Migfilin and its binding partners: from cell biology to human diseases

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Summary

Links between the plasma membrane and the actin cytoskeleton are essential for maintaining tissue integrity and for controlling cell morphology and behavior. Studies over the past several decades have identified dozens of components of such junctions. One of the most recently identified is migfilin, a widely expressed protein consisting of an N-terminal filamin-binding domain, a central proline-rich domain and three C-terminal LIM domains. Migfilin is recruited to cell-matrix contacts in response to adhesion and colocalizes with β-catenin at cell-cell junctions in epithelial and endothelial cells. Migfilin also travels from the cytoplasm into the nucleus, a process that is regulated by RNA splicing and calcium signaling. Through interactions with multiple binding partners, including Mig-2, filamin and VASP, migfilin links the cell adhesion structures to the actin cytoskeleton. It regulates actin remodeling, cell morphology and motility. In nuclei, migfilin interacts with the cardiac transcriptional factor CSX/NKX2-5 and promotes cardiomyocyte differentiation. It probably functions as a key regulator both at cell adhesion sites and nuclei, coordinating multiple cellular processes, and is implicated in the pathogenesis of several human diseases.

Key words: Migfilin, Filamin, Mig-2, Kindlin, CSX/NKX2-5, Cell-cell junctions, Cell-extracellular matrix adhesions

Introduction

The cellular environment, which includes the extracellular matrix (ECM) and neighboring cells, provides instructive cues that control cell morphology, motility and fate (e.g. proliferation, differentiation or apoptosis). These are frequently transduced by cell-ECM and cell-cell adhesions, key components of which include proteins that link the actin cytoskeleton to the plasma membrane (Burridge and Chrzanowska-Wodnicka, 1996; Yap et al., 1997; Adams and Nelson, 1998; Liu et al., 2000; Geiger et al., 2001; Hynes, 2002; Jamora and Fuchs, 2002). These protein complexes provide anchorage sites for transmembrane adhesion receptors (e.g. integrins and cadherins) and the actin cytoskeleton, which are essential for force transmission, shape modulation, motility and tissue integrity. In addition, they play important roles relaying signals between the extracellular, cytoplasmic and nuclear compartments and therefore actively participate in the regulation of gene expression, cell-cycle progression, differentiation and survival.

Migfilin (also known as FBLP-1A or Cal) is a recently identified, widely expressed component of actin-cytoskeleton-membrane junctions that is emerging as a key regulator of a variety of fundamental cellular processes, including shape modulation, motility and differentiation (Tu et al., 2003; Takafuta et al., 2003; Akazawa et al., 2004). Here, I review our current understanding of the molecular activities and cellular functions of migfilin. I discuss the underlying mechanisms and their implications for the pathogenesis of several human diseases, ranging from the rare skin disorder Kindler syndrome to common diseases such as cancer and congenital heart disease.

Molecular activities of migfilin

Migfilin was identified and cloned following yeast two-hybrid screens for binding proteins of mitogen-inducible gene 2 (Mig-2), a component of cell-ECM adhesions (Tu et al., 2003). Migfilin contains an N-terminal region that exhibits no obvious sequence motifs, a central proline-rich region and a C-terminal region consisting of three LIM domains (Fig. 1). LIM domains are cysteine-rich sequences of ~50 residues that fold into a specific two-zinc-finger structure (Schmeichel and Beckerle, 1994; Dawid et al., 1998; Jurata and Gill, 1998). To date, seven migfilin-binding proteins, which belong to four different protein families, have been identified, and each region of migfilin mediates at least one protein-protein interaction (Fig. 1). The C-terminal LIM region mediates the interaction with Mig-2 (Tu et al., 2003), whereas the central proline-rich domain interacts with vasodilator-stimulated phosphoprotein (VASP) (Y. Zhang, Y. Tu and C.W., unpublished), which is an actin-binding protein (Krause et al., 2003). Yeast two-hybrid screens using the migfilin N-terminal fragment as bait revealed filamin A and C, members of an actin-crosslinking protein family (Stossel et al., 2001; van der Flier and Sonnenberg, 2001; Stossel and Hartwig, 2003), as migfilin-binding partners (Tu et al., 2003). An independent study has shown that the mouse homolog of migfilin (termed Cal) interacts with CSX/NKX2-5, a transcriptional factor that is crucial for heart development (Komuro and Izumo, 1993; Lints et al., 1993; Lyons et al., 1995), through its C-terminal LIM region (Akazawa et al., 2004).

Other studies using a filamin B fragment as bait have identified a splice variant of migfilin – filamin-binding LIM

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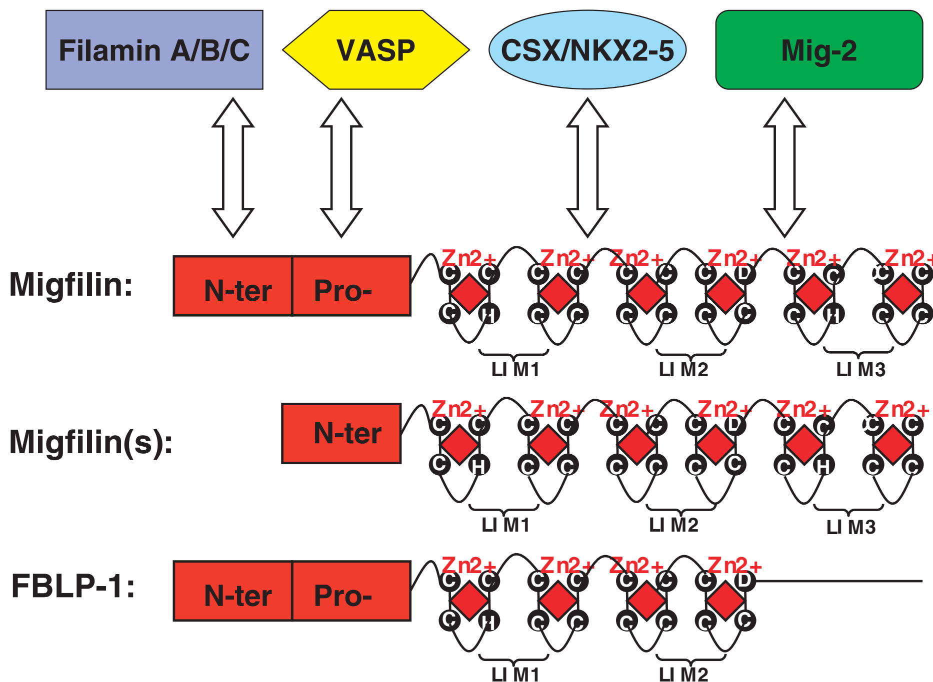

Fig. 1. Migfilin and its binding partners. The figure depicts migfilin domain structure, splicing variants and its binding partners. Double arrows represent direct interactions of migfilin with filamin A, B or C (mediated by the N-terminal domain of migfilin and the 21st repeat of filamin A/C (Tu et al., 2003) or the 10-13th repeats of filamin B (Takafuta et al., 2003], VASP (mediated by the central proline-rich domain of migfilin and the EVH1 domain of VASP) (Y. Zhang, Y. Tu and C.W., unpublished data), CSX/NKX2-5 (mediated by the C-terminal LIM region of migfilin and the homeodomain of CSX/NKX2-5) (Akazawa et al., 2004) and Mig-2 (mediated by the C-terminal LIM region of migfilin) (Tu et al., 2003).

protein 1 (FBLP-1), which shares the N-terminal region, the central region and the first two LIM domains with migfilin but lacks the third LIM domain (Takafuta et al., 2003). FBLP-1 binds to filamin B (Takafuta et al., 2003) and probably also interacts with filamin A/C and VASP given its sequence similarity with migfilin. Because FBLP-1 differs from migfilin in the C-terminal LIM region, it probably lacks Mig-2-binding activity. A second splice variant lacks the central proline-rich domain and therefore is much shorter [hence the name migfilin(s) (Tu et al., 2003)] (Fig. 1). Migfilin(s) interacts with Mig-2 (Tu et al., 2003), filamin (Tu et al., 2003) and probably CSX/NKX2-5 but not VASP (Y. Zhang, Y. Tu and C.W., unpublished data). Migfilin(s) is co-expressed with migfilin in several cell types (Tu et al., 2003).

Subcellular localizations of migfilin and its regulation

Staining with a monoclonal anti-migfilin antibody has revealed that, in fibroblasts, migfilin is highly concentrated at cell-ECM adhesions (Fig. 2), although a fraction of migfilin is also detected along the actin stress fibers linking the cell-ECM adhesions (Tu et al., 2003). Expression of FLAG- or green fluorescent protein (GFP)-tagged migfilin proteins has shown that both migfilin and migfilin(s) cluster at cell-ECM adhesions and associate with the actin filaments. The Mig-2-binding C-terminal LIM region is both necessary and sufficient for localization to cell-ECM adhesions. Furthermore, depletion of Mig-2 from human Hela cells diminishes migfilin localization to cell-ECM adhesions (Tu et al., 2003). This suggests that Mig-2 recruits migfilin to cell-ECM adhesions (Fig. 3).

Although the interaction with Mig-2 draws migfilin towards cell-ECM adhesions, the interaction with filamin attracts migfilin to actin filaments (Fig. 3). The subcellular localization of migfilin in fibroblasts appears to reflect largely the balance of these two forces. Thus, recombinant migfilin mutants that contain the filamin-binding site but lack the Mig-2-binding site, as well as the naturally occurring splice variant FBLP-1, exhibit a striking subcellular distribution mimicking that of actin filaments (Takafuta et al., 2003; Tu et al., 2003).

In epithelial and endothelial cells, migfilin localizes not only to cell-ECM adhesions but also to cell-cell adhesions (see this issue pp. 697-710) (Gkretsi et al., 2005). It is concentrated at discrete clusters, which are readily detectable by fluorescence microscopy. These appear to bridge neighboring cells and are filamentous, actin-containing, electron-dense compact structures that are often closely (within 100 nm) associated with β-catenin. Interestingly, the LIM2 domain, which is required for localization of migfilin to cell-ECM adhesions, is also required for its localization to cell-cell junctions. This suggests that two mutually exclusive interactions compete for the same or at least partially overlapping site on migfilin and therefore dictate the relative distribution of migfilin between cell-cell and cell-ECM adhesions in epithelial and endothelial cells (Fig. 3). This is reminiscent of the mechanism that regulates the distribution of β-catenin to different subcellular compartments. β-catenin interacts with cadherins, members of the LEF/TCF-family of transcription factors, and the tumor suppressor product APC through overlapping sites in its armadillo repeat region, and these mutually exclusive interactions control, at least in part, its subcellular distribution (Gottardi and Gumbiner, 2001; Huber and Weis, 2001).

In addition to clustering at cell-ECM and cell-cell adhesions, and associating with the actin cytoskeleton, migfilin has been detected in nuclei (Akazawa et al., 2004). The nuclear localization of migfilin appears to be tightly regulated. There are at least two mechanisms by which its nuclear localization is regulated (Fig. 3). The Ca^{2+} ionophore A23187 promotes nuclear localization of migfilin (Akazawa et al., 2004), which suggests that this process is regulated by intracellular Ca^{2+}. Migfilin contains a nuclear export sequence (NES) in its proline-rich domain (Akazawa et al., 2004; Takafuta et al., 2003). Deletion of the NES results in accumulation of the migfilin mutant in nuclei (Akazawa et al., 2004). As mentioned above, migfilin(s) lacks the proline-rich domain. Consequently, high levels of migfilin(s), but not full-length migfilin, are

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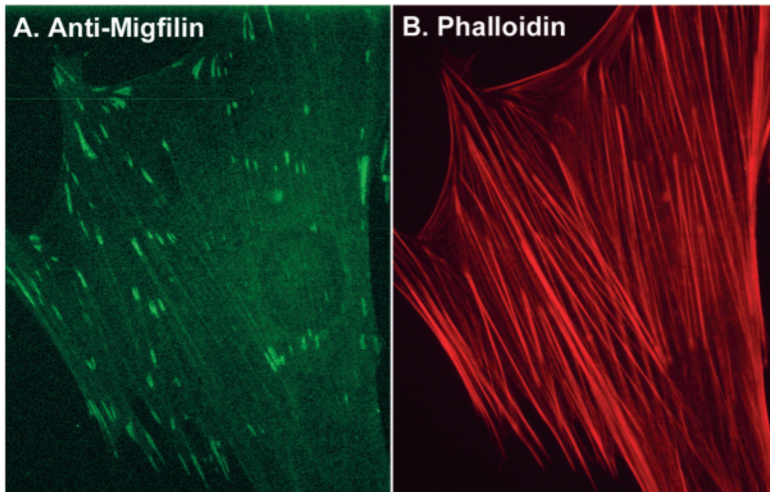

Fig. 2. Migfilin is clustered at cell-ECM adhesions. Human WI-38 fibroblasts were dually stained with (A) a mouse monoclonal anti-migfilin antibody (clone 43) and (B) rhodamine-phalloidin. The mouse monoclonal anti-migfilin antibody was detected with a secondary FITC-conjugated anti-mouse IgG antibody. Figure reproduced with permission from Elsevier (Tu et al., 2003).

present in nuclei (see this issue pp. 697-710) (Gkretsi et al., 2005). Thus, the nuclear localization of migfilin is also regulated by alternative RNA splicing.

Cellular functions of migfilin

Cell-ECM adhesion is an important determinant of cell morphology. Recent studies have suggested that migfilin functions as a key regulator of cell morphology. Depletion of migfilin from Hela cells significantly impairs changes in cell shape induced by cell-ECM adhesion; this defect is identical to that induced by depletion of Mig-2 (Tu et al., 2003). Migfilin is thus likely to serve as a scaffold linking the Mig-2-containing cell-ECM adhesions to filamin-containing actin filaments.

Given its crucial role in cell shape modulation, migfilin probably also controls motility. Indeed, loss of migfilin significantly reduces motility in Hela cells (Y. Zhang, Y. Tu and C.W., unpublished). Surprisingly, however, elevated levels of migfilin can also reduce cell motility (Y. Zhang, Y. Tu and C.W., unpublished). This suppression of cell motility requires the proline-rich domain of migfilin, which suggests that it exerts its inhibitory effect through VASP, an important suppressor of cell motility (Krause et al., 2002). It is worth noting that migfilin(s) lacks the VASP-binding proline-rich domain. Because different types of cells often exhibit vastly different motilities, the presence of two naturally occurring migfilin variants, migfilin and migfilin(s), which have vastly different motility-regulating activities, could provide a versatile system by which cells control this process.

Work by Akazawa and coworkers has revealed an important role of migfilin in transcriptional regulation (Akazawa et al., 2004). They have found that migfilin, through its interaction with CSX/NKX2-5, strongly promotes the transcriptional activity of this cardiac transcription factor. Indeed, overexpression of a migfilin mutant lacking an NES significantly promotes cardiomyocyte differentiation (Akazawa et al., 2004). These results, together with the fact that CSX/NKX2-5 is critically involved in the heart development (Komuro and Izumo, 1993; Lints et al., 1993; Lyons et al., 1995), suggest that migfilin has a role in the regulation of cardiac gene expression. Since the migfilin mutant lacking an NES has increased transcription- and differentiation-promoting activities (Akazawa et al., 2004), migfilin(s) probably also has higher transcription- and differentiation-promoting activity.

It has been shown that several other LIM-containing focal adhesion proteins, including zyxin (Nix and Beckerle, 1997), LPP (for ‘lipoma preferred partner’) (Petit et al., 2000), Ajuba (Kanungo et al., 2000), Trip6 (for ‘thyroid hormone interacting protein 6’) (Wang and Gilmore, 2001), Hic-5 (for ‘hydrogen peroxide-inducible clone 5’) (Shibanuma et al., 2003; Shibanuma et al., 1997; Yang et al., 2000), PINCH-1 (Campana et al., 2003) and PINCH-2 (Zhang et al., 2002), can also be transported to the nucleus. Although much remains to be learned about their functions in the nucleus, currently available data suggest that they probably recognize different sets of nuclear targets and therefore mediate diverse nuclear activities. Additionally, it appears that distinct upstream signals are involved in the regulation of their nuclear localization. For

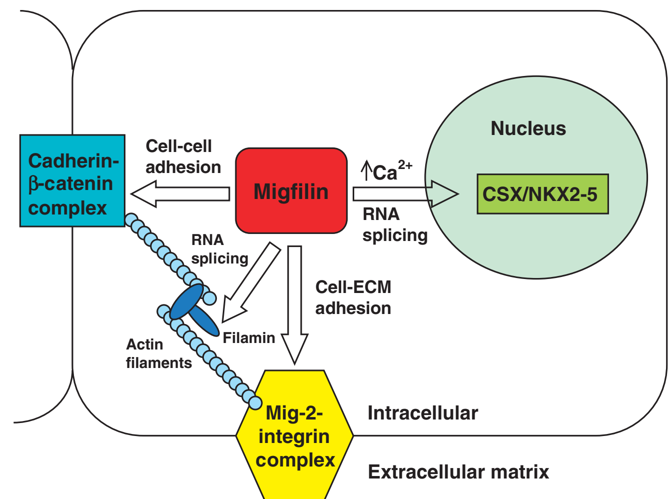

Fig. 3. Control of the subcellular localization of migfilin. The figure shows a model for the mechanism that controls migfilin localization to cell-ECM adhesions, cell-cell junctions, actin filaments and nuclei. Migfilin is recruited to cell-ECM adhesions through its interaction with Mig-2, a component of cell-ECM adhesions. In epithelial and endothelial cells, migfilin is also recruited to adherens junctions in response to cadherin-mediated cell-cell adhesion through an interaction with a yet-to-be-identified protein, which is probably a component of the cadherin–β-catenin complex. Migfilin associates with actin filaments through its interaction with filamin, although the associations of migfilin with cell-ECM and cell-cell adhesions appear to dominate. FBLP-1, a migfilin splicing variant lacking LIM3 and therefore the ability to localize to cell-ECM or cell-cell adhesions, predominantly associates with the actin filaments. Travel of migfilin to the nucleus is regulated both by intracellular Ca^{2+} signaling and a nuclear export signal located within the proline-rich domain that is subjected to alternative splicing.

example, whereas the nuclear localization of migfilin is enhanced by treatment of cells with a Ca^{2+} ionophore, the nuclear localization of Ajuba is promoted by treatment of cells with retinoic acid (Kanungo et al., 2000). Nevertheless, despite their diverse nuclear functions, they do share certain common structural features such as the presence of a leucine-rich NES. Thus, given the role of the migfilin NES in promoting cardiomyocyte differentiation and its regulation by alternative splicing, it will be interesting to determine whether RNA splicing plays a similar role in the regulation of nuclear-cytoplasmic shuttling of other related LIM-containing focal adhesion proteins.

Migfilin-binding partners in human diseases

Recent studies have demonstrated that mutations in the genes encoding migfilin-binding partners cause several human disorders. Mutations in the *FLNa* gene that encodes filamin A cause periventricular heterotopia (Fox et al., 1998), an X-linked dominant disorder in which neurons fail to migrate into the cerebral cortex. Most hemizygous affected males die early during embryogenesis (Fox et al., 1998). Furthermore, missense mutations in *FLNa* lead to a broad range of congenital malformations, affecting craniofacial structures, the skeleton, brain, viscera and urogenital tract, which cause X-linked human disorders including otopalatodigital syndrome types 1 and 2, frontometaphyseal dysplasia and Melnick-Needles syndrome (Robertson et al., 2003). Recent genetic studies have also linked mutations in the gene encoding filamin B to several human skeletal disorders, including spondylocarpotarsal syndrome, Larsen syndrome and the perinatal lethal atelosteogenesis type I and III phenotypes (Krakow et al., 2004). Given the genetic heterogeneity of some of the disorders (Sheen et al., 2004) and the existence of other related human diseases, it will be interesting to determine whether mutations in the genes encoding migfilin or other proteins that are physically and functionally associated with filamin are involved in a subset of these or other related human diseases.

Another exciting finding is that loss of kindlin-1 (also known as kindlerin-1 or URP-1), which is structurally closely related to Mig-2 (62% identical at the amino acid level), causes Kindler syndrome (Jobard et al., 2003; Siegel et al., 2003), an autosomal-recessive skin disorder characterized by cleavage at the dermal-epidermal junction, cytoskeletal alterations and marked basement membrane reduplication. Both Mig-2 and kindlin-1 are highly similar to UNC-112, a *Caenorhabditis elegans* protein that is essential for the assembly of integrin-containing muscle attachment structures (Rogalski et al., 2000). Kindlin-1 is expressed at a high level in basal keratinocytes (Ashton, 2004) and perhaps also in some other types of epithelial cell, which might explain why the clinical lesions resulting from loss of kindlin-1 are primarily in the skin and in some cases also involve mucosal membranes of the digestive and urinary tracts. In keratinocytes, clusters of kindlin-1 have been detected at cell-ECM adhesions (Kloeker et al., 2004; Siegel et al., 2003), to which actin filaments are anchored. Thus, the skin blistering observed in the Kindler syndrome patients is probably caused by, at least in part, the weakening of the actin-cytoskeleton-membrane link in the basal keratinocytes.

How kindlin-1 is linked to actin filaments remains to be determined. However, given the high level of structural similarity between kindlin-1 and Mig-2, migfilin, which is also clustered at cell-ECM adhesions in keratinocytes (see this issue pp. 697-710) (Gkretsi et al., 2005), might link kindlin to filamin-containing actin filaments. Kindlin-1, like Mig-2, possesses migfilin-binding activity (Y. Tu and C.W., unpublished data), which supports this notion. In this regard, it is also worth noting that, although loss of kindlin-1 clearly causes Kindler syndrome, some Kindler syndrome patients do not carry mutations in the *KIND1* gene (Kloeker et al., 2004; Siegel et al., 2003); mutations in other genes encoding proteins that are physically or functionally linked to kindlin-1 could thus also lead to diseases resembling Kindler syndrome. Since migfilin localizes to both cell-ECM adhesions and adherens junctions in keratinocytes, loss of kindlin-1 could alter the distribution of migfilin between the basal cell-ECM adhesions and the lateral adherens junctions, which might also contribute to the clinical manifestations of Kindler syndrome.

Interestingly, whereas loss of kindlin-1 causes epithelial blistering, cytoskeletal alterations and atrophy, an increase in kindlin-1 expression in epithelia is associated with lung and colon carcinomas (Weinstein et al., 2003). Treatment of human mammary epithelial cells with transforming growth factor β1, a promoter of tumor progression and metastasis, results in a significant increase in the levels of kindlin-1 and a reduction in E-cadherin levels (Kloeker et al., 2004). The mechanism underlying this opposing regulation is probably complex. Nevertheless, given that migfilin localizes to cell-ECM adhesions and adherens junctions, it will be interesting to test whether it participates in the coordinated regulation of cell-ECM and cell-cell adhesions and, consequently, the control of carcinoma invasion or related processes such as the epithelial-mesenchymal transition.

There is now strong evidence that CSX/NKX2-5, to which migfilin binds, not only is essential for heart development but also is crucially involved in the pathogenesis of cardiac diseases. For example, mutations in *CSX/NKX2-5* cause human cardiac malformations and atrioventricular conduction abnormalities (Schott et al., 1998). The level of CSX/NKX2-5 is increased in cardiac hypertrophy (Thompson et al., 1998; Saadane et al., 1999). CSX/NKX2-5 may therefore participate in its development (reviewed by Akazawa and Komuro, 2003), which is known to be greatly influenced by cell-ECM adhesions. The interaction of migfilin with CSX/NKX2-5 and its marked effect on transcription-promoting activity and cardiac differentiation, together with its prominent role in cell adhesion and cytoarchitecture, strongly suggest that migfilin has an important role in the development and function (or malfunction) of the heart.

Conclusion

Although migfilin was discovered only two years ago, accumulating evidence points to crucial roles for the protein in several fundamental cellular processes, including shape modulation, motility and differentiation. The identification of multiple binding partners of migfilin, including Mig-2, filamin, VASP and CSX/NKX2-5, and the demonstration that it localizes to several key subcellular compartments (i.e. cell-ECM adhesions, cell-cell junctions and nuclei), provide

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important insights into the molecular and cellular mechanisms by which it functions. The molecular, cell biological and genetic studies discussed above have allowed us to postulate several hypotheses. Testing these will undoubtedly help us understand the molecular and cellular basis of a variety of human diseases, ranging from skin disorders to cancers and cardiac abnormalities. Given that migfilin possesses five different protein-protein interaction domains, and localizes to three different subcellular localizations, additional migfilin-binding partners probably exist. Identifying these and determining how the formation of the migfilin complex is regulated represent important challenges for future research. Finally, migfilin, particularly migfilin(s), localizes to nuclei not only in cardiomyocytes but also in other cell types, including fibroblastic and epithelial cells. Thus, migfilin is probably a transcriptional regulator that not only controls cardiomyocyte differentiation but also gene expression, proliferation or differentiation in other cell types. Despite recent progress in this area, much remains to be discovered. Future studies will undoubtedly provide important information about the cell biology of key subcellular compartments, ranging from adhesion sites to the nucleus, as well as the pathogenesis of human diseases in organs, ranging from the skin to the heart.

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