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REVIEW

Spectraplakin family proteins – cytoskeletal crosslinkers with versatile roles

Jamie Zhang*, Jiping Yue* and Xiaoyang Wu‡

ABSTRACT

The different cytoskeletal networks in a cell are responsible for many fundamental cellular processes. Current studies have shown that spectraplakins, cytoskeletal crosslinkers that combine features of both the spectrin and plakin families of crosslinkers, have a critical role in integrating these different cytoskeletal networks. Spectraplakin genes give rise to a variety of isoforms that have distinct functions. Importantly, all spectraplakin isoforms are uniquely able to associate with all three elements of the cytoskeleton, namely, F-actin, microtubules and intermediate filaments. In this Review, we will highlight recent studies that have unraveled their function in a wide range of different processes, from regulating cell adhesion in skin keratinocytes to neuronal cell migration. Taken together, this work has revealed a diverse and indispensable role for orchestrating the function of different cytoskeletal elements *in vivo*.

KEY WORDS: Spectraplakin, F-actin, Microtubule, Intermediate filament, Cytoskeletal coordination, Cell–matrix adhesion, Cell–cell junction

Introduction

The cytoskeletal network is composed of filamentous actin (F-actin), microtubules (MTs) and intermediate filaments (IFs), and orchestrates an integrated cytoplasmic scaffold that is responsible for signaling, intracellular trafficking, polarization, migration, adhesion, cell division, mechanical strength and cellular shape. The cytoskeleton is highly dynamic and has the ability to remodel itself quickly during normal developmental morphogenesis or upon tissue injury. Spectraplakins function in these remodeling processes through their unique ability to crosslink and integrate different cytoskeletal networks to regulate various cellular processes (Broderick and Winder, 2005; Liem, 2016; Röper et al., 2002). Spectraplakins are part of the spectrin superfamily and are large, evolutionarily conserved cytoskeletal proteins that can bind to different cytoskeletal networks to coordinate cytoskeletal dynamics (Broderick and Winder, 2005; Liem, 2016; Röper et al., 2002). Currently, two genes are known to encode the members of the mammalian spectraplakin family, microtubule and actin crosslinking factor 1 (*MACF1*; also known as actin crosslinking factor 7, *ACF7*) and bullous pemphagoid antigen 1 (*BPAG1*; also known as dystonin, *DST*). Zebrafish has a single spektraplakin gene, *MACF1a* (*Magellan*), and there is also a single gene in *Drosophila*, *shot*, encoding for Short stop, also known as Kakapo, as well as the single *vab-10* gene in *Caenorhabditis elegans* (see Table 1 for alternative names). Spectraplakins generate further diversity from their differential promoter usage and splicing, which results in a plethora of possible proteins that are able to selectively link and coordinate many biological functions within different cell types (Fig. 1; Box 1) (Brown et al., 1995; Duchen et al., 1964; Green et al., 1990; Guo et al., 1995; Jefferson et al., 2006; Künzli et al., 2016; Leung et al., 2001b; Poliakova et al., 2014; Ruhrberg and Watt, 1997; Sawamura et al., 1991b; Stanley et al., 1981; Wiche et al., 1991). In this Review, we will discuss the structure and function of spectraplakin proteins and summarize recent studies that unveil their role in many physiological and pathological processes.

Ben May Department for Cancer Research, University of Chicago, Chicago, IL 60637, USA.
*These authors contributed equally to this work

‡Author for correspondence (xiaoyangwu@uchicago.edu)

X.W., 0000-0001-6378-3207

Domain structure of spectraplakins

Spektraplakins have a conserved domain structure consisting of an actin-binding domain, a number of spectrin repeats, a tandem EF-hand and a GAR domain (see Fig. 1A), as discussed in the following sections.

Actin-binding domains

Most spectraplakins contain a conserved sequence motif in their N-terminal domain that enables them to associate directly with the actin cytoskeleton, and this F-actin-binding domain consists of two calponin homology (CH) domains, CH1 and CH2 (Broderick and Winder, 2005) (Fig. 1). CH domains are also found in many other F-actin-binding proteins, such as α-actinin, filamin, utrophin and dystrophin (Sjöblom et al., 2008). The crystal structure of the F-actin binding domain of MACF1 has been recently resolved (Yue et al., 2016). As shown in that study, the two tandem CH domains in MACF1 contain only α-helices connected by short loop sequences, and they adopt a closed conformation with extensive intramolecular contacts between CH1 and CH2. Although the protein conformation upon binding of F-actin is unknown, biophysical studies with tandem CH domains from other spectrin proteins, such as α-actinin and dystrophin, strongly suggest that tandem CH domains undergo significant conformational changes when they associate with F-actin (Broderick et al., 2012; Lin et al., 2011; Singh and Mallela, 2012; Sjöblom et al., 2008).

Although a single CH domain can associate with F-actin, the presence of a tandem CH domain can greatly enhance the binding affinity (Sjöblom et al., 2008). For instance, the use of alternative transcription start sites gives rise to isoforms of *BPAG1* and *MACF1* (discussed below) that only have the CH2 domain, which has a very weak affinity for F-actin (Karakesisoglou et al., 2000; Leung et al., 1999; Yang et al., 1999). The functional relevance of this splicing event remains unclear.

Plakin domain

Another signifying feature of spectraplakins is their plakin domain. Plakins consist of six to nine spectrin-like repeats in a tandem array with a Src-homology-3 (SH3) protein–protein-interacting domain

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Table 1. Nomenclature of spectraplakin genes

Full protein name	Species	Official gene name	Protein aliases
Microtubule-actin crosslinking factor 1	Mammalian	*MACF1*	ACF7, MACF, OFC4, ABP620
Bullous pemphagoid antigen 1 or dystonin	Mammalian	*DST*	BP240, BPA, BPAG1, CATX-15, CATX15, D6S1101, DMH, DT, EBSB2, HSAN6, MACF2
Short Stop	*Drosophila*	*Shot*	Shortstop, Kakapo, Grv, Shot, Su(Mir)1, grv, kak, kop
Microtubule-actin crosslinking factor 1a	Zebrafish	*MACF1a*	Magellan
VAB-10	*C. elegans*	*VAB-10*	VAB-10A, VAB-10B

embedded within the fifth spectrin repeat (Choi and Weis, 2011; Sonnenberg et al., 2007) (see Fig. 1).

Whereas spectraplakins are involved in coordinating dynamic microtubule (MT) functions, plakins link the intermediate filament (IF) cytoskeleton and membrane–protein complexes to adhesive junctions (Jefferson et al., 2004). For example, the spektraplakin isoform BPAG1e is epidermally expressed and links the keratin IF network to hemidesmosomes (a specific cell–extracellular matrix junction) through α6β4 integrin and collagen XVII to strengthen the mechanical integrity at the base of the epidermis (Guo et al., 1995; Hamill et al., 2011; Koster et al., 2003).

Spectrin repeats

Spectrin repeats are a major component of spectraplakins. Each spectrin repeat consists of ∼100 residues, which fold into three α-helices. These helices have a heptad periodicity, enabling them to fold into an antiparallel coiled-coil (Djinovic-Carugo et al., 2002; Jefferson et al., 2004; Speicher and Marchesi, 1984). These spectrin repeats enable proteins to respond elastically to mechanical forces and act as a spacer region between functional domains at the N- and C-termini (Djinovic-Carugo et al., 2002; Röper and Brown, 2003; Sonnenberg et al., 2007).

The spectrin repeats in MACF1 associate with various signaling proteins involved in the Wnt pathway, such as Axin proteins, LRP6 and GSK3β (Chen et al., 2006). The spectratrin repeat domain also associates with the MT minus-end-binding-protein calmodulin-regulated spectrin associated protein family member 3 (CAMSAP3) (Noordstra et al., 2016). Speculations of other yet undiscovered interactions of spectrin repeats have been sparked due to the presence of a SH3 domain residing in the central spectrin repeat of α-spectrin (Sonnenberg et al., 2007). Another notable example of such a potential interaction domain, is the erzin/radixin/moesin (ERM) family protein-interacting domain that is located within the spectrin repeat region of BPAG1a1 (also known as BPAG1n4), through which BPAG1a1 interacts with dynactin to regulate retrograde axonal transport in sensory neurons (Liu et al., 2003).

EF-hand and GAR domain

The EF-hand and a GAS2-related protein domain (GAR) domain are encoded in the 3′ (C-terminal) exons of spectraplakins. The GAR domain is only found in a few proteins in addition to spectraplakins, such as growth arrest specific protein 2 (GAS2) and the GAS2-like proteins (GAS2L1, GAS2L2 and GAS2L3) (Akhmanova and Steinmetz, 2008; Jiang et al., 2012; Lander et al., 2001; Röper et al., 2002; Stroud et al., 2011, 2014). It was documented that the GAR domain in spectraplakins associates with and stabilizes MTs, thus helping to link MTs to other parts of the cytoskeleton (Lee and Kolodziej, 2002; Leung et al., 1999; Sun et al., 2001). However, there is new evidence to suggest that the GAR domain only has an accessory role in MT interactions, and there are other domains in spectraplakins that mediate MT binding, as well as plus-tip tracking (Alves-Silva et al., 2012; Applewhite et al., 2010). Interestingly, deletion of the GAR domain in GAS2 causes apoptotic-like rearrangements of the actin cytoskeleton (Brancolini et al., 1995), leaving open the possibility that this domain could have other functions besides binding to MTs.

The C-termini of spectraplakins also harbor the Ser-x-Ile-Pro (SxIP) motif. SxIP motifs exist in multiple MT plus-end-tracking proteins (+TIPs), including the spectraplakins MACF1 and BPAG1 (Akhmanova and Steinmetz, 2008; Gupta et al., 2010; Honnappa et al., 2009). MACF1 directly binds to EB1 (also known as MAPRE1) through its SxIP motif and exhibits MT plus-end tracking (Slep et al., 2005). The presence of SxIP motifs in the C-terminal region of BPAG1 controls its MT plus-end localization (Honnappa et al., 2009).

The EF hand is a helix-loop-helix structural motif present in many Ca^{2+}-binding proteins (Lewit-Bentley and Réty, 2000; Schäfer and Heizmann, 1996). The tandem EF-hand domains of spectraplakins are localized adjacent to the GAR domain and contain a proximal Ca^{2+}-dependent EF hands and a distal Ca^{2+}-independent EF (Fig. 1). It has been recently shown that the EF-hand motifs play a critical role in MT binding of BPAG1 (Kapur et al., 2012).

Plakin-repeat domain

Plakin repeat domains (PRDs) are unique to the plakin superfamily and a few spectraplakin isoforms. They consist of a conserved central core region, the plectin module, which is bound by less-conserved linker sequences (Janda et al., 2001). The plectin module is a globular domain consisting of 4.5 tandem repeats of a 38-amino-acid motif, called the plakin or plectin repeat (Janda et al., 2001; Jefferson et al., 2004), which forms a β-hairpin followed by two antiparallel α-helices (Choi et al., 2002). PRDs function in IF binding, as seen in BPAG1e and desmoplakin (Choi et al., 2002; Guo et al., 1995), plectin (Nikolic et al., 1996) and VAB-10A in *C. elegans* (Bosher et al., 2003).

Interestingly, *Drosophila* lacks cytoplasmic IFs, but flies have the spectraplakin isoform Shot B, which contains conserved plakin/plectin repeats (Röper and Brown, 2003; Röper et al., 2002) (see Fig. 1A). However, the plakin/plectin repeats in Shot B are not organized into groups of 4.5 tandem repeats, suggesting that they do not form such a PRD globular domain structure (Röper et al., 2002). Thus, the PRD may have other functions beyond IF binding.

Regulation of spectraplakins by cellular signaling networks

As unique cytoskeletal crosslinkers, the activity of spectraplakins must be spatiotemporally regulated to achieve specific cytoskeletal orchestration, and guidance of polarized and directional cellular processes as discussed below.

Cytoskeletal crosslinking and regulation by intramolecular inhibition

The cytoskeletal cross-linking ability of Shot has been suggested to be regulated by intramolecular inhibition (Applewhite et al., 2013, 2010) (Fig. 2A). In its inactive conformation, Shot takes on a closed conformation arising from interactions between its N-terminal

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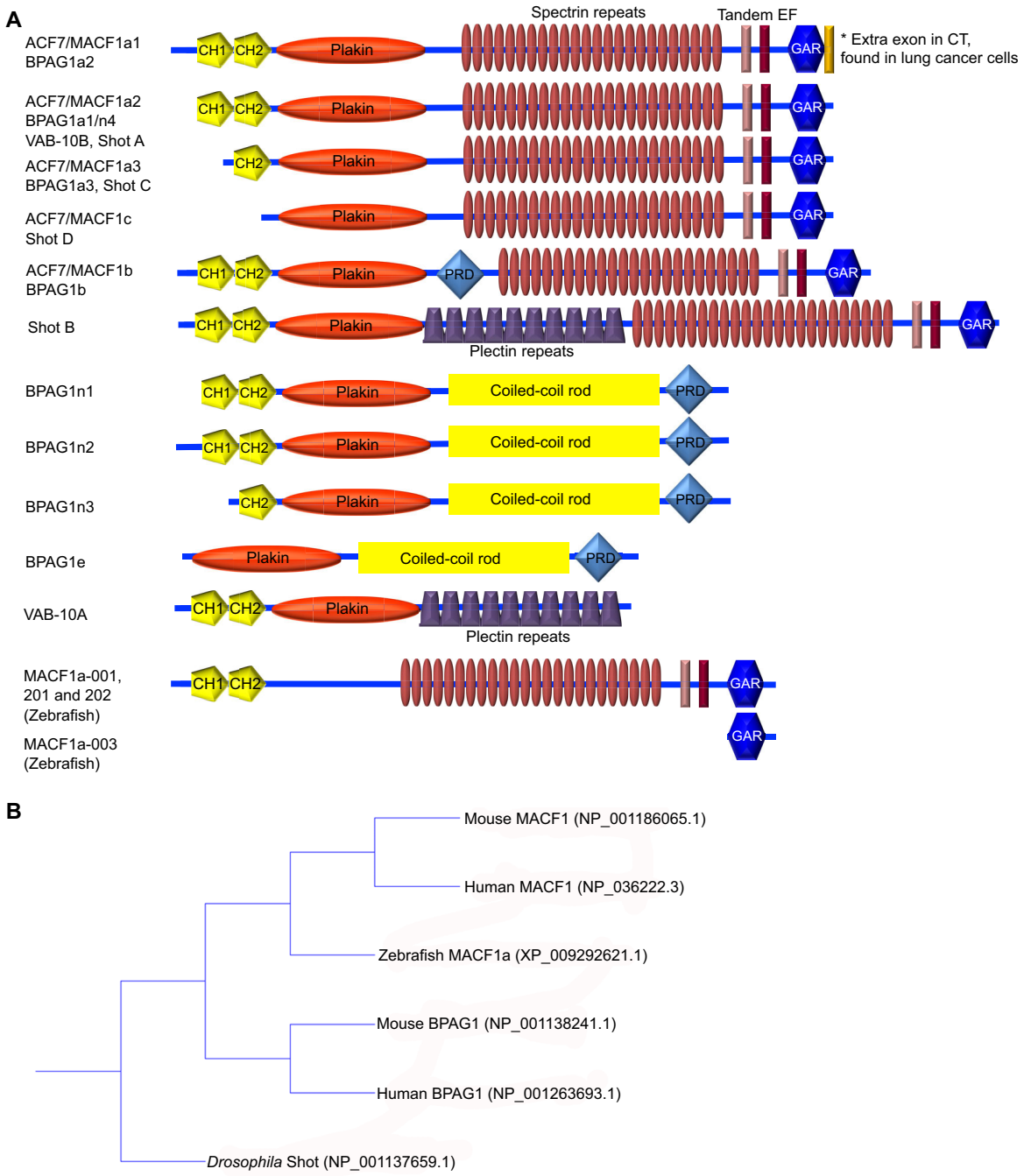

Fig. 1. Isoforms and domain structures of mammalian and invertebrate spectraplakins. (A) Major isoforms of the spectraplakin proteins MACF1, BPAG1, Shot, VAB-10 and MACF1a are classified according to their domain structure similarity. Many functional domains can be identified in spectraplakin family proteins, including: calponin homology (CH) domains for F-actin binding, a plakin domain, an α-helical spectrin repeat domain, a PRD, plectin repeats, tandem EF-hand motifs (the distal and proximal motif to the GAR domain are differentially colored), and a GAS2-related (GAR) domain for MT interaction. The number of spectrin repeats and plectin repeats are descriptive in nature and may vary in different spectraplakins. The figure is not drawn to scale with respect to the actual spectraplakin proteins. This panel has been adapted from Suozzi et al. (2012) with permission. (B) The similarity of the spectraplakin members. The homology tree was generated using MAFFT version 5 with annotated sequences of spectraplakin proteins retrieved from NCBI genebank database (accession numbers given in parenthesis) (Katoh et al., 2005).

F-actin-binding domain and the C-terminal EF-hand GAR domain. In this auto-inhibited conformation, Shot cannot associate with either F-actin or MTs, but is able to bind the +TIP EB1 and so to localize to the plus-ends of growing MTs. It is thought that in response to unknown activating signals at the cell cortex, this intramolecular inhibition will be disrupted, mediating the activation of Shot. In its active conformation, Shot is able to crosslink cytoskeletal networks by binding to the MT through its C-terminal GAR domain and simultaneously to F-actin with its N-terminal tandem CH domains (Applewhite et al., 2013, 2010).

Phosphorylation of MACF1

During cell migration, the turnover of focal adhesions, which allows cell retraction, is an essential step (Wozniak et al., 2004). MACF1

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Box 1. Isoforms originating from splicing

Differential splicing of the mammalian *BPAG1* gene gives rise to several proteins, with the first *BPAG1* isoforms reported being *BPAG1e* and *BPAG1n* (Li et al., 1992; Sawamura et al., 1990). Additional *BPAG1e* isoforms, including *BPAG1eA*, *BPAG1eS* and *BPAG1eB*, are found in muscle (Okumura et al., 2002). There are two *BPAG1n* isoforms, which are derived from the use of distinct neuronal transcriptional start sites that produce different short peptides preceding the F-actin-binding domain (Bernier et al., 1996; Lee, 2000; Yang et al., 1999). Furthermore, there is also neuronal *BPAG1n3*, whose transcription start site begins within the F-actin-binding domain (Liu et al., 2003). Besides the above-mentioned isoforms, *BPAG1a* and *BPAG1b* are two other major isoforms encoded by the *BPAG1* gene (Leung et al., 2001b). These isoforms derive from expression of the same N-terminal exons as BPAG1n1 to BPAG1n3, but their C-terminal segments originate from the exons encoding the spectrin repeat region, the EF hand downstream and the GAR domain. BPAG1a (BPAG1n) is highly expressed in the nervous system, whereas BPAG1b, which contains plakin/plectin repeats, is the major isoform found in muscles. BPAG1b contains an additional binding domain for IFs compared to BPAG1a. Moreover, additional alternative splicing within the first exon of *BPAG1a* creates three further isoforms (*BPAG1a1*, *BPAG1a2* and BPAG1*a3*) (Jefferson et al., 2006). These exhibit a differential tissue-distribution pattern and different binding capabilities to bind to the F-actin network (Jefferson et al., 2006).

At least three transcription start sites have been described for *MACF1* (Roper et al., 2002), giving rise to the three isoforms *MACF1a1*, *MACFa2* and *MACF1a3*. Similar to *BPAG1b*, there is also a MACF1b isoform, which contains a plakin repeat domain in the middle of the protein (Lin et al., 2005). The MACF1b isoform is highly expressed in the lung, and has been shown to mainly localize to the Golgi in cultured cells (Lin et al., 2005). A novel MACF1c isoform has been described in a conditional knockout study in the nervous system (Goryunov et al., 2010) and was shown to differ from MACF1a by the absence of the N-terminal actin-binding domain. A recent alternative splicing profiling in lung cancer identified another differential splicing isoform within the MT-binding domain of *MACF1*, which was upregulated in lung cancer cells (Misquitta-Ali et al., 2011).

The *vab-10* locus in *C. elegans* gives rise to two sets of isoforms that result from alternative splicing at a common 5′-start site, but entail different 3′ regions (Bosher et al., 2003). VAB-10A has a C-terminus that contains plakin repeats, whereas VAB-10B has a C-terminus with spectrin repeats (see Fig. 1) (Bosher et al., 2003). These two isoforms also have different distributions in the epidermis; VAB-10A is essential for epidermis attachment to the extracellular cell matrix, whereas VAB-10B maintains a connection between the apical and basal epidermis and plasma membranes during morphogenesis (Bosher et al., 2003). EMSEMBL (ID# WBGene00006876) reports seven other isoforms of the *vab-10* gene whose functions are as yet unclear. In *Drosophila*, there are at least 22 different isoforms of Shot/Kakapo (EMSEMBL gene # FBgn0013733). Among them, Shot A and Shot B, which harbor F-actin-binding domains, are expressed in the central nervous system, whereas Shot C and Shot D are not (Lee et al., 2000). Potential differential splicing of the zebrafish *MACF1a* gene results in four isoforms (MACF1a-001, -201, -202 and -003, EMSEMBL gene # ENSDARG00000028533). The zebrafish spectraplakins are shorter than their mammalian counterparts and do not contain plakin or PRD domains (Fig. 1).

promotes focal adhesion dynamics by targeting MT plus-ends to focal adhesions. Recent structural analysis indicates that the tandem CH domains in MACF1 adopt a closed conformation with extensive intramolecular contacts; this precludes F-actin binding, as this typically requires the transition to an open conformation between the CH domains (Lin et al., 2011; Moores et al., 2000; Sjöblom et al., 2008). Focal adhesion kinase (FAK, also known as PTK2) and Src tyrosine kinases are two prominent signaling molecules that are activated by integrin receptors in focal adhesions (Brunton and Frame, 2008). Indeed, our recent study identified a key tyrosine residue in the CH domain of MACF1, whose phosphorylation by Src or FAK is essential for the conformational switch of the tandem CH domains and thus F-actin binding (Fig. 2B) (Yue et al., 2016). Using skin epidermis, we demonstrated that the phosphorylation of MACF1 plays an important role in focal adhesion dynamics and epidermal migration both *in vitro* and *in vivo* (Yue et al., 2016).

In addition to tyrosine phosphorylation, we have previously shown that glycogen synthase kinase 3 (GSK3) can directly phosphorylate MACF1 within its MT-binding domain (Wu et al., 2011). This phosphorylation can disrupt the interaction between MACF1 and MTs (Fig. 2B), and has a role in the regulation of skin bulge stem cell migration and skin wound repair (Wu et al., 2011). Consistent with these findings, ErbB2-induced inhibition of GSK3 kinase activity has been found to be essential for plasma membrane localization of adenomatous polyposis coli protein (APC) and CLASP2, which regulate MT dynamics and recruit MACF1 to the plasma membrane for the capture and stabilization of MTs in breast carcinoma cells (Zaoui et al., 2010).

Regulation of spectraplakin activity by Ca^{2+}

EF-hand motifs function as Ca^{2+}-dependent molecular switches (Atkinson et al., 2001; Gifford et al., 2007; Yap et al., 1999), and the presence of tandem EF-hand motifs in spectraplakins raises the possibility that cytoskeletal cross-linking mediated by them could be regulated by Ca^{2+}. In fact, a recent study of the mammalian spectraplakin BPAG1n4 demonstrated that increased calcium led to a rapid switching between the binding of the C-terminal MT-binding domain of BPAG1n4 from MT plus-ends to the MT lattice (Fig. 2B) (Kapur et al., 2012). This regulation was shown to be EF-hand- and Ca^{2+}-dependent. This calcium-dependent regulatory mechanism might regulate other spectraplakins with EF hand motif (such as MACF1) and have a critical role in different MT-associated processes.

The various binding partners of spektraplakins

As mentioned above, spectraplakins associate with EB proteins and so localize at the plus-ends of MTs. EB proteins influence MT dynamic instability, which is essential for MT reorganization during apico-basal epithelial differentiation (Goldspink et al., 2013). In this context, it has been shown that the expression of *EB2* (also known as *MAPRE2*) and not *EB1*, is crucial for initial MT reorganization, and that downregulation of *EB2* can promote the association of MACF1 with the MT lattice and its co-aligment with actin filaments, as well as MT bundle formation (Goldspink et al., 2013).

The engulfment and cell motility (ELMO) proteins are adaptor proteins that function as part of a protein complex to form guanine nucleotide exchange factors (GEFs) for Rho family small GTPases (Côté and Vuori, 2007). MACF1 has recently been shown to interact with ELMO1. Co-expression of ELMO1 with MACF1 promotes the formation of long membrane protrusions during integrin-mediated cell spreading, and the binding of ELMO1 to MACF1 increases the persistence of the protruding activity (Margaron et al., 2013). Thus, ELMO1 play a role in the recruitment of MACF1 to the membrane to promote its function in MT capture and stability.

In epithelial cells, MTs form parallel arrays along the apico-basal axis. Recent work has shown that members of the calmodulin-regulated spectrin-associated protein (CAMSAP)/Patronin family control apical tethering of MT minus ends. Work in mammalian intestinal epithelial cells, 3D cultures and short-term epithelial

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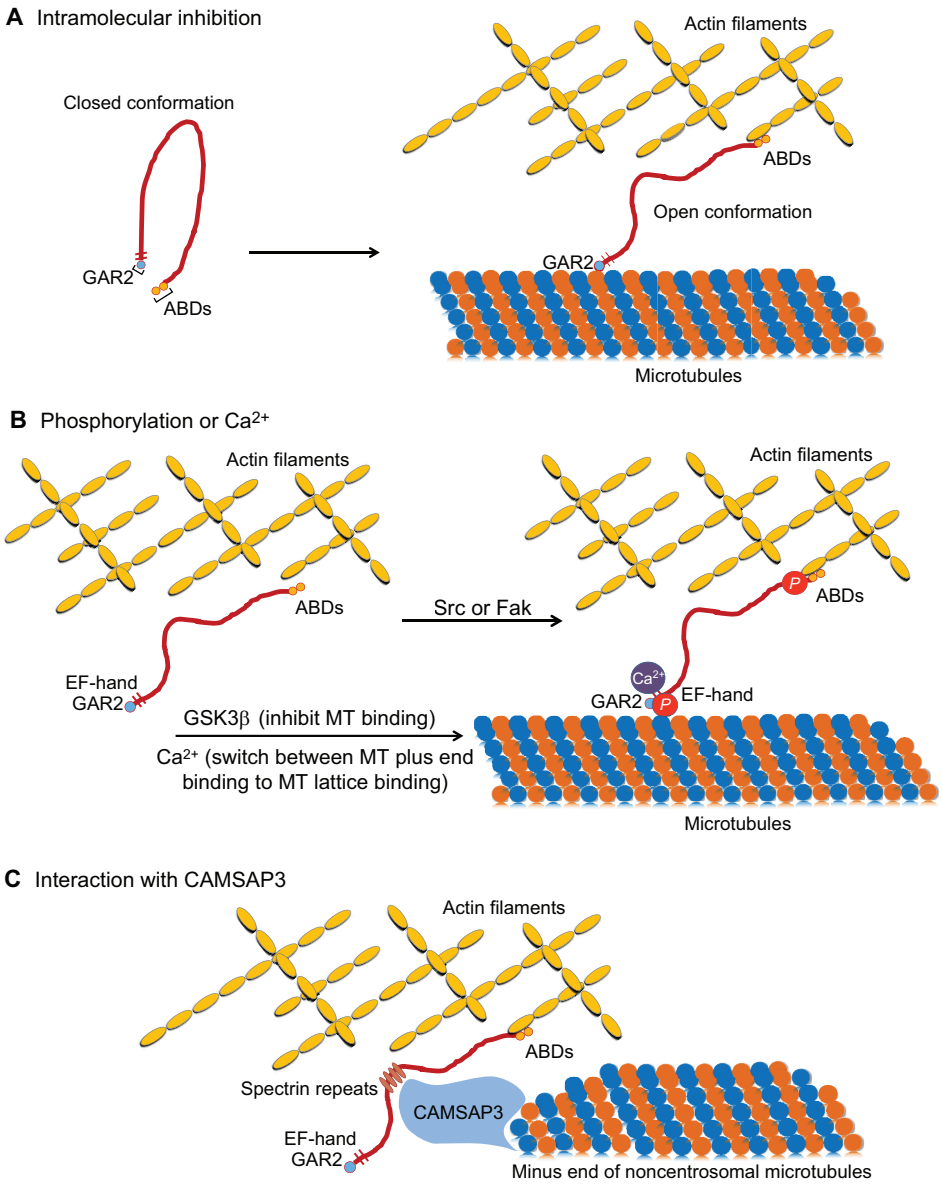

Fig. 2. Molecular mechanisms of spectraplakin regulation. (A) The *Drosophila* spectraplakin Shot is regulated by intramolecular inhibition. An intramolecular auto-inhibitory interaction occurs between the N-terminal actin-binding domains (ABDs) and the C-terminal MT-binding GAR domain. In response to activating signals at the cell cortex, this intramolecular inhibitory interaction is disrupted and Shot is able to crosslink cytoskeletal networks by binding to both MTs and F-actin. (B) The cytoskeletal crosslinking activity of mammalian spectraplakins can be regulated by protein phosphorylation. Tyrosine phosphorylation (*P*) at the N-terminal F-actin-binding domain of MACF1 can enhance its association with F-actin, and promote focal adhesion dynamics in cultured skin keratinocytes. In addition, phosphorylation of its C-terminal MT-binding domain by GSK3 kinase can disrupt its interaction with MTs. Ca^{2+} can also regulate BPAG1n4 through its C-terminal EF-hand motifs. An increase in Ca^{2+} can lead to a rapid switching between the MT-plus-end and lattice-binding of the C-terminal domain of BPAG1n4. (C) The spectraplakin MACF1 interacts with CAMSAP3 at the minus end of noncentrosomal MTs and anchors them to actin filaments.

polarization assays has demonstrated that MACF1 regulates apico-basal polarity in mammalian intestinal cells through binding with CAMSAP3 and anchoring MT minus-ends to F-actin (Ning et al., 2016; Noordstra et al., 2016).

In CaCO2 epithelial cells, MACF1 interacts with CAMSAP3 at the minus-end of noncentrosomal MTs and anchors them to actin filaments (Fig. 2C), which contributes to the regulation of focal adhesion size and cell migration (Ning et al., 2016; Noordstra et al., 2016). In *Drosophila* epithelia, the MT-binding proteins Patronin, which has three human homologs, CAMSAP1, CAMSAP2 and CAMSAP3, and Shot are recruited to the apical membrane domain, where they polarize MTs along the apical-basal axis to enable apical transport of Rab11-containing endosomes and so contribute to the biogenesis of actin microvilli through the delivery of key microvillar determinant Cadherin 99C (Khanal et al., 2016). In *Drosophila* oocytes, Shot interacts with the cortex through its actin-binding domain and recruits Patronin to form the cortical noncentrosomal MT-organizing centers, where the Shot–Patronin complex can capture and stabilize existing MT minus-ends and promote new MT growth (Nashchekin et al., 2016).

Spectraplakin functions in vertebrates

Based on genetic studies in vertebrate models including mice, it is clear that spectraplakins perform essential functions in various tissue and organs, including muscle, neurons, and skin epithelium, where extensive remodeling and coordinated dynamics of cytoskeletal networks are required for tissue development, homeostasis, and wound repair.

Cellular functions of BPAG1

A number of findings suggest that a key function of BPAG1 is to regulate the organization and stabilization of the MT network of sensory neurons to maintain axonal transport (Liu et al., 2003; Yang et al., 1999). Loss of BPAG1 function in mice results in a lethal movement disorder known as dystonia musculorum (*dt/dt*), which is likely caused by rapid sensory neuron degeneration (Duchen et al., 1964). The *dt/dt* mice display skin blistering, and sensory neuron and muscle degeneration, with each of these cell types exhibiting defects in cytoskeletal organization (Dalpé et al., 1998; Liu et al., 2003; Ryan et al., 2012b,c). Consistent with this notion, the loss of BPAG1 in *dt/dt* neurons results in short,

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disorganized and unstable MTs that are defective in axonal transport (Yang et al., 1999).

It is not completely understood how exactly loss of *BPAG1* contributes to neuronal degeneration. It has been shown that the BPAG1a2 isoform (also known as dystonin-a2) has a role as a mediator of normal ER structure and function. For example, *BPAG1* mutant mice show defects at the ER in sensory neurons, which corresponds to the induction of ER-stress proteins (Young and Kothary, 2008). ER stress leads to sensory neurodegeneration through the induction of a proapoptotic caspase cascade (Nakagawa et al., 2000). BPAG1a2 potentially regulates the ER response by linking actin filaments with the ER membrane, which is critical for maintaining neuronal integrity (Young and Kothary, 2008). Moreover, BPAG1a2 associates with microfilaments that surround the nucleus and Golgi, and thus may also be involved in cytoskeletal organization and membrane attachment in the nuclear region (Young et al., 2006) (see Fig. 3). Recently, it has been found that BPAG1a2 also has a role in protein trafficking by promoting MT acetylation through its association with the MT-associated protein 1B (MAP1B) around the centromere (Ryan et al., 2012a).

Epidermal BPAG1e interacts with the hemidesmosomal proteins α6β4 integrin and collagen XVII in basal keratinocytes and mediates epidermal–dermal cohesion (Borradori and Sonnenberg, 1999; Hopkinson and Jones, 2000; Koster et al., 2003; Leung et al., 2001b; Litjens et al., 2006; Sawamura et al., 1991a,b). In addition, BPAG1e also binds to the epidermal cytokeratin network that is formed by keratins 5 and 14 (K5, K14) in basal keratinocytes and thereby connects them to the extracellular matrix (Fontao et al., 2003; Guo et al., 1995). In the epidermis, loss of *BPAG1e* leads to a selective fragility of the base of the columnar basal cells, resulting in blisters and compromised wound healing (Guo et al., 1995). The phenotype of mice with an epidermal-specific deletion of *BPAG1* resembles that of bullous pemphigoid, a human skin disease in which patients produce autoantibodies against BPAG1 (Labib et al., 1986; Mueller et al., 1989; Diaz et al., 1990). Mutations in *BPAG1* have also been found in humans with an inherited skin fragility disorder (Groves et al., 2010). Additionally, it has been showed that BPAG1e acts as an essential component of the signaling pathway by which β4-integrin regulates front-to-rear cell polarity and cell migration in skin (Michael et al., 2014).

Cellular function of MACF1

MACF1 has a role in MT stabilization and cell motility (Drabek et al., 2006) (Fig. 3). Accordingly, the loss of MACF1 in cultured endodermal cells results in less stable and long MTs with skewed cytoplasmic trajectories. Indeed, a full *MACF1* knockout in the developing mouse embryo caused preimplantation lethality (Kodama et al., 2003). In cultured skin keratinocytes, MACF1 deficiency impairs focal adhesion dynamics, as MT ends are unable to converge normally at the peripheral focal adhesion and focal adhesions thus become highly stabilized and refractory to the normal dynamics required for efficient cell migration (Wu et al., 2008).

Another cellular process that relies on MACF1 is the transport of vesicles from the trans-Golgi network (TGN) to the cell periphery as the TGN protein p230 (also known as GOLGA4), which is anchored to TGN membranes, was shown to interact with MACF1, allowing for transport of glycosylphosphatidylinositol (GPI)-anchored proteins, along the MT and actin cytoskeleton (Kakinuma et al., 2004) (Fig. 3).

Interestingly, MACF1 also plays a role in the Wnt signaling pathway (Chen et al., 2006). *MACF1*-deficient mouse embryos have defects in the formation of the primitive streak, node and mesoderm (Chen et al., 2006), which is similar to the phenotype observed upon loss of *Wnt3*, the earliest-acting Wnt in mammals (Liu et al., 1999), or loss of the Wnt co-receptors *LPR5* and *LPR6* (Kelly et al., 2004). There is evidence suggesting that MACF1 binds to the Axin Wnt signaling inhibitor proteins, which promotes β-catenin phosphorylation and degradation, and so inhibits Wnt signaling (Chen et al., 2006). Thus, MACF1 may act as a positive regulator of Axin proteins by helping to transport them to LRP6 at the cell membrane.

MACF1 is also important in nervous system development (Goryunov et al., 2010). *MACF1* depletion in mouse primary cortical neurons caused a variety of defects and a significant decrease in axon length (Sanchez-Soriano et al., 2009). Defects in axon outgrowth suggest that MACF1 plays a role in axon guidance (Sanchez-Soriano et al., 2009).

In the peripheral nervous system, MACF1 tethers acetylcholine receptors to the actin cytoskeleton (Antolik et al., 2007). Results from *in vitro* co-transfection studies suggest that acetylcholine receptors are anchored to the membrane in mammalian skeletal muscle by binding to rapsyn (also known as RAPSN), which interacts with the actin-binding domain of MACF1 (Antolik et al., 2007).

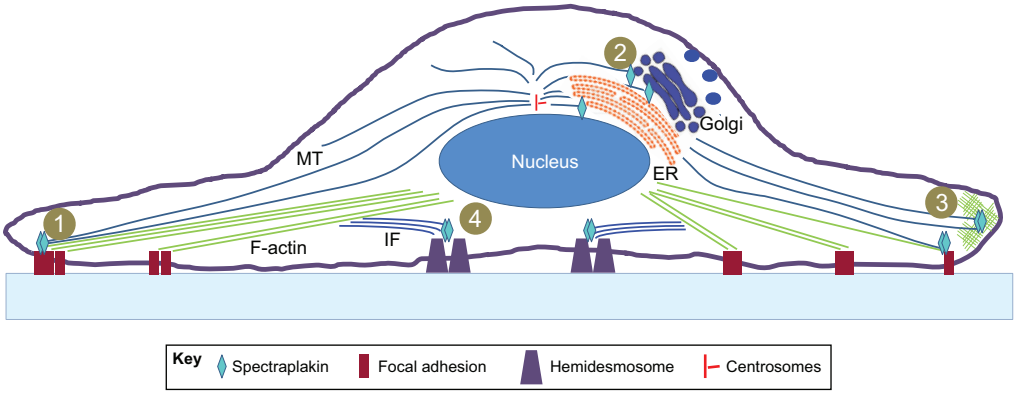

Fig. 3. Localization and cellular function of spectraplakins. Spectraplakins orchestrate various cytoskeletal interactions and mediate crosslinking in different cellular compartments. Therefore, they are able to regulate different cellular processes as illustrated here. 1, spectraplakins mediate the MT targeting of MTs to focal adhesions, which promotes focal adhesion turnover and cell motility; 2, spectraplakins have been shown to regulate the integrity of the nucleus, ER and the Golgi complex, as well as and vesicle transport; 3, spectraplakins also associate with adaptors such as ELMO proteins and mediate cytoskeletal crosslinking at the cortical membrane; 4, spectraplakins connect hemidesmosomes with IFs and strengthen the integrity of the basal layer.

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MACF1 also regulates the migration of pyramidal neurons, dendritic aborization and axon outgrowth. Neuronal migration and differentiation are critical for establishing functional neural circuitry in the developing brain. MACF1 plays a role in determining neuronal positioning by regulating MT dynamics and mediating GSK3 signaling during brain development, which was demonstrated using MACF1 conditional knockout mice and *in utero* gene manipulation (Ka et al., 2014).

MACF1 also has a critical role in skin wound repair. The deletion of the *MACF1* gene in skin epidermal cells resulted in a significant delay in wound repair due to impaired focal adhesion dynamics and epidermal migration (Wu et al., 2008, 2011).

A role for MACF1 in the colon and intestine has recently been demonstrated *in vivo* (Liang et al., 2013) based on conditionally knockout of *MACF1* in the intestinal mucosa of mice. Here, *MACF1* deficiency resulted in colonic epithelial cell rearrangement and decreased colonic paracellular permeability (Liang et al., 2013).

Furthermore, MACF1 has been found to be involved in MT distribution in cardiomyocytes and their adaptation to hemodynamic overload. Expression of *MACF1* was shown to increase significantly in response to transverse aortic constriction (TAC), and, accordingly, *MACF1* conditional knockout exacerbated transverse aortic constriction (TAC)-induced heart hypertrophy, ventricular dilation and contractile dysfunction. Based on this, it has been suggested that MACF1 is important for spatial regulation of several signaling proteins, including PKC-α and β1 integrin, during heart hypertrophy (Fassett et al., 2013).

In both mouse and zebrafish vestibular and cochlear hair cells, hair bundles integrate actin and MT network. A recent electron tomography study showed that MACF1 is interwoven into the cuticular plate (CP) and encircles the basal body of the kinocilium, which connects MTs to the CP. These observations are consistent with a role for MACF1 as a linker protein that helps to shape the cytoskeleton of the hair cell early during hair-bundle genesis (Antonellis et al., 2014).

Spectraplakin in zebrafish

MACF1a (also named *Magellan*), the spectraplakin found in zebrafish (Dosch et al., 2004), plays a critical role in forming the animal vegetal axis (Gupta et al., 2010). *MACF1* regulates the size and localization of the Balbiani body – an aggregate found in developing oocytes, which consists of organelles, including the mitochondria, ER and Golgi. This parallels the localization of Shot on the fusome during fly oogenesis (Röper and Brown, 2004).

Spektraplakin functions in invertebrates

VAB-10

Analyses of *C. elegans vab-10* mutants show that VAB-10A is essential in fibrous organelles, which mediate muscle connection to the cuticle across the epidermis (Bosher et al., 2003). The loss of *VAB-10A* reduces the number of fibrous organelles and the ability of the epidermis to attach to the underlying basement membrane. By contrast, the loss of *VAB-10B* leads to an increase in epidermal thickness (Bosher et al., 2003). Here, VAB-10B protects cells from internal shearing forces, as it likely anchors cortical actin to the MT network (Bosher et al., 2003). VAB-10B also directs nuclear translocation and cell migration in gonadal distal tip cell by mediating actin filament and MT crosslinking (Kim et al., 2011). Furthermore, a recent study demonstrated that VAB-10A can link the *C. elegans* fusogenic protein epithelial fusion failure 1 (EFF-1) to the F-actin network and so enhance cell–cell fusion (Yang et al., 2017).

Functions of Shot

Mutations of *shot* in *D. melanogaster* lead to a wide variety of defects, including pertubations in cytoskeletal organization, cell adhesions and integrin-mediated cell attachment (Röper and Brown, 2003), developmental abnormalities in the foregut and tracheal tubes (Fuss et al., 2004; Lee et al., 2003), defects in neuronal growth (Lee and Kolodziej, 2002), defects in MT association with the fusome during oogenesis (Röper and Brown, 2004), and aberrant formation and maintenance of tendon cells (Alves-Silva et al., 2008; Strumpf and Volk, 1998; Subramanian et al., 2003). As these effects have been well documented previously (Leung et al., 2001a; Röper et al., 2002; Suozzi et al., 2012), we will focus here on the more recent findings. It has been recently shown that Shot regulates filopodia formation in a manner that is dependent on the function of its EF-hand domains (Sanchez-Soriano et al., 2009). The EF-hand motifs of Shot associate with the translational regulator Kra/eIF5C, which might regulate the translation of actin and actin regulators that govern the polarized induction of new filopodia (Sanchez-Soriano et al., 2009).

Shot is also indispensable for photoreceptor morphogenesis, and is required for the correct positioning and/or targeting of the apical domain, adherens junctions and stable microtubules during photoreceptor development (Mui et al., 2011). Furthermore, in the *Drosophila* oocyte, Shot associates with Patronin and serves as the cortical anchor for noncentrosomal MT-organizing centers, which functions in a similar manner to MACF1 (Nashchekin et al., 2016). Shot also functions in regulating MT reorganization, which is required for the localization of axis-determining mRNAs during oogenesis. Shot also links MTs with the actomyosin network during tubulogenesis of the salivary gland epithelia in embryos (Booth et al., 2014).

Moreover, together with Tau protein, Shot promotes synapse formation and maintenance through Jun kinase (JNK)-mediated neuronal trafficking. Here, Tau and Shot act upstream of a three-step regulatory cascade, in which MTs activate JNK signaling, which in turn regulates kinesin-3-mediated axonal transport to ensure adequate delivery of synaptic proteins. This cascade acts both during development (synapse formation) and aging (synapse maintenance) (Voelzmann et al., 2016). These findings might explain the intellectual disability in Tau-deficient individuals, as well as early synapse loss in patients suffering dementia, such as those with Alzheimer's disease.

Finally, dual leucine zipper-containing kinase (DLK, also known as Leucokinin, Lk) in *Drosophila* is activated in the absence of Shot. DLK is a component of the axonal injury response pathway and promotes the degeneration of distal axons and regeneration of proximal axons. The activation of DLK in the absence of Shot suggests that DLK is activated by cytoskeletal instability, which is a common feature shared between spectraplakin mutants and injured axons (Valakh et al., 2013). In agreement with this, disruption of MTs by nocodazole treatment in mammalian sensory neurons activates the DLK signaling pathway (Valakh et al., 2015).

Spectraplakins in human disease

As coordinated cytoskeletal dynamics are critically involved in many biological processes, accumulating recent evidence has begun to unveil the potential role of spectraplakin proteins in pathogenesis of different human diseases as discussed below.

Spectraplakins in neuronal degeneration and Parkinson's disease

It is well known that loss-of-function mutations in *Bpag1* in mice leads to a lethal movement disorder, dystonia musculorum, which is

likely caused by degeneration of sensory neurons (Ferrier et al., 2013; Guo et al., 1995; Lynch-Godrei and Kothary, 2016; Yang et al., 1996; Young and Kothary, 2008). Consistent with this, the hereditary and sensory autonomic neuropathy type VI in humans potentially associates with mutations in the human *BPAG1* gene (Edvardson et al., 2012; Ferrier et al., 2014). Mutations in the major neurodegeneration-associated genes TAR DNA-binding protein (*TARDBP*), fused in sarcoma (*FUS*) and leucine-rich repeat kinase 2 (*LRRK2*) are associated with alternative splicing of *BPAG1*, which may be involved in development of neurodegeneration conditions such as Parkinson's disease (Elliott et al., 2012).

BPAG1 in epidermolysis bullosa simplex

In the epidermis, loss of BPAG1e results in fragility of the base of the columnar basal cells, and even internal cell degeneration, blisters and compromised wound healing (Guo et al., 1995). As noted above, the epidermis of *dt/dt* mutant mice resembles that of bullous pemphigoid, a human disorder where autoantibodies against BPAG1 are produced (Diaz et al., 1990; Labib et al., 1986; Mueller et al., 1989). A *BPAG1e*-specific mutation has been found in humans with an inherited skin fragility disorder (Groves et al., 2010). This mutation within the coiled-coil domain of BPAG1e is associated with the loss of only the hemidesmosomal inner plaque. In parallel to skin blistering in *BPAG1*-null epidermis (Guo et al., 1995), this mutation also resulted in the fragility of the basal cells and an autosomal recessive form of epidermolysis bullosa simplex (EBS) (Groves et al., 2010; Liu et al., 2012). Recent studies in keratinocytes that harbor the naturally occurring mutations have addressed the underlying mechanisms and demonstrated a key role for BPAG1e in regulating keratinocyte adhesion and migration (Hamill et al., 2009; Michael et al., 2014).

BPAG1 in infection – a role in viral capsid transportation

Upon cell infection by herpes simplex virus 1 (HSV-1), the viral capsid is transported throughout the cytoplasm along the MT network. It has been shown that BPAG1 is recruited to HSV-1 capsids by the capsid-bound tegument protein pUL37 to promote efficient cytoplasmic transport of capsids (Pasdeloup et al., 2013). A second study concluded that BPAG1 has a specific role in plus-end transport of capsids from the centrosome to the nucleus during the entry of HSV-1 (McElwee et al., 2013). As centrosomes are the principal MT-organizing centers, capsids first travel to the centrosome by minus-end-directed transport, before they switch polarity to plus-end-directed transport in order to travel to the nucleus. BPAG1 depletion inhibited capsid transport to the centrosome and also significantly impaired capsid transport away from the centrosome; this resulted in the accumulation of virus capsids in the vicinity of the centrosome, thereby adversely affecting virus replication (McElwee et al., 2013).

Duplication of *MACF1* in neuromuscular dysfunction

A recent study showed that a heterozygous duplication of the *MACF1* locus on human chromosome 1p34.4 can lead to a diminished *MACF1* gene product *in vivo*, which was found to cause a novel type of muscular dystrophy (Jørgensen et al., 2014). Here, the reduced levels of MACF1 in the affected patients leads to periodic hypotonia, facial weakness, lax muscles, diminished motor skills, contractures and muscle pains, all indicative of muscular dystrophy. These findings strongly suggest that MACF1 is potentially implicated in the development of this novel neuromuscular condition.

Spectraplakins in human cancer

A BPAG1 autoantibody has been identified as a marker for human melanoma (Shimbo et al., 2010). Although BPAG1 was initially found to be expressed in keratinocytes, it is now known to be expressed in many cell types, including melanoma cell lines (AA375 and G361) and tumors, as well as in normal melanocytes (Shimbo et al., 2010). However, this raises the question as to whether there are specific isoforms of BPAG1 that are expressed in melanocytes and might be involved in melanoma.

In addition, MACF1 has also been implicated in human cancers as it was identified as a candidate cancer gene for breast cancer (Sjöblom et al., 2006). Although this finding has been questioned after reanalysis (Rubin and Green, 2007), a more recent study detected alternative exons in MACF1 transcripts in adenocarcinoma tumors from patients with non-small cell lung carcinoma (Misquitta-Ali et al., 2011). Because MACF1 functions in the Wnt signaling pathway (Chen et al., 2006), the increased inclusion of the alternative exon could lead to altered Wnt signaling, which is often associated with lung cancer and other types of cancers (Moon et al., 2004); however, this needs to be validated directly.

Conclusion and prospects

Recent research on spectraplakins has highlighted the cellular roles of this fascinating group of multifunctional cytoskeletal linker proteins. The complexity of their gene structure allows for a significant diversity in promoter usage and exon splicing, thus creating a plethora of isoforms that exhibit spatiotemporal specificity to satisfy the cytoskeletal-junctional requirements in different cell types. They not only govern normal cellular processes, but can also contribute to a variety of abnormal phenotypes when mutated. Future studies will be essential to fully understand the spatiotemporal regulation of the cytoskeletal crosslinking function mediated by spektraplakins and to unravel any novel roles in both physiological and pathological settings.

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