Review

Susanne Dietrich, Sabine Weiß, Sandra Pleiser and Eugen Kerkhoff*

Structural and functional insights into the Spir/formin actin nucleator complex

Abstract: The diversity of cellular actin functions is attained by the activation of actin nucleator complexes, which initiate the polymerization of actin monomers into a helical double-stranded filament at defined subcellular compartments. Next to actin functions at the cell membrane, including different forms of membrane protrusions and invaginations, actin dynamics at intracellular membranes has recently become a research focus. Experiments addressing the vesicle-associated Spir WH2 domain containing actin nucleators have provided novel mechanistic insights into the function of actin dynamics at intracellular membranes. Spir proteins are targeted by a modified FYVE zinc finger motif toward endosomal and vesicle membranes, where they interact and cooperate with the distinct nucleators of the FMN subfamily of formins in the nucleation of actin filaments. The function of the Spir/formin actin nucleator complex is closely related to the Rab11 small G protein, which is a key regulator of recycling and exocytic transport processes. Together with the actin motor protein and Rab11 effector myosin Vb, Spir/formin nucleated actin filaments mediate actin-dependent vesicle transport processes. Drosophila and mouse genetic studies as well as cell biology experiments point toward an important role of the Spir/formin complex in oocyte maturation and in the structure and signaling of the nervous system.

Keywords: actin nucleation; myosin Vb; oocyte maturation; Rab11; vesicle transport.

Introduction

The motility and morphological plasticity of cells throughout evolution requires force-generating molecules that enable the cellular diversity and motion of living beings. Among those force-generating systems, the dynamic actin cytoskeleton and associated actin motor proteins of the myosin family are of special importance. The actin/myosin forces are generated either just by the polymerization of monomeric globular actin proteins into a double-stranded helical filament or by myosin motor proteins pulling these filaments or sliding along a filament (Pollard and Cooper, 2009; Akhmanova and Hammer, 2010). The forces generated by the actin cytoskeleton are involved in many different cell biological processes, including cell membrane pushing and pulling force during mammalian cell migration or neurite outgrowth. The actin cytoskeleton, however, also provides the forces for cell divisions or the fission of organelles, just to name some actin functions.

Spontaneous actin polymerization in cells is prevented by actin monomer binding proteins such as profilin and β-thymosin and by the relative instability of the actin dimer and trimer (Pollard and Cooper, 2009). The diversity of cellular actin structures is regulated by the spatial and temporal regulation of actin nucleation factor complexes, which help to overcome the kinetic barrier of filament initiation. Different nucleation mechanisms have been discovered, which all have in common that the nucleator complex stabilizes at least an actin trimer by binding monomeric actin or as it is the case in the best studied nucleator complex, actin-related proteins 2/3 (Arp2/3), where a dimer of the Arp2/3 is stabilized in a multiprotein complex (Firat-Karalar and Welch, 2011). Arp2/3 is activated by nucleation-promoting factors of the Wiskott-Aldrich syndrome protein (WASp) superfamily, which bind the Arp2/3 complex and activate the complex by a structural rearrangement and providing additional actin monomer binding sites such as the so-called WASp homology 2 (WH2) domains (Robinson et al., 2001). Proteins of the WASp superfamily of nucleation-promoting factors are regulated by distinct signal transduction...
cascades, which target the proteins to different subcellular compartments and thereby enable regulation of multiple actin structures by the Arp2/3 nucleator complex (Pollard and Insall, 2009; Padrick and Rosen, 2010; Rotty et al., 2013). Next to the Arp2/3 complex, and the formin family of actin nucleators, proteins that contain one or multiple WH2 actin monomer binding domains, and can nucleate actin filaments independent of the Arp2/3 complex, were identified as actin nucleators (Pollard and Cooper, 2009; Schonichen and Geyer, 2010; Carlier et al., 2011). Among the latter family are the mammalian Spir proteins, the cordon bleu (Cobl) protein and a nucleator of muscle actin, leiomodin (Lmod) (Quinlan et al., 2005; Ahuja et al., 2007; Chereau et al., 2008). The mammalian genome encodes 15 different formins, which are characterized by two common domains, the formin homology 1 (FH1) and 2 (FH2) domains (Schonichen and Geyer, 2010). The FH2 domain can form a ring-shaped dimer, where each FH2 subunit can bind two actin monomers (Otomo et al., 2005). The FH2 dimer is sufficient to nucleate an actin filament (Pruyne et al., 2002; Sagot et al., 2002). The FH1 domain is a proline-rich sequence motif that can bind profilin/actin, which is thought to be the major source of actin used for filament elongation in cells (Schonichen and Geyer, 2010). As it is the case for the different WASp superfamily nucleation-promoting factors, the 15 different mammalian formins respond to very different cellular signals and can thereby regulate a huge variety of distinct actin structures in cells (Schonichen and Geyer, 2010). Next to their ability to nucleate actin filaments, the formins were also found to act as actin filament elongation factors (Romero et al., 2004). The dimeric FH2 domains were found to stay associated with the fast-growing barbed end of actin filaments and increase the polymerization rate of profilin bound actin by binding profilin/actin to multiple FH1 domains adjacent to the FH2 domains (Paul and Pollard, 2009; Courtemanche and Pollard, 2012). In addition, the FH2 decorated fast-growing barbed ends are protected from being terminated by capping protein binding.

In this review, we will address another interesting feature of actin nucleation – the cooperative mechanism of two distinct actin nucleators: the WH2 domain containing nucleators of the Spir family (dSpir, PEM-5, Spir-1, and Spir-2) and the formins of the FMN subgroup of formins (dCapu, Fmn-1, and Fmn-2). The proteins of these two families interact directly with each other and cooperate in the regulation of cellular actin structures (Figure 1).

Within the Spir/FMN actin nucleator complex, the formin even might act as nucleation-promoting and elongation factor rather than as a nucleator itself (Figure 2). We will report what is known about this actin nucleator complex and what are the cell biological processes regulated by the Spir/FMN function (Table 1).

**Actin nucleation by the Spir/FMN complex**

Spir proteins encode in the central region a cluster of four WH2 actin monomer binding sites (WH2-A, -B, -C, and -D) (Otto et al., 2000) (Figure 1). They share the WH2 domain with the nucleation-promoting factors of the WASp superfam- 


et al., 2007). The same was also shown for the mammalian homologues formin-1 and -2 (Fmn-1 and -2) and Spir-1 and -2 (Pechlivanis et al., 2009). The interaction is mediated by an acidic cluster on the surface of the kinase noncatalytic C-lobe domain (KIND) at the N-terminus of Spir and basic sequences at the very C-terminal ends of the FMN formins, the formin Spir interaction (FSI) motif (Pechlivanis et al., 2009; Vizcarra et al., 2011; Zeth et al., 2011) (Figure 1). The electrostatic interaction mediates a nanomolar affinity of the two distinct nucleators. Sequence alignment studies showed that KIND domain evolved from the catalytic protein kinase fold into a protein interaction domain (Ciccarelli et al., 2003). The close similarity of the KIND domain and the C-lobe of kinases was recently confirmed experimentally by X-ray crystallography (Vizcarra et al., 2011; Zeth et al., 2011). The crystal structure of the Spir-1-KIND/Fmn-2-FSI complex showed also a 1:1 ratio of KIND/FSI complex formation (Vizcarra et al., 2011; Zeth et al., 2011). An interaction of the Spir proteins with the formin FH2 dimer therefore should dimerize the Spir proteins (Figure 1). Consistent with the findings that enforced dimerization of the N-terminal Spir-WH2 domains increases Spir nucleation activity, the interaction of a Spir-KIND-WH2 protein with a nucleation incompetent C-terminal Capu dimer markedly increased the Spir nucleation activity (Quinlan et al., 2007; Namgoong et al., 2011).

In vitro pyrene actin polymerization studies showed that the interaction of the KIND domain with the formin Cappuccino or its mammalian homologue Fmn-2 inhibits actin nucleation by the formin protein (Quinlan et al., 2007). Further in vitro studies employing total internal reflection
fluorescence (TIRF) microscopy, to monitor actin filament elongation and filament annealing, indicate that Spir-KIND binding promotes dissociation of Capu from the barbed end of actin filaments (Vizcarra et al., 2011). A potential mechanism of the Spir-KIND formin inhibition could be a steric hindrance of actin binding in the presence of the Spir-KIND domain (Figure 1C). We have employed structural data of the formin FH2 dimer alone and in complex with actin and of the Spir-1-KIND/Fmn-2-FSI complex to generate a structural picture of this potential hindrance drawn to scale (Figure 1C).

The FSI motif is located at the very C-terminal end of the FMN subfamily proteins (Pechlivanis et al., 2009) (Figure 1A). Other formins of the Dia, Daam, FMNL, and FHOD subfamilies encode at this position a diaphanous autoregulatory domain (DAD), which can interact with N-terminal sequences and thereby mediate a backfolded autoinhibited conformation (Schonichen and Geyer, 2010). The FMN subfamily protein Capu was recently also shown to be autoinhibited (Bor et al., 2012). N-terminal Capu sequences (Capu-NT) inhibited actin nucleation by the C-terminal Capu-FH1-FH2-FSI (Capu-CT) (Bor et al., 2012). Addition of the Capu-FSI peptide partially released the inhibition of actin nucleation activity by Capu-NT, suggesting that the autoinhibition is at least partially mediated by the FSI motif (Bor et al., 2012). Up to now,
no structural domain could be identified in the N-terminal part of the FMN subfamily formins and the N-terminal parts of the FMN formins do not show any conserved sequence motifs (Schonichen and Geyer, 2010). It therefore remains to be analyzed if the mammalian Fmn-1 and Fmn-2 proteins are also autoregulated.

The mDia1 DAD domain not only mediates the autoinhibition but also works in concert with the FH2 domain to enhance actin nucleation (Gould et al., 2011). A C-terminal mDia1 protein (mDia1-C, FH1-FH2-DAD) has a high actin assembly-promoting activity, whereas deletion of the DAD domain (mDia1-C-DDAD) highly reduced the actin assembly activity. The DAD domain alone had very low actin monomer binding activity and no actin monomer binding activity was detected for the FH2 domain in these studies. A mDia1 FH2-DAD protein, in contrast, showed a high affinity for monomeric actin (Gould et al., 2011), suggesting that the DAD domain contributes to the actin nucleation activity of the formin by cooperatively binding actin monomers and thereby stabilizing a multimeric actin nucleus. In this respect, it is interesting to note that also the C-terminal Capu-FSI motif was found to be essential for actin nucleation (Vizcarra et al., 2011; Bor et al., 2012). Deletion of the Capu-FSI motif from a C-terminal Capu protein encoding the FH1 and FH2 domains dramatically reduced the actin nucleation activity of the formin. In analogy to the results obtained for the mDia1-DAD domain, it is tempting to speculate that the inhibition of FMN actin nucleation activity by the Spir-KIND domain may result from overlapping interaction sites of the KIND domain and monomeric actin at the FSI motif. Actin-binding assays with the FSI motif have not yet been performed, and it therefore still has to be analyzed if the FSI motif has an affinity for actin at all.

A model has been proposed that the interaction of Spir protein with Fmn proteins dimerizes the Spir proteins and the two strands of the helical actin filament can be nucleated by the two Spir-WH2 clusters, where each is able to bind four actin monomers (Quinlan and Kerkhoff, 2008) (Figures 1 and 2). The model is based on (1) analytical ultracentrifugation data showing that the Capu-CT (FH1-FH2-FSI) dimer forms a heterotetrameric complex with the KIND domains (2:2) (Quinlan et al., 2007) and on experimental findings that dimerization of several FYVE domains was shown to be required membrane binding. The crystal structure of the EEA1 FYVE domain shows a dimerization of the zinc finger and the N-terminal extensions (Dumas et al., 2001; Hayakawa et al., 2004). (2) Electron microscopy, small-angle X-ray scattering (SAXS), and crystallographic data support that the Spir WH2 domain cluster forms a stable longitudinal-like complex with actin loosely positioned along the stretch of WH2 repeats (Quinlan et al., 2005; Ducka et al., 2010; Sitar et al., 2011). (3) Capu-CT (FH1-FH2-FSI) Spir-NT (KIND-WH2)
and actin coelute in one complex in gel filtration experiments (Vizcarraraz et al., 2011). Subsequently, a dissociation of the Spir-KIND/Fmn-FSI has been proposed and the elongation of the nucleated actin filament should be driven by a processive association of the formin with the fast-growing barbed end of the actin filament (Figure 2). The model, however, leaves open what triggers the dissociation of the nanomolar KIND/FSI interaction, and in addition, it is still on debate if Spir proteins stay bound to the pointed end of the actin filament or if they are associated to the fast-growing barbed end, for both assumptions experimental data were obtained (Quinlan et al., 2005; Bosch et al., 2007; Ito et al., 2011). Data showing that Spir did not cosediment with actin filaments from the steady-state phase of an in vitro actin nucleation assay suggested a third possibility that the Spir protein dissociates from actin filaments following nucleation (Sitar et al., 2011) (Figure 2). Detailed TIRF actin nucleation and elongation assays employing Spir and Fmn proteins and single-molecule imaging will be necessary to solve the discrepancy. The model of a cooperative nucleation and a subsequent filament elongation by a formin has recently been experimentally confirmed for the adenomatous polyposis coli (APC) protein and the formin mDia1 (Breitsprecher et al., 2012). By employing single-molecule imaging TIRF microscopy, the authors showed that upon actin nucleation the APC/mDia1 complex dissociated. The APC protein stayed associated with the pointed end of the actin filament, whereas the formin mDia1 was processively bound to the fast-growing barbed end (Breitsprecher et al., 2012). A similar mechanism has been proposed also for the yeast formin Bni1 and its interaction partner Bud6, which cooperate in the nucleation of actin polymerization (Graziano et al., 2011). In the Spir/Fmn, APC/mDia1, and Bud6/Bni1 complexes, the different formins all interact with actin-binding proteins, which cooperatively promote the nucleation activity. It is a tempting assumption that this might be a general mechanism for formins and that there are many more of formin-interacting actin-binding proteins/nucleators, meaning that for most of the 15 mammalian formins we are missing out essential information about the functional regulation. In addition to actin nucleation, sequestering of actin monomers and severing of actin filaments has been described as a Spir function (Bosch et al., 2007; Sitar et al., 2011) (Figure 2).

**Spir/FMN cooperation in oocyte maturation**

The cooperation of Spir and FMN subfamily formins in actin organization has been convincingly demonstrated both in fly and mouse oocytes by showing that the proteins regulate common actin structures (Dahlgaard et al., 2007; Pfender et al., 2011). As already described above, the dSpir and Capu proteins are both essential to form a network of thin actin filaments in the ooplasm of the Drosophila egg (Dahlgaard et al., 2007). Both spire and cappuccino mutant flies lack the ooplasmic actin network. Mouse genetics revealed a function of the mammalian Capu homologue formin-2 in the maturation of mammalian oocytes (Leader et al., 2002). Formin-2-deficient mice (Fmn2−/− mice) exhibit female hypofertility. The Fmn2−/− oocytes cannot correctly position the metaphase spindle during meiosis I and form the first polar body (Leader et al., 2002; Dumont et al., 2007; Azoury et al., 2008; Li et al., 2008; Schuh and Ellenberg, 2008). A Fmn2-dependent mouse ooplasmic actin meshwork, similar to the Spir/Capu-regulated meshwork in fly oocytes, was discovered (Azoury et al., 2008; Li et al., 2008; Schuh and Ellenberg, 2008) and it was later shown to be regulated by the Fmn-2 protein in cooperation with the mammalian Spir-1 and Spir-2 proteins (Pfender et al., 2011). The knockdown of both mammalian Spir proteins was necessary to inhibit the formation of the ooplasmic actin meshwork in in vitro mouse oocyte maturation experiments, indicating a redundant function of the two mammalian Spir isoforms in mouse oocyte maturation (Pfender et al., 2011). In a yet not completely understood mechanism, the actin meshwork is required for the positioning of the meiotic spindle to the oocyte cortex. A pulling (Schuh and Ellenberg, 2008) and pushing (Li et al., 2008) model has been proposed as well as recently a model that reconciles both (Azoury et al., 2011). In the latter study, a correlation between formin-2 protein levels and the dynamic ooplasmic actin meshwork remodeling was discovered, indicating that the regulation of Fmn-2 protein expression is a critical parameter in oocyte maturation (Azoury et al., 2011). In addition, the mouse Spir-1 and -2 and Fmn-2 proteins were also found to accumulate at the cleavage furrow and promote the assembly of the cleavage furrow during the first polar body extrusion (Pfender et al., 2011) (Table 1).

**Functional cooperation of Spir/formin-2, Rab11, and myosin Vb in vesicle transport processes**

In a recent study, the ooplasmic actin meshwork in mouse oocyte cells was analyzed in detail and identified to be originating from Rab11 vesicle membranes (Schuh, 2011)
In somatic cells, the small G protein Rab11 is a master regulator of recycling and exocytic vesicle transport processes (Wang et al., 2008; Goldenring and Nam, 2011). The Spir proteins encode a modified FYVE zinc finger membrane-binding domain at the C-terminal end (Kerkhoff et al., 2001). FYVE zinc fingers are typically found in proteins involved in membrane trafficking (Hurley, 2006). Early on after the discovery of Spir proteins, transient expression studies in NIH 3T3 fibroblast cells showed that the actin nucleators accumulate at vesicle membranes where they induce the accumulation of filamentous actin (Otto et al., 2000). The integrity of the FYVE zinc finger and the N-terminal adjacent Spir-box, which is a potential small G protein interaction site, were shown to mediate the distinct subcellular localization of the Spir proteins (Kerkhoff et al., 2001). Spir proteins were found to colocalize with the Rab11 protein in somatic cells. A functional correlation of Rab11 and Spir resulted from overexpression experiments of a dominant interfering Spir mutant, which could localize to vesicle membranes, but lacked the N-terminal actin and formin-binding sequences (Spir-1-CT) (Kerkhoff et al., 2001). Transient Spir-1-CT expression in NIH 3T3 mouse fibroblasts and monkey Vero kidney epithelial cells blocked the exocytosis of the vesicular stomatitis virus G protein from the trans-Golgi apparatus to the plasma membrane, a process that was shown before to be regulated by Rab11 (Chen et al., 1998; Kerkhoff et al., 2001). In human HeLa cells, RNA interference knockdown experiments showed a function of Spir-1 in transport processes beyond early endosomes (Morel et al., 2009).

First mechanistic evidence upon the function of the Spir actin nucleators in vesicle transport processes came...
from the work of Melina Schuh in oocytes (Schuh, 2011). This study confirmed the functional correlation of Spir and Rab11 shown before in somatic cells. Melina Schuh showed that Spir-1 and Spir-2 cooperate with formin-2 to nucleate actin structures on Rab11 vesicles that enable a myosin Vb actin motor protein-dependent long-range transport of vesicles toward the oocyte cortex (Schuh, 2011) (Figure 3D and E). The myosin Vb actin motor protein is an effector of the Rab11 protein (Lapiere et al., 2001; Hales et al., 2002). The processive actin motor protein myosin Vb is targeted toward vesicle membranes by interacting with Rab11-GTP and the adaptor protein FIP2 (Figure 3).

The metaphase oocyte employed in this study differs from somatic cells in that they lack microtubule tracks, which are usually employed for long-range transport processes in cells (Figure 3A and B). The microtubules in the oocytes, however, are trapped in the mitotic spindle. The actin-dependent long-range transport mediated by the Spir/Fmn actin nucleator complex and the Rab11/myosin Vb actin motor protein thereby overcomes the need of microtubule for long-range membrane transport (Schuh, 2011). Somatic cells have an extended network of microtubule tracks, yet the function of the Spir/FMN complex may be quite similar in these cells as in metaphase oocytes. In somatic cells, two types of Spir vesicles can be detected, round vesicles, which are detached from the microtubule network, and elongated tubular vesicles, which slide along the microtubule tracks with high velocity, up to 2 μm/s (Figure 3B and C). The detached round vesicles, however, make very thin protrusions, which enable the vesicles to contact the microtubule network. It is an intriguing idea that these protrusions could be driven by Spir/formin nucleated actin polymerization and the myosin Vb motor. In this case, Spir/Fmn function would not be to mediate actin-dependent long-range transport of vesicles to the cortex but rather to mediate an actin-dependent short-range transport toward the microtubule tracks. This, however, is still a model that has to be addressed experimentally. Another obvious difference between oocyte cells and somatic cells is that whereas Spir proteins accumulate at the oocyte cortex, in somatic cells, Spir proteins are only rarely localized at the cell cortex (Figure 3A and B).

**Spir and Fmn functions in the nervous system**

A functional cooperation of Spir and FMN formins was further supported by gene expression analysis of the *spir-1* and *formin-2* genes during mouse embryogenesis and in adult mouse tissues (Schumacher et al., 2004). The genes of the cooperating actin nucleators have a very restricted but highly overlapping expression pattern. Next to oocytes and testis, the *spir-1* and *formin-2* genes are highly expressed in developing nervous system and neuronal cells of the adult nervous system (Schumacher et al., 2004; Pfender et al., 2011). Despite the predominant expression throughout the nervous system, mouse genetic analysis of formin-2 and Spir-1 function did not reveal any gross neural abnormalities (Leader et al., 2002; Peleg et al., 2010; Sandra Pleiser, Mumna Al Banchaabouchi, and Eugen Kerkhoff, unpublished data) (Table 1). Only detailed behavioral studies of formin-2-deficient mice (*Fmn2*−/− mice) and Spir-1 mutant mice, which were generated by a genetrap approach, finally revealed a phenotype in emotional fear learning (Peleg et al., 2010) (Sandra Pleiser, Mumna Al Banchaabouchi, and Eugen Kerkhoff, unpublished data). The function of the formin-2 and Spir-1 proteins in emotional learning is still unclear. Rab11 and myosin Vb are major regulators of AMPA receptor recycling in the postsynapse, which is thought be an essential mechanism in the formation of memory through long-term potentiation of neuronal signaling (Wang et al., 2008). In analogy to the functional relation of Rab11, myosin Vb, and the Spir/Fmn complex in oocytes, one could speculate about a neuronal function of the Spir/FMN complex in postsynaptic signaling.

A neuronal Spir function has recently also been addressed in the fly nervous system (Gates et al., 2011). In situ hybridization experiments found the *Drosophila spire* gene to be expressed broadly in the embryonic central and peripheral nervous system of the fly. Neural development in *spire* zygotic mutant flies was examined. Whereas the central nervous system axon scaffold appeared grossly normal, axon of the ISNb motoneurone often failed to innervate the ventrolateral musculature (Gates et al., 2011) (Table 1). A mechanistic analysis of Spir protein function in the formation of neuromuscular junctions, however, was not included in the study.

**Spir/FMN dysfunction and human disease**

Considering the important function of the Spir-1 and -2/Fmn-2 complex in mouse oocyte maturation, one might speculate that the perturbation of Fmn-2 or Spir function in humans might be a cause of female sterility. A mutation analysis of the human *formin-2* gene in women with unexplained female sterility has been performed (Ryley et al., 2005). The study was performed in 2005 and suffered
from a low number of analyzed patients. Polymorphisms were not analyzed by sequencing, as it is standard today but by denaturing gradient gel electrophoresis, detecting fragment melting polymorphisms. Only one tested patient was homozygous for the high allele in these assays and raising the possibility that this is a recessive mutant allele of human formin-2. The authors concluded that the low incidence or potential absence of formin-2 gene mutations, similar to the absence of gonadotropin-releasing hormone (responsible for the release of follicle-stimulating hormone) gene mutations, might be a consequence of their importance for species propagation (Ryley et al., 2005). A more detailed study with higher patient numbers and including women with polycystic ovary syndrome will be necessary to further investigate a role of the FMN/Spir complex in female infertility.

Spir-1 was linked to human disease by detecting anti-Spir-1 antibodies in the blood sera of human breast cancer patients (Sioud and Hansen, 2001). Five of 30 breast cancer patients had antibodies against the Spir-1 protein. Only 1 of 28 samples of the healthy control group was positive for anti-Spir-1 antibodies (Sioud and Hansen, 2001). Anti-Spir-1 antibodies were detected in the blood sera of breast cancer patients more frequently than antibodies against the p53 tumor suppressor protein (4 of 30). Why breast cancer patients show an immunoreactivity against the Spir-1 protein remained an unsolved question. The functional relation of the Spir/FMN complex with Rab11, however, suggests a potential function of the vesicle-associated actin organizers in oncogenic cell transformation. The Rab11 family protein Rab25 (Rab11c) is associated with aggressive cancers (Cheng et al., 2004; Caswell et al., 2007; Goldenring and Nam, 2011). The regulation of cofilin integrin transport by Rab25 has been identified as the critical function of Rab25 in cancer progression (Caswell et al., 2007; Dozynkiewicz et al., 2012). The transmembrane proteins of the integrin family connect cells to the extracellular matrix and are key functions in cell migration. In this respect, it is interesting to note that studies employing Fmn-1 knockout fibroblasts have shown a function of Fmn-1 in the regulation of cell migration (Dettenhofer et al., 2008) (see below).

**Perspectives**

Since the discovery of Spir proteins in the year 1999 (Wellington et al., 1999; Otto et al., 2000), many outstanding findings were made in respect to structural domains (KIND domain) (Ciccarelli et al., 2003), actin nucleation mechanisms (WH2 domain containing nucleators) (Quinlan et al., 2005), nucleation factor cooperation (Spir/Fmn complex) (Quinlan et al., 2007), discovery of an ooplasmic actin meshworks (Dahlgaard et al., 2007; Azoury et al., 2008; Li et al., 2008; Schuh and Ellenberg, 2008; Pfender et al., 2011), and actin/myosin-dependent vesicle transport (Spir/Fmn-2, Rab11, and myosin Vb cooperation) (Schuh, 2011). These data added invaluable knowledge to the field of cell biology. The cell biological processes regulated by the Spir/FMN complex are still arising. It is well established that Spir/FMN function is essential for oocyte maturation (Pfender et al., 2011). We got a first glance on the functions of the Spir/FMN actin nucleator complex in the nervous system and a very recent paper also reports a spire function in cardioblast differentiation during Drosophila heart development (Xu et al., 2012) (Table 1). The Fmn-1 protein was found to be localized at microtubule tracks in mouse embryo fibroblast cells by electron microscopy, further supporting a role of Fmn subgroup proteins in vesicle transport processes (Dettenhofer et al., 2008). In the same study, a function Fmn-1 in the regulation of cell migration was shown by findings that Fmn-1 contributes to cell protrusions and focal adhesion formation (Dettenhofer et al., 2008). mRNA localization studies in the ascidian egg (Satou and Satoh, 1997) or the frog early embryo (Le Goff et al., 2006) support a Spir function in egg and embryonic polarity (Table 1). On the background of Spir/FMN function in the regulation of intracellular membrane transport processes, which we think is the general cellular role of the Spir/Fmn complex, the upcoming years will refine its mechanism in actin organization and identify its role in different cell biological processes as well as the regulatory signals.

**Acknowledgments:** We thank Melina Schuh (University of Cambridge, Cambridge, UK) for providing a picture of a Spir-2/microtubule-stained mouse oocyte cell. The picture of the Spir-2/microtubule stained somatic cell has been generated at the “Summer School on Actin Dynamics”. We thank Gert Sonntag and Ronald Gerstl of the Carl Zeiss company for help. The work was supported by the German Research Foundation Priority Programme SPP 1464 and the Bavarian Geneome Research Network (BayGene).

Received May 13, 2013; accepted July 13, 2013; previously published online July 16, 2013
References


Susanne Dietrich studied biology at the University of Regensburg (Germany) with main subject molecular biology. She did her PhD thesis on the topic of mutational and kinetical analyses of a (βα)8-barrel at the Department of Biophysics and Physical Biochemistry of the University of Regensburg under the supervision of Prof. Reinhard Sterner. In 2011 she started as a post-doc in the molecular cell biology laboratory at the Department of Neurology, University Hospital Regensburg, Germany. There, she set her focus on biochemical and bioinformatics research of the actin nucleation factor Spir.

Sabine Weiß studied Biology at the Julius-Maximilians-University Würzburg (Germany) and graduated in 2011. The doctoral thesis encompasses the function of the Spir actin nucleators in vesicle transport processes under the tutelage of Prof. Dr. Eugen Kerkhoff. As a postdoctoral researcher she is investigating the molecular mechanisms that underlie intracellular transport focussing on the mechanisms of protein transport between the ER and Golgi complex in the research group of Prof. Dr. Charles Barlowe in the Department of Biochemistry, Geisel School of Medicine, Dartmouth College, USA.

Sandra Pleiser studied Biomedical Science at the University of Würzburg in Germany. She completed her Master’s thesis in 2007 analysing the function of B-Raf in migration of cortical neurons in the group of Prof. Dr. Ulf R. Rapp (University of Würzburg, Germany). She then joined the group of Prof. Dr. Eugen Kerkhoff at the University Hospital Regensburg and accomplished her PhD in 2012. Her research interest focused on the analysis of the cell biological function of the actin nucleation factor Spir and the characterization of Spir-1 mutant mice.

Eugen Kerkhoff studied chemistry at Cologne University (Germany) and graduated in biochemistry in 1993. As a post doctoral fellow he continued his work on the Myc, Ras and Raf onco-proteins in the labs of Edward B. Ziff (NYU Medical Center, USA), Hartmut Land (ICRF London, UK) and Ulf R. Rapp (University of Würzburg, Germany). In 1998 he became an independent research group leader at the University of Würzburg. His research interest since then is the regulation of cell morphology by signalling cascades, with a specific emphasis on actin organization at vesicle membranes by the Spir actin nucleators. In 2008 he became a professor at the University of Regensburg (Germany) where he heads the molecular cell biology laboratory at the Department of Neurology, University Hospital Regensburg, Germany.