special attention, since it is a small[2] actin-sequestering protein that readily forms actin/profilin complex with monomeric G-actin. Thanks to its very high concentration in eukaryotic cells, profilin is responsible for binding a large percentage of otherwise free actin units (reviewed in Aspenström, 2010). Profilin binds actin in a way that prevents its

[2] Molecular mass ~14–16 kDa

spontaneous nucleation and polymerization onto a pointed end of actin filament. Ability of monomers to bind onto barbed end is only very slightly affected. All this together neccesitates existence of factors that can facilitate actin monomer nucleation and filament elongation even in presence of high concentration of profilin – Those factors are of course FH1 and FH2 domains (reviewed in Higgs, 2005).

2.2.1. STRUCTURE OF FH1 DOMAIN

Excluding the polyproline helices, the rest of FH1 domains is not considerably preserved in any way and is expected to be disordered (Paul and Pollard, 2008).

2.2.2. FH1 AND ITS ROLE IN ACTIN RECRUITMENT

Ability to effectively nucleate actin polymerization into filaments depends on close co-operation of both FH2 and FH1 domains. In vitro studies showed that under some circumstances the C-terminal FH2 domain may be by itself necessary and sufficient for promoting actin nucleation and filament elongation (Pruyne et al., 2002). Unlike other protein families responsible for nucleation of actin (in particular the Arp2/3 complex), formins do not allow uncontrolled growth on free barbed end. Instead, they remain attached to the barbed end of growing filament – this is known as processive association (Paul and Pollard, 2008). This formin-specific feature prevents capping proteins such as capZ homologues or gelsolin (reviewed in Zigmond, 2004) from binding the barbed end of the filament, therefore ensuring the possibility of further actin monomer addition even in presence of such proteins (Otomo et al., 2005).

FH1 domain serves as a recruitor of profilin-actin complexes. Its polyproline strips bind profilin-acting heterodimers and thus increase local actin concentration. Since it is located in tandem with FH2 domain, this change in concentration dramatically increases rate of actin incorporation. Rate of nucleation also depends on number of the polyproline strips – the more the higher rate of elongation (Paul and Pollard, 2009).

3. NUCLEATION AND ELONGATION OF ACTIN FILAMENTS

Nucleation of actin is a process that occurs spontaneously *in vitro* and doesn't necessarily require any additional asssembly machinery. It describes binding of the first 3 actin monomers into a very short bundle, which is the minimum needed for further barbed end elongation. *In vivo,* however, spontaneous nucleation of G-actin is inhibited, because the actin

monomers are sequestered by association with actin-binding proteins, such as profilin (Dominguez, 2009). These prevent the monomers from binding to each other and without specialized molecular machinery, nucleation and subsequent elongation of actin filaments is not thermodynamically favored (Breitsprecher et al., 2011). FH2 domain offers a solution to this problem. Crystalography study of Bni1p FH2 domain interacting with tetramethyl-rhodamine-labeled actin (TMR actin) showed that the actin filament sits inside the hole of the parallellogram-shaped FH2 dimer, which associates with 3 actin monomers - upper actin binds the *knob* of one subunit, middle actin binds *post+lasso* of the same subunit and the *knob* of the half of the dimer. Third actin monomer binds to the *post* of the second FH2 subunit. The spatial relation of actin monomers in the crystal closely resembles arrangement of subunits in actin filament. Two neighboring actin monomers bound to FH2 domain at the end of filament are related by 180° rotation and translated by ~28Å. In Holmes model of F-actin, these values slightly differ - rotation is only 166° and translation 27.5Å (Otomo et al., 2005). The spatial relation in which actin monomers are bound to the bridge of FH2 prevents more additions to the barbed end, effectively capping it (Fig. 2). The FH2 domain has to move to remove the obstruction and to provide additional actin binding spot. For incorporation of additional actin monomer, dissociation of only one actin binding region of FH2 dimer is necessary - this setup makes it possible for the dimer to move along the barbed end during elongation. Model of actin nucleation and filament elongation derived from structure of Bni1p FH2+TMR-actin data is called nucleating ratchet model.

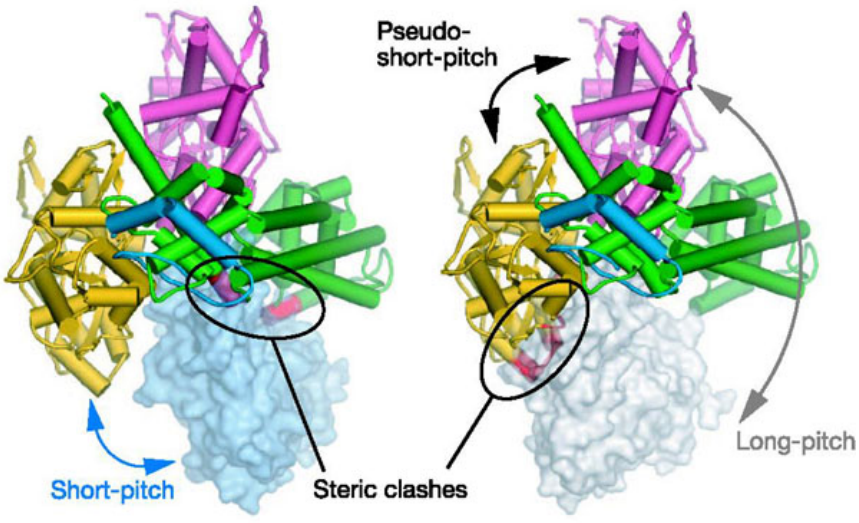

Figure 2 *- Bridge prevents addition of actin and acts as capping protein.*

2 actin monomers (yellow and pink) are shown bound to the bridge of FH2 domain (green and blue). Third actin monomer is vizualized in a surface rendition mode attached to the barbed end. Steric clashes are shown in red and demonstrate that addition of the monomer is impossible.

Modified from (Otomo et al., 2005)

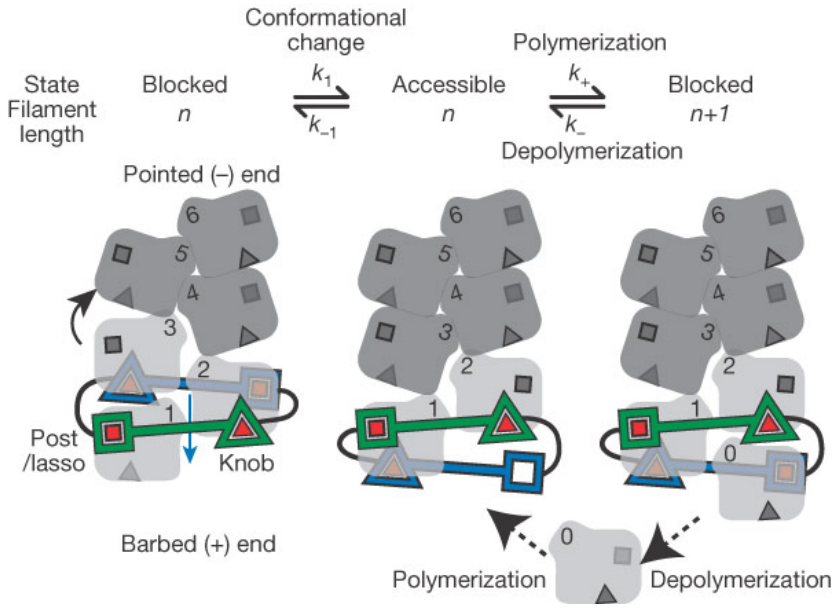

Figure 3 *- Conformational change of FH2 allows for filament elongation*

Actin binding sites on FH2 dimer and their respective partners on actin are shown: knob (▲-bound, △-unbound) and post/lasso (■-bound, □-unbound). In blocked state, actin cannot be added. In accesible state, the blue bridge moves towards the +end and exposes one of its binding sites. After addition all 4 binding sites are once again used and the complex is blocked.

Modified from (Otomo et al., 2005)

3.1. NUCLEATING RATCHET MODEL

The model of nucleating ratchet describes FH2 dimer associated with actin monomers as a system, which is in rapid equilibrium between blocked and accessible conformation and switches as a brownian ratchet. In blocked conformation, FH2 dimer binds 3 actins with all 4 binding sites. Position of the dimer bridges sterically prevents addition of monomers to the barbed end. During conformational change, one FH2 domain frees its two binding sites and moves toward the barbed end. Here, the *knob* site reattaches to terminal actin monomer and FH2 dimer binds 2 actins, leaving one *post/lasso* site unbound. The complex is now in accessible conformation and additional actin subunit can access and bind near the barbed end. By binding onto the *post/lasso* site, the complex once again becomes blocked for another addition until FH2 bridge moves and so on (Otomo et al., 2005). The equilibrium between blocked and accessible state $(K_{o/c})$[3] attains very diverse values for various FH2 domains (reviewed in Kovar, 2006). Actins behind the ratchet acquire ideal spatial conformation and eventually form the filament (Fig. 3). Model of nucleating brownian ratchet predicts that depending on concentration of free actin subunits in cytoplasm, the FH2 domain can either processively elongate the filament or trail at its end disassembling it into separate actins. The higher the concentration of actin, the less favorable the dissociation of actin monomer and vice versa (Otomo et al., 2005). When taking into account that *in vivo* most G-actin subunits are actually sequestered by profilin or other actin-binding proteins (therefore lowering concentration of free G-actin dramatically), the importance of neighboring FH1 domain

[3] open/closed equilibrium, open = accessible, closed = blocked

becomes clear. (Otomo et al., 2005). Comparative study showed that a positive correlation exists between number of polyproline strips on FH1 domain and speed of filament elongation. Explanation for this is that FH1 binds actin-profilin units directly from cytoplasm and delivers them straight to the barbed end of the filament. After the subunit attaches to the barbed end, both FH1 and profilin disengage and are ready to bind more actin units. This theory is supported by structure of FH1. It contains longer unspecified regions between proline strips, which are flexible enough to reach the barbed end of filament (Paul and Pollard, 2008). Presence of profilin dramatically changes level of activity of FH1FH2 domains. E.g. fission yeast Cdc12p FH1FH2 without profilin essentially functions as a capping protein (~0,0 μM^{-1} sec^{-1}), however in presence of profilin, it becomes an active elongation factor (~12,5 μM^{-1} sec^{-1}). Even though our knowledge of the functional mechanism of FH2 domain has greatly increased in the past couple of years, there are still issues to resolve. Under the current model, helical character of actin filament would be expected to cause rapid spinning of polymerizing FH2 domain or undergo supercoiling itself. None of this has been observed, so nucleating apparatus deals with this in some way - an explanation has been proposed (Shemesh et al., 2005).

4. FORMIN FAMILIES:

Formin proteins across eukaryotic systems have variable architecture and function. As first homologs to the original formin were discovered, FH1 and FH2 common sequence domains were defined. Studies based on phylogenetic analysis of domains and their arrangement in polypeptide chain now recognize several separate formin families with unique conserved domain arrangements (Chalkia et al., 2008; Higgs and Peterson, 2005).

4.1. METAZOAN FORMIN FAMILIES

4.1.1. FMN (FORMIN) - is a group containing FMN1 and FMN2 proteins. They are the largest known formins at sizes of 158 and 180 kDa respectively. FMN1 was first recognized in 1990 as a product of *limb deformity* gene (Woychik et al., 1990). Loss-of-function study in murine fibroblasts showed involvement of FMN1 in binding of microtubules during interphase (Zhou et al., 2006) and role in cell-spreading and formation of focal adhesion (Dettenhofer et al., 2008). FMN2 is involved in positioning of metaphase 1 spindle in murine oocytes and lack of expression leads to improper chromosome separation, polyploidy and subsequent infertility (Leader et al., 2002). FH1 and FH2 domains occupy about one third of

the sequence and are located near the C-terminus. A short Spir protein binding sequence was discovered between FH2 and C-terminus. Spir is an actin-nucleating factor containing 4 WH2 motifs as the actin binding regions (Pechlivanis et al., 2009a).

4.1.2. DIA (DIAPHANOUS) - Dia1 was first detected in 1994 in *Drosophila* as a product of a gene *diaphanous*. Proteins in this formin family contain C-terminal *Diaphanous autoregulatory domain* (DAD) that binds to a *Diaphanous inhibitory domain* (DID) - a region C-terminal to *GTPase binding domain* (GBD), located near N-terminus, rendering the formin inactive. The protein requires competetive binding of GTPase RhoA to GBD to undergo a change in conformation and become an active nucleator of actin (Alberts, 2001). Small GTPases of the Rho clade are known cytoskeletal regulators (see below chapter small GTPases) Dia1 is involved in formation of stress fibers and regulation of cell morphology and invasion (Alberts, 2002). Mutation in human Dia2 was linked to premature ovarian failure (Bione et al., 1998).

4.1.3. FMNL / FRL (FORMIN-LIKE / FORMIN-RELATED) - Original member of this family - FRL (now FMNL1) was first described in 2000 as FH1, FH2 domains-containing protein highly expressed in lymphatic tissue of mice (Yayoshi-Yamamoto et al., 2000). Its structure contains both DID and DAD domains - classifying it as a member of Diaphanous-related formins (DRF) group. FMNL is autoinhibited unless associated with GTPase Cdc42. A study found co-localization of FMNL1 and actin-rich cores of primary macrophage podosomes (Mersich et al., 2010). Overexpression of FMNL1 was observed in patiens with several kinds of lymphoid cancer (DeWard et al., 2010). FMNL2 is ubiquitous in human tissues and its expression was elevated in in colorectal metastatic cancer cells compared to non-metastatic, suggesting its involvement in metastasis (Zhu et al., 2008). FMNL3 stimulates filopofia assembly and is present in large quantities in filopodia tips (Harris et al., 2010).

4.1.4. DAAM (DISHEVELED-ASSOCIATED ACTIVATOR OF MORPHOGENESIS) formins are related to Diaphanous. Daam1 was first discovered in *Xenopus* as binding partner to Disheveled, a protein involved in a Wnt signalling pathway (Habas et al., 2001a). Daam formins contain both DID and DAD domains and like in other formins, interactions between the two regions inhibit the protein from nucleating actin. RhoGTPase binding does not suffice in relieving the autoinhibited state - Wnt-stimulated interaction of Disheveled to DAD relieves the autoinhibition in Daam1 (Li et al., 2011). Daam1 was implicated in RhoA GTPase activation. Functional studies showed that Daam1 plays a role in trachea formation in

Drosophila (Matusek et al., 2006) gastrulation in *Xenopus* (Habas et al., 2001a) and notochord development in Zebrafish (Kida et al., 2007). Daam1-deficient mice exhibit multiple serious cardiac defects leading to embryonic and neonatal lethality (Li et al., 2011). Daam1 and Daam2 are structurally very similar to each other and thus probably serve similar roles.

4.1.5. DELPHILIN is a protein with a very specific expression localization. It was discovered as interactor of GluRδ2[4] in cerebellar Purkynje cells. The interaction is facilitated by a PDZ domain[5] and C-terminus of GluRδ2 (Miyagi et al., 2002). There are two isoforms of Delphilin - original S-Delphilin is shorter and palmitoylated at the N-terminal, and has 1 PDZ domain involved in binding of GluRδ2. Is is expressed mainly in dendritic spines of neurons in hippocampus. The alternative splicing isoform L-Delphilin is longer, contains one extra PDZ domain, lacks the N-terminal palmitoylation and can be found in soma and dendritic shafts (Matsuda et al., 2006).

4.1.6. FHOD (FORMIN HOMOLOGY DOMAIN-CONTAINING PROTEIN) is another family belonging to the DRF group. FHOD1 is highly expressed in mammalian spleen and binds to RhoGTPase called Rac1. Binding of Rac1 is not sufficient for relieving autoinhibitory conformation of the formin but turns on transcription from *serum response element* (SRE) (Westendorf, 2001). Association with Rho effector kinase ROCK1 leads to phosphorylation at 3 sites on DAD domain, which disrupts the autoinhibition. FHOD1 plays a role in formation of actin stress fibers and promotes Src-dependent plasma membrane blebbing (Hannemann et al., 2008).

4.1.7. INF1 (INVERTED FORMIN-1) has a very unique structure among formins - FH1 and FH2 domains are located near N-terminus. INF1 is implicated in microtubule organization enabled by presence of special microtubule binding domains (MTBD) in C-end region. INF1 expression in mouse fibroblasts leads to formation of actin stress fibers, alignment of microtubules and actin filaments, tubulin acetylation and microtubule bundling. INF1 ablation leads to decrease in microtubule acetylation (Young et al., 2008).

[4] Glutamate receptor, ionotropic, *δ*2

[5] PDZ is acronym of 3 proteins first discovered to share the domain — post synaptic density protein (PSD95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (zo-1)

4.1.8. INF2 (INVERTED FORMIN-2) belongs to the DRF group. The name is actually a historical misnomer caused by misaligned start of the open reading frame, which caused omission of both FH3 and FH1 domains from the predicted protein product (reviewed in Schönichen and Geyer, 2010). Full-length INF2 has traditional formin domain arrangement. Unlike other Diaphanous related formins, INF2 substitutes the DAD domain with WH2 domain, which seems to serve the same autoinhibitory role by binding to region within FH3 domain. INF2 has unique ability to accelerate both polymerization and depolymerization of actin filament. Cooperation of actin-monomer-binding WH2 domain and filament-severing ability of C-terminus is necessary for depolymerization (Chhabra and Higgs, 2006). Autoinhibited INF2 cannot depolymerize actin filaments but retains the nucleating ability (Chhabra et al., 2009). RhoGTPase Cdc42 relieves the autoinhibited state (Boyer et al., 2011). Posttranslational C-terminal farnesylation coupled with ionic interactions associate INF2 with cytosolic side of endoplasmatic reticulum (ER) (Chhabra et al., 2009). INF2 is strongly expressed in Schwann-cell cytoplasm and podocytes (Boyer et al., 2011). In human T-lymphocytes INF2 regulates MAL-mediated transport of lymphocyte-specific protein tyrosine kinase (Lck) to the plasma membrane (Andres-Delgado et al., 2010).

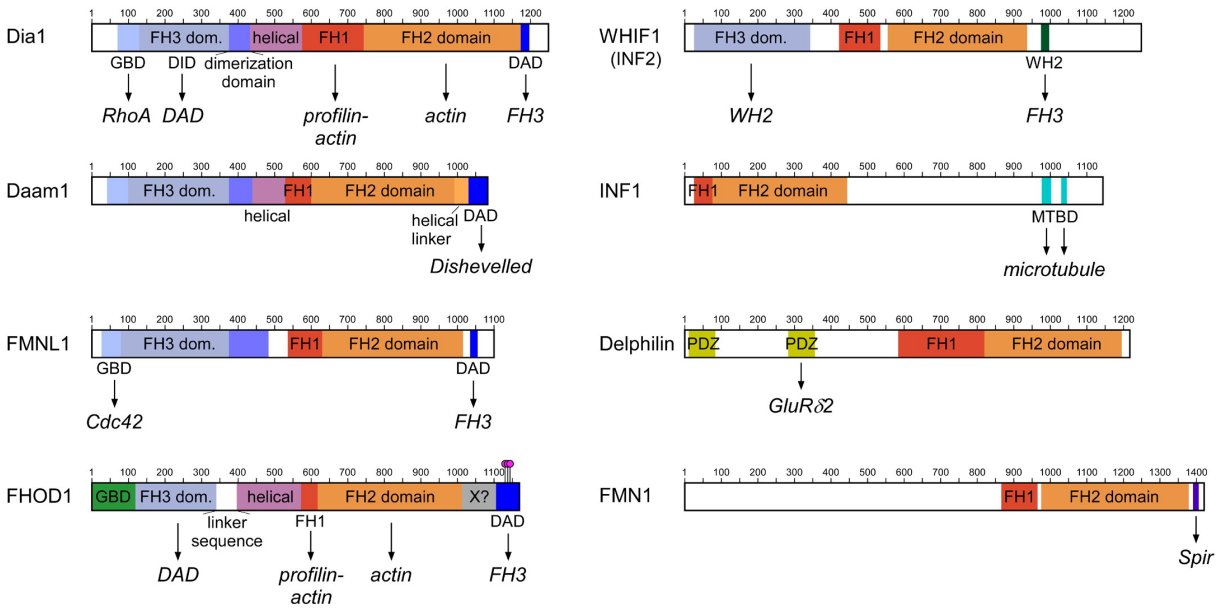

Figure 4 *- Representatives of seven metazoan formin classes with their specific domain arrangements.* Modified from (Schönichen, 2010)

4.2. YEAST FORMIN FAMILIES

Budding yeasts contain 2 different FH2 domain-containing proteins, Bni1p and Bnr1p.

4.2.1. BNI1P (BUD NECK INVOLVED PROTEIN) is classified as a member of the Diaphanous-related formins group - and it is autoinhibited unless activated by one of various RhoGTPases (Kohno et al., 1996). Unlike mammalian DRFs, it seems that Bni1p can also be activated differently - phosphorylation of 3 specific threonines (located within GBD, FH1 and the very C-terminus) by actin regulatory kinases Prk1p and (to a lesser effect) Ark1p seems to be enough to relieve the autoinhibitory conformation (Wang et al., 2009). GFP-linked Bni1p was found to form small speckles in the cytoplasm, with fraction of them being associated with actin cables (Buttery et al., 2007). Bni1p takes part in nucleation and elongation of actin cables that start at the neck and line the bud cortex and serve as tracks for delivery of secretory vesicles during growth (Pruyne et al., 2004).

4.2.2. BNR1P (BNI1 RELATED PROTEIN) has domain arrangement very similar to that of Bni1p and as such also belongs to DRF. During cytokinesis, Bnr1p is expressed in the mother cell and elongates actin cables that stretch from the neck into the mother cell, therefore establishing polarized growth (Pruyne et al., 2004).

Fission yeasts possess 3 formins, each with different properties and specific roles.

4.2.3. CDC12P (CELL DIVISION CYCLE 12 PROTEIN) is a non-DRF protein acting in cytokinesis. It is necessary for assembly of the actin ring circumscribing the cell. Special characteristic of this formin is its de-facto capping activity in absence of profilin. Cdc12p nucleates a filament, but totally blocks monomer addition on the barbed end, leaving only the pointed end free for elongation. Addition of profilin disables the capping activity and allows barbed end elongation (Kovar et al., 2003).

4.2.4. FUS1P (CELL FUSION PROTEIN) is structurally similar to Cdc12p. Role of Fus1p in fission yeast is in conjugation and it localizes to projection tips of mating cells (Nelson et al., 2004). Mutant phenotype cannot succesfully degrade cell walls between mating partners, therefore preventing conjugation (Frazier and Field, 1997).

4.2.5. FOR3P (FORMIN-3 PROTEIN) has a role in polar growth and is required for formation of actin cables during interphase. It was detected associating with cortex at poles of the cell

and extending actin cables along the axis between the poles (Martin and Chang, 2006). The cables function as tracks for myosin-facilitated transport of cargo (Pruyne et al., 1998).

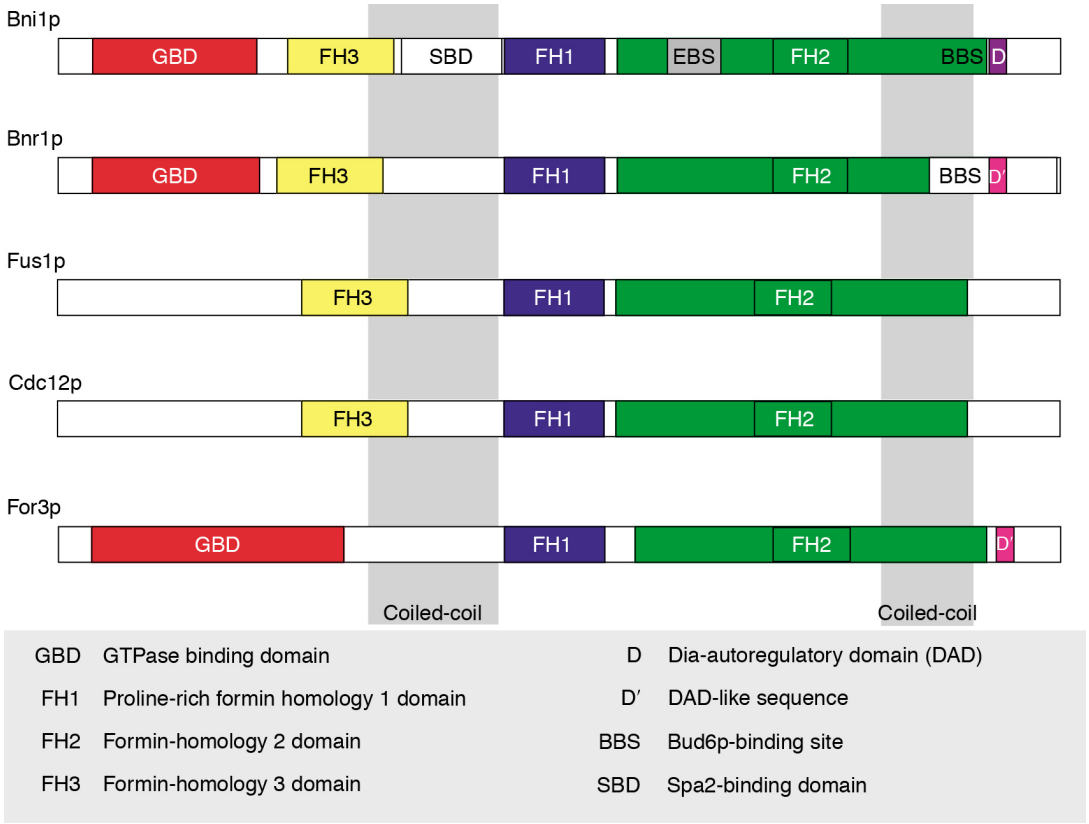

Figure 5 *- Domain arrangement of yeast formins*

Modified from (Wallar and Alberts, 2003)

4.3. PLANT FORMIN FAMILIES

4.3.1. CLASS I formins contain both FH2 and FH1 domain. Specific membrane secretion targeting sequence near N-terminal and amphipathic transmembrane helix were predicted and in some cases confirmed to bind class I formins into membranes (Banno and Chua, 2000; Cheung and Wu, 2004; Cheung et al., 2010; Cvrčková, 2000; Deeks et al., 2005; Favery et al., 2004; Martiniere et al., 2011). In *Arabidopsis thaliana* 11 different isoforms exist (reviewed in Blanchoin and Staiger, 2010).

4.3.2. CLASS II formins do not possess the transmembrane domain and are of cytosolic character. Aside from standard FH1FH2 combination, majority of them share a mammalian-related PTEN domain, which is most likely non-functional from the enzymatic

standpoint[6] (Cvrčková et al., 2004) but seems to be important for recruiting Class II formins to cell cortex by binding PI(3,5)P_2. In moss, Class II formins were implicated in sites of membrane remodeling (van Gisbergen et al., 2012).

4.3.3. CLASS III formins are specific for their N-terminal located RhoGAP-homologous domain. They were detected only in non-seed plants (see chapter small GTPases) (Grunt et al., 2008).

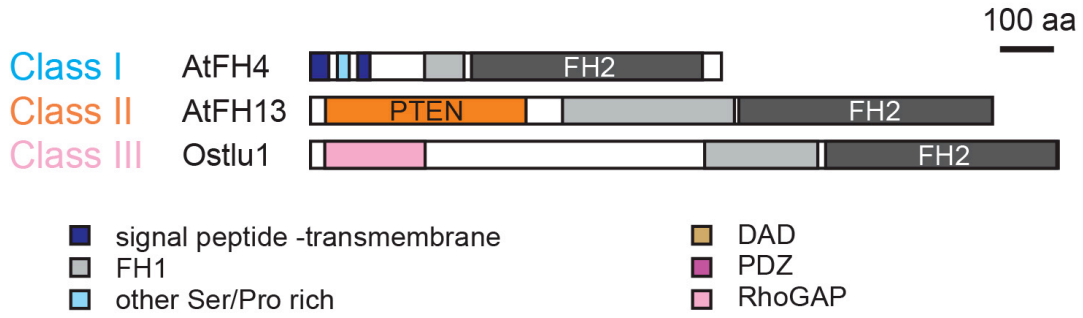

Figure 6 *- Representatives of plant formins with their specific domain arrangements.*
Modified from (Grunt et al., 2008)

5. FORMIN INTERACTORS AND ACTIVITIES BY TYPE

5.1. ACTIN

Outside of general abilities of formins, which are 1) nucleation of actin; 2) elongation of filaments; 3) blocking the barbed ends from (other) capping proteins, certain formins were discovered to interact with actin in non-traditional manner. A subset of those is described below.

5.1.1. DEPOLYMERIZING AND SEVERING

INF2 is a mammalian diaphanous related protein. In addition to standard formin functions it has an distinctive ability of depolymerizing existing actin cables. This is dependent on presence of unique C terminal domain, containing WASP homology 2 motif (WH2). The domain can sequester actin monomers from cytoplasm in 1:1 ratio and is also necessary for

[6] phosphatase and tensin homology domain - In humans, it is a lipid phosphatase with anti-oncologic properties. By converting PI(3,4,5)P_3 to PI(4,5)P_2, it lowers amounts of the former, which through couple of intermediary steps slows down cell growth and division.

filament severing. During actin filament elongation, ATP-bound G-actin is recruited and after binding into the filament, the ATP-F-actin eventually hydrolyzes into ADP-F-actin and a phosphate. By unknown mechanism, release of the phosphate unit enables the depolymerization and severing facilitated by WH2 - this system of activation logically preferably depolymerizes older filaments, since they contain higher percentage of ADP-actin. Depolymerization and severing activity seems to be greatly reduced by addition of profilin (Chhabra et al., 2009).

5.1.2. BUNDLING OF FILAMENTS

FRL1 is a mammalian formin capable of binding sides of actin filaments by interaction with FH2 domain. *In vitro* experiments demonstrated that its FH2 by itself not only binds, but also bundles the filaments - probably thanks to its dimeric structure. Bundling is competitive with binding of barbed ends. *mDia2* is another formin that also bundles F-actin with its FH2 domain, but in non-competitive manner in relation to barbed end association. Both formins assemble filament bundles of mixed orientation. Ionic interactions seem to be important for the binding effect. (Harris et al., 2006). Recent study showed that fragments of FH2 of mDia1-3 lacking the linker and lasso domains were able to induce F-actin bundling (Machaidze et al., 2010). In *Arabidopsis thaliana*, AtFH1 overexpression in pollen tubes resulted, among other things, in formation of actin cables. *In vitro* experiments confirmed the bundling activity. Comparison of multiple AtFH1 fragments points at involvement of FH1 domain (Michelot et al., 2005). *In vivo*, the occurrence of bundling depended on additional factors - AtFH1 was found to contain specific extracellular-residing domain located N-terminal from the amphiphatic transmembrane helix. It contains SPPPP motif - homologous sequence can be found in cell-wall associated proteins called expansins. Experiments with GFP-marked construct suggest its role as cell wall anchor preventing lateral movement of AtFH1. Anchoring in the cell wall is necessary for actin bundling and provides a stable stationary point for binding of actin filaments (Martinière et al., 2011). AtFH3 was observed to cause appearance of actin cables in pollen tubes. Unlike short bundles formed by AtFH1, AtFH3 induces longer cables. (Ye et al., 2009). AtFH8 FH1FH2 was also recognized as potent bundler and nucleator (Xue et al., 2011).

5.1.3. ASSOCIATION WITH OTHER ACTIN NUCLEATORS

Cooperation between formins and other actin nucleators has been discovered. Cappucino formin in *Drosophilla* was shown to bind to *kinase noncatalytic C-lobe domain* (KIND) of

Spire. Actin nucleation activity of Spire was enhanced in the complex, but formin-mediated nucleation was strongly inhibited. (Quinlan et al., 2007). Existence of similar complex was observed in mammals between Spir1 and 2 and FMNs, but the association was between KIND domain and a special conserved Spir binding sequence located near C-terminus of FMNs (Pechlivanis et al., 2009b).

Arp2/3, actin-nucleator with the ability to branch from existing actin filaments can be linked to formins by means of several adaptory proteins. IQGAP1 is one such example - it was demonstrated as a viable link between mDia1 and Arp2/3 complex (Brandt et al., 2007; Brandt and Grosse, 2007). Dia-interacting proteins such as SPIN90 are also thought to be able to closely co-ordinate FH2-Arp2/3 activities (reviewed in Aspenstrom, 2010).

5.2. MICROTUBULES

Formins have been recognized as important modulators of structure and dynamics of microtubule cytoskeleton.

5.2.1. BINDING AND BUNDLING OF MICROTUBULES

Elaborate studies undertaken on NIH/3T3 fibroblasts of mice show FMN1 binding microtubules during interphase (Zhou et al., 2006). FMN2 associates with MTs during cell division in oocytes (Leader et al., 2002).

At high concentration, FH1FH2mDia2 was observed to form small bundles composed of overlapping microtubules of variable lengths (Bartolini et al., 2008; Gaillard et al., 2011). Same concentrations of FH1FH2mDia1 showed no bundling activity (Gaillard et al., 2011). INF1 formin has two C-terminal motifs capable of binding microtubules both *in vitro* and *in vivo*. Overexpression of C-terminal fragment leads to microtubule bundling and full INF1 protein aligns along MT bundles in cells (Young et al., 2008). INF2 formin was also found to associate microtubules into bundles. *In vitro* study comparing FH1FH2INF2 with and without the C-terminal motifs showed significant bundling in the former and no bundle formations in the latter. Bundles formed by INF2 fragment were composed of microtubules of antiparallel or random orientation. Presence of actin monomers strongly inhibited the bundling activity of INF2 (Gaillard et al., 2011).

5.2.2. MICROTUBULE DYNAMICS

Microtubules both *in vitro* and *in vivo* are characterized by behaviour termed dynamic instability. They alternate between phases of slow tubulin addition (MT growth) and rapid removal (MT shrinkage). Transitions between these two phases are known as *rescue* (shrinkage → growth) and *catastrophe* (growth → shrinkage). Formins were found to affect the relative ratio of these two activities.

Constitutively active FH1FH2 fragment of mDia2 was found to be sufficient for binding microtubules *in vitro*. MTs associated with the FH1FH2mDia2 are protected against cold- and dilution- induced disassembly. FH1FH2mDia2 was observed to slow down both MT assembly and disassembly rates by 30% and 50% respectively. Stoichiometric ratio of mDia2:tubulin in complex was measured as 1:4.7. This suggests that the stabilizing effect of mDia2 is caused by binding multiple tubulin subunits along the MT lattice. The localization of the protein on MTs was confirmed *in vitro.* (Bartolini et al., 2008). INF1 was observed *in vivo* to stabilize MT network against nocodazole[7] in concentrations as high as 10 μM (Young et al., 2008).

5.2.3. MICROTUBULE-ACTIN CROSS-TALK

Formins are implicated in microtubule-actin cross-talk in both yeast and higher eukaryotes. In yeast, no evidence exists for direct contact between formin and MTs, but they nevertheless play role in MTs-actin interaction.

Budding yeast formins Bnr1 and Bni1 establish actin cables, necessary for polarized growth. Type V myosins Myo2 and Myo4 use these filaments for transporting cargo towards the bud. Attachment of MTs to the myosins through BIM1[8] and KAR9 proteins allows for control of MT position, which subsequently helps to establish proper orientation of nucleus and spindle along the mother-bud axis (Fig. 7) (Pruyne et al., 2004).

[7] Nocodazole is an anti-neoplastic agent interfering with tubulin assembly into MTs.
[8] BIM1 is a yeast homolog of mammalian microtubule associating EB1.

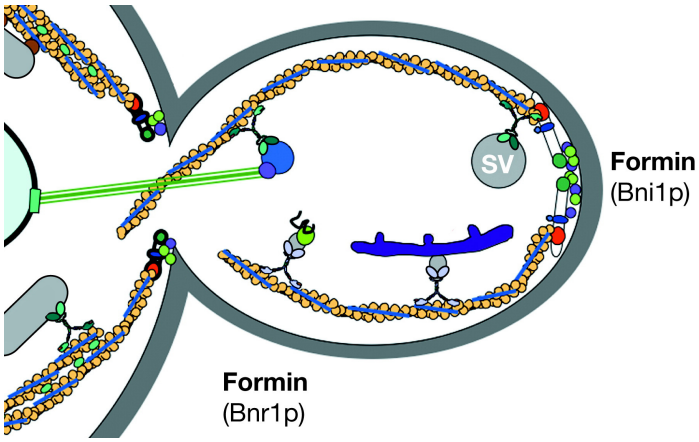

***Figure 7** - different localization of Bnr1 and Bni1 in budding yeast.*

Modified from (Bretscher, 2003)

Fission yeast formin for3p is involved in polarized growth in the rod-shaped cells of *Schizosaccharomyces pombe*. Before cell division takes place, the mother cell undergoes growth at opposite tips - here actin cables are present as tracks for cargo transport. After cell division for some time growth continues only from the pre-existing tip and the newly formed tip doesn't start growing until after the cell reaches a certain point in G2 phase of the cell cycle. Then, the growth of the inactive tip is initiated in a process called New End Take Off (NETO) and yeast then grows at both opposite ends. For the growth to take place, redistribution of actin cables is essential. Formation of a complex ensuring assembly of actin cables at the new end was discovered - MT +TIP tea1p and tea4p proteins bind to growing ends of MTs and are deposited to the tip cortex. Here, tea4p recruits for3p and assembly of actin cables can take place (fig. 8). Cooperation of actin and MTs is therefore critical for establishing cell polarity (Martin et al., 2005).

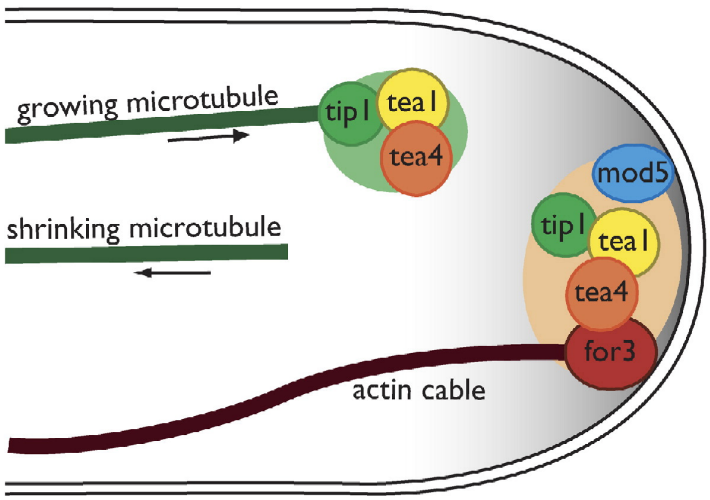

Figure 8

Model for establishment of cell polarity at the new end. MT+TIP proteins Tip1p and tea4p are attached to the growing MT and after deposition to the polar cell cortex recruit for3p and other factors. For3p then produces actin filaments necessary for transport of cargo and polarized growth.

(Adapted from Martin et al., 2005)

In higher eukaryotes, formins were found to interact with both MTs and actin directly. Constitutively active FH1FH2mDia1 fragment was found to induce bipolar elongation in HeLa cells. It causes parallel co-alignment of MTs and actin filaments. Co-expression of FH1FH2mDia1 and FH2mDia1 eliminates the cell elongation and co-alignment of F-actin and MTs. FH2 domain seems to be responsible for binding both actin and MTs (Ishizaki et al., 2001).

Another formin exhibiting F-actin and microtubule co-alignment activity is FHOD1. Activation of this formin by Rac1 GTPase *in vivo* leads to formation of stress fibers. Microtubules were found to align parallel to the actin fibers. As in mDia1, the expression in HeLa cells leads to elongation. Both FH1 and FH2 domains seem to be necessary for inducing the co-alignment. Actin filaments formed by FHOD1 are prerequisite for MT coordination (Gasteier et al., 2005).

Cappuccino (Capu) is a *Drosophilla melanogaster* formin. It is expressed in early oocyte development and is partly responsible for regulating start of a developmental process known as ooplasmic streaming. This activity is MT based, but it was thought that F-actin probably plays role in its timing. *In vitro* studies suggested Capu and certain Spire isoforms as being responsible for crosslinking of F-actin filaments and MTs. Two regions taking part in the interaction were described – FH2 domain was found to facilitate crosslinking of both cytoskeletal systems, while another domain located C-terminal to FH1 caused actin filament bundling (Rosales-Nieves et al., 2006). More recent study argues against the role of Capu as a direct crosslinker *in vivo*. Instead, it proposes model, where Capu together with Spire form an isotropic actin mesh throughout the ooplasm, regulating the arrangement of microtubules in another way (Dahlgaard et al., 2007).

Considering the high number of formins isoforms in plants, it should come as no surprise that microtubule-binding ones were discovered among them. Membrane-associated Class1 formin AtFH4 of *Arabidopsis thaliana* contains a newly identified domain termed the GOE motif, located N-terminal of FH1. *In vitro* experiments showed direct interaction between the domain and microtubules. FH1 seems to also play minor role – as a fragment it does not associate with MTs by itself, but increases the MT binding potential in the full-lenght formin. Since AtFH4 is also a potent actin nucleator, this formin can serve as a common interactor between lipid membranes, microtubules and actin filaments (Deeks et al., 2010). Binding to

microtubules was demonstrated also for the Class II formins AtFH14 from Arabidopsis and FH5 from rice (Li et al., 2010; Yang et al., 2011; Zhang et al., 2011).

5.2.4. ROLE IN ASSEMBLY OF MITOTIC SPINDLE

Metaphase chromosome oscillation is a process during which sister kinetochores move back and forth while attached to microtubules. The movement is facilitated by distinct microtubule dynamics on either kinetochore – the leading one being attached to shrinking and the trailing one to polymerizing microtubules. How kinetochores stay continuously attached to these dynamically changing structures remained elusive. Recent studies suggest involvement of formins. In HeLa cells mDia3 was found colocalizing with kinetochores and its presence was deemed critical for proper chromosome alignment during mitosis (Yasuda et al., 2004). Mutated mDia3 formin defective for actin nucleation still facilitates normal chromosome alignment, showing that the function is independent of its nucleation ability. Depletion of mDia3 leads to slight decrease of stability of the kinetochore MTs, but overall kinetochore assembly is not disrupted in any major way (Cheng et al., 2011). Two modes of mDia3 affecting MT stability have been proposed. One describes the formin being attached directly to the microtubule (Bartolini et al., 2008), the other depicts the mDia3 interacting with *end-binding protein 1* (EB1) and its binding partner, adenomatous polyposis coli (APC) tumor-suppressor protein (Fig. 9). EB1 is a MT plus-end tip tracking protein promoting MT polymerization and has been previously shown located at the trailing kinetochore. Its expression as well as interaction with a region inside FH2 domain of mDia3 was observed to be necessary for kinetochore-MT association and subsequent metaphase chromosome alignment (Cheng et al., 2011). mDia3 affinity towards MTs can be effectively regulated. Specific interaction sites for Aurora B kinase were discovered on mDia3 and their phosphorylation leads to dramatic decrease of MT binding. Stabilization properties against cold-induced depolymerization are too negatively affected (Cheng et al., 2011).

5.3. MEMBRANE AND LIPIDS

Actin dynamics and membrane remodelling are closely linked processes often occuring in tandem. Just as other actin-related proteins, also formins were found to associate with plasma membranes.

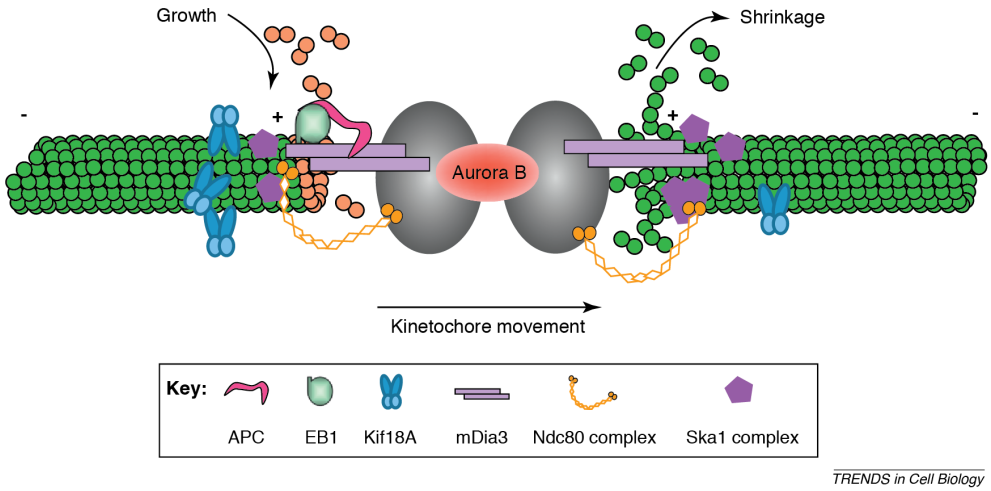

Figure 9 *- Role of mDia3 in binding of kinetochores and microtubules during metaphase chromosome oscillation - mDia3 is attached to kinetochore and binds with APC and EB1. Using the MT tracking ability of EB1, the complex is able to stay attached to the fluctuating end of MT.*

(adapted from Mao, 2011)

Class I formins of plants carry specific N-terminal membrane-targeting signal peptide and transmembrane domain. This suggests their localization into plasma membrane. Presence near cell cortex or near membraneous structures such as ER or cell plate was shown for numerous class I homologs, supporting the notion of transmembrane localization (Cheung et al., 2010; Deeks et al., 2010; Favery et al., 2004; Ingouff et al., 2005; Xue et al., 2011).

Class II formins with their specific PTEN-homologous domain are cytoplasmic, but recent studies suggest that they can be recruited to membrane. Formin For2A was localized in sites of membrane remodelling and PTEN domain presence was necessary for its targeting. PTEN domain has high affinity to $PI(3,5)P_2$. For2A co-localized with membrane spots high in $PI(3,5)P_2$ content. Overexpression of alternative $PI(3,5)P_2$ binders led to decrease in density of cortical For2A spots. This shows that Class II formins can be recruited to specific membrane locations (van Gisbergen et al., 2012).

Interaction of formins and membranes can also be facilitated by adaptory proteins. Studies of *Schizosaccharomyces pombe* showed Cdc12p binding to F-BAR[9] protein Cdc15p - both were required for assembly of the contractile ring during cytokinesis (Wu et al., 2006) In budding yeast, Hof1p (another F-BAR protein) was seen to bind Bnr1p (Kamei et al., 1998).

[9] BAR domain dimers have crescent-shaped surface covered with positively charged residues, allowing for inducing and sensing of membrane curvature.

5.4. SMALL GTPASES

Diaphanous-related formins (DRFs) have specific domain arrangement, with extra regulatory domains located both sides to the FH1FH2 region. N-terminal to FH1 is GTPase binding domain (GBD) and diaphanous inhibitory domain (DID). C-terminal to FH2 lies diaphanous autoregulatory domain (DAD). Interactions between DID and DAD put DRFs into autoinhibited state. Inactive DRFs require association with specific factors (small GTPases) to attain their cytoskeleton-remodelling activity. GTPases can bind to GBD domain and competitively disrupt the bond between DID and DAD, essentially turning the DRFs on (fig. 10) (Seth et al., 2006).

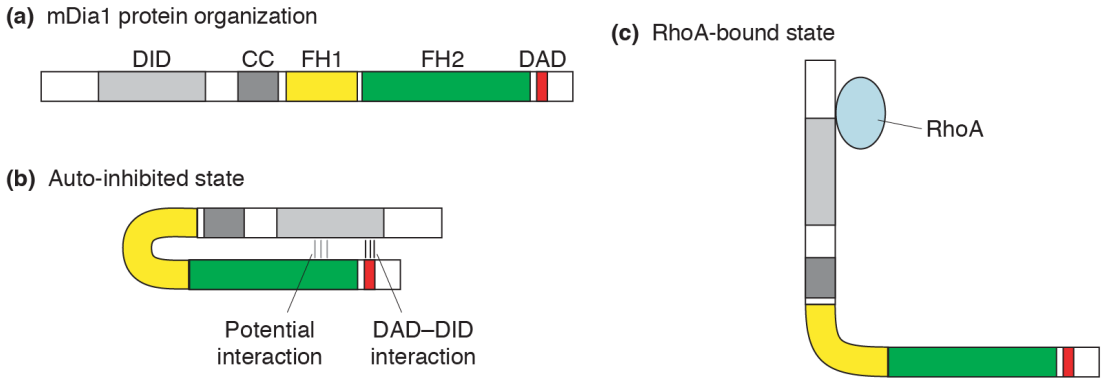

Figure 10 - *DRF formin is autoinhibited, but binding of RhoGTPase can disrupt the autoinhibitory bond between DID and DAD*

Modified from (Higgs, 2005)

RhoGTPases switch between "on" and "off" state by binding either GTP or GDP[10] respectively. Their state is modified by RhoGEFs or RhoGAPs, which can be further controlled. RhoGTPases contain membrane-associating motif, but can rest in inactivated GDP-bound state in cytosol, the motif being sequestered by RhoGDI[11]. After GDP → GTP exchange by GEF, they require membrane association to become active regulators (reviewed in Bustelo et al. 2007).

Diaphanous-related formins have been shown to interact with a range of different RhoGTPases. Since the GTPases not only activate the formins, but also regulate other pathways, diverse programs of actin cytoskeleton remodelling can be achieved by

[10] bound GTP hydrolyzes into GDP + Pi

[11] RHO protein GDP dissociation inhibitor - prevents release of GDP and subsequent unwanted activation by GTP.

combination of single formin isoform as a result of other co-operating proteins (Young and Copeland, 2010a). Outcomes of specific combinations of RhoGTPases and DRFs are beyond the scope of this thesis.

Formins mDia1, 2 and 3 have been observed to function also as downstream activators of RhoGTPases. FH2 domain of mDia2 was found to associate with and trigger RhoGEF called LARG. This RhoGEF subsequently activated RhoA GTPase. This resulted a positive feedback loop with the GTPase subsequently activating more DRFs (Kitzing et al., 2007). Activated Daam1 was also spotted to bind to induce Rho activation by binding to specific GEF (Habas et al., 2001b).

Class III plant formins contain a RhoGAP-related domain, which seems to have lost its ability of assisting GTP hydrolysis. However, the structure is conserved enough to be considered a putative Rho GTPase binding site (Grunt et al., 2008). More research into the possible interaction of small GTPases with class III formins is necessary.

5.5. NUCLEUS

Unique FH2 domain-containing protein is without doubt *fozi-1* (formin zinc finger protein-1). It is a nucleus-located *Caenorhabditis elegans* formin, containing two zinc-finger motifs and one partially degenerated FH2 domain. In development of postembryotic mesoderm of hermaphroditic *C. elegans*, there is a phase, in which 18 identical cells originating from mesoblast stop following the same path of development and separate into groups with distinct fates. 14 of these cells become the basis for striated body wall muscles (BWM), 2 develop into non-muscle coelomocytes (CC) and 2 take part in development of sex myoblasts (SM). fozi-1 together with 2 other transcriptional regulators[12] ensures the proper fate for the BWMs. Several randomly selected BWMs and both CCs transform into SMs in individuals lacking functional fozi-1 (Amin et al., 2007). Fozi-1 was also implicated in control of differentiation of two asymmetric gustatory neurons ASEL (primary Na^+ sensor) and ASER (primary Cl^- and K^+ sensor). Its role in ASER is prevention of expression of effector genes specific for ASEL. Mutation in fozi-1 can lead to existence of ASER with certain ASEL-specific expression patterns (Johnston et al., 2006).

Neither of these two fozi-1 regulated events suggest any direct relation to actin monomers or filaments. The notion that FH2 domain is present mainly for its dimerization abilities, is

[12] other two factors being Hox factor MAB-5 and HLH-1

supported by the fact that regions responsible for dimerization remain intact and capable, while actin binding sites are deteriorated to non-functional state (Amin et al., 2007; Johnston et al., 2006).

Interesting association between nucleus-located activity and formins stems from discovery of two formin binding proteins (FBPs) FBP11 and FBP21 in mice. Special tyrosine-rich WW domains were identified as part of both proteins. These WW domains are highly similar to ones exhibited in yeast splicing factor PRP40. Further inquiry showed that both proteins actually associate with other splicing factors and FBP21 was found to co-localize in spliceosomes on pre-mRNAs in nucleus. All these and more findings strongly suggest that both FBPs function as components of pre-mRNA splicing machinery in mammals (Bedford et al., 1998).

5.6. SIGNALLING PATHWAYS

Outside of direct regulation of actin assembly and managing cross-talk of various cell structures, formins are also implicated in transcriptional regulation and are potent modulators in several pathways.

Diaphanous-related formins were found to take part in Rho-dependent activation of serum response factor (SRF) - a transcription factor with a wide range of target genes, many of which are cell-shape and morphogenesis related. Cytoplasm-localized SRF- co-factors MAL16 and MAL22 contain 3 specific N-terminal RPEL motifs with affinity for G-actin (Mouilleron et al., 2008). Binding of G-actin to those regions represses actin/MAL/SRF pathway (MAL is prevented from entering the nucleus and cooperating with SRF). Formin-induced polymerization of actin and subsequent depletion of free cytoplasmic G-actin has been demonstrated to relieve inhibition of MALs and allow for their translocation into nucleus. (reviewed in Young and Copeland, 2010b).

Wnt signalling pathway plays important role in cell to cell communications. It serves as a link between receptors located on the cell surface and DNA expression regulation in the nucleus. Dishevelled - a downstream effector of transmembrane Wnt-receptor Frizzled was linked to Daam formins as their potent activator. This allows for Wnt signaling to modulate cytoskeleton. Eg. in *Xenopus*, Daam1 was shown to form actin stress fibers in response to Wnt signalling and its presence was necessary for Wnt induced gastrulation (Sato et al., 2006).

Src tyrosine kinases, responsible for signalling and cell fate determination, contain conserved SH3 domain with binding preference for proline-rich sequences (Rickles et al., 1994). Polyproline helices of FH1 domain are suitable targets for Src. Delphilin was detected to interact with n-Src SH3 domain. Delphilin/GluRδ2 association through PDZ suggests that it might serve as a contributing factor to signal transduction by modulating the pathway involving Src protein tyrosine kinase (Miyagi et al., 2002). Src association with formins was also observed in case of mDia 1 and 2. Src tyrosine kinase associated and co-localized with them in endosomes and mid-bodies of cells undergoing division (Tominaga et al., 2000).

6. CONCLUSION

Discovery of formins little over 20 years ago marks an important milestone in scientific research of eukaryotic cytoskeleton dynamics and remodeling. FH2 domain in tandem with FH1 function as a well performing nucleating and processive capping machinery that seems to elegantly get around the problem of working in cytoplasm containing variety of capping proteins and mosty sequestered actin monomers. Involvement of formins in bundling of actin filaments, binding/bundling of microtubules and mediating their interactions all point to the extraordinary multi-purpose nature of both FH2 domain and formins as a whole. Formins have also been observed to facilitate membrane/cytoskeleton association and can co-operate with other actin-nucleating factors such as WH2-containing Spire or Arp2/3 complex. Autoinhibitory nature of Diaphanous-related formins, their regulation by small GTPases and connection to major signalling pathways allows for rapid response to various extracellular and intracellular stimuli. Formins themselves function not only as downstream effectors, but are also capable of triggering large-scale responses such as activation of SRF.

Our knowledge of formins and their functions has greatly increased since the actin-nucleating ability was discovered. However, most of the more detailed knowledge is obtained by working with just fragments of the molecules and interactions in full-length structures still need to be researched. Specific mechanisms of formin regulations and related pathways also seem like interesting topics for future study.

During writing of this thesis, I have gained basic overview of the non-traditional functions of formins.

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