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The role of the drebrin/EB3/Cdk5 pathway in dendritic spine plasticity, implications for Alzheimer's disease.

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Highlights

- The cytoskeleton underlies cell shape changes in response to guidance cues
- The drebrin/EB3/Cdk5 pathway couples dynamic microtubules to actin filaments
- Microtubule insertion into dendritic spines facilitates spine plasticity
- Microtubule insertion into dendritic spines requires drebrin and EB3
- Loss of drebrin contributes to cognitive decline In Alzheimer's disease

Abstract

The drebrin/EB3/Cdk5 intracellular signaling pathway couples actin filaments to dynamic microtubules in cellular settings where cells are changing shape. The pathway has been most intensively studied in neuronal development, particularly neuritogenesis and neuronal migration, and in synaptic plasticity at dendritic spines in mature neurons. Drebrin is an actin filament side-binding and bundling protein that stabilizes actin filaments. The end-binding (EB) proteins are microtubule plus-end tracking proteins (+TIPs) that localize to the growing plus-ends of dynamic microtubules and regulate their behavior and the binding of other +TIP proteins. EB3 binds specifically to drebrin when drebrin is bound to actin filaments, for example at the base of a growth cone filopodium, and EB3 is located at the plus-end of a growing microtubule inserting into the filopodium. This interaction therefore forms the basis for coupling dynamic microtubules to actin filaments in growth cones of developing neurons. Appropriate responses to growth cone guidance cues depend on actin filament/microtubule co-ordination in the growth cone, although the role of the drebrin/EB3/Cdk5 pathway in this context has not been directly tested. A similar cytoskeleton coupling pathway operates in dendritic spines in mature neurons where the activity-dependent insertion of dynamic microtubules into dendritic spines is facilitated by drebrin binding to EB3. Microtubule insertion into dendritic spines drives spine maturation during long-term potentiation and therefore has a role in synaptic plasticity and memory formation. In Alzheimer's disease and related chronic neurodegenerative diseases, there is an early and dramatic loss of drebrin from dendritic spines that precedes synapse loss and neurodegeneration and might contribute to a failure of synaptic plasticity and hence to cognitive decline.

Keywords: dendritic spines, synaptic plasticity, actin, microtubules, drebrin, EB3, ADF/cofilin, myosin II, Alzheimer's disease

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1. Introduction

Drebrin is a filamentous (F)-actin side-binding and bundling protein with roles in neuronal development and synaptic plasticity (reviewed in: Dun and Chilton, 2010; Shirao and González-Billault, 2013; Gordon-Weeks and Fournier, 2014; Gordon-Weeks, 2016). Drebrin is highly expressed in neurons, where it has been intensively studied, but there are also high levels in other cell types including mesangial cells, muscle, lymphocytes, podocytes, seminiferous epithelium, endothelial cells and some metastatic tumours (Dun and Chilton, 2010; Dart and Gordon-Weeks, 2016). In the mammalian nervous system, an embryonic form, drebrin E, is replaced by an alternatively spliced adult form, drebrin A, distinguished by a 46 amino acid insert of unknown function (Kojima et al., 2016). In embryonic rat hippocampus, for example, drebrin E predominates whereas in early postnatal hippocampus, during the time of maximum synaptogenesis, both forms are present and by adolescence drebrin A predominates (Kojima et al., 2016). Drebrin E is distributed throughout the developing neuron, although highly concentrated in growth cones, whereas drebrin A is particularly concentrated in the dendritic spines of excitatory (glutamatergic) synapses in the cerebral cortex and hippocampus. The sub-cellular distribution of drebrin in growth cones is well documented whereas its distribution in dendritic spines is less clear because of their very small size. In growth cones, drebrin E is mainly localised to the transition zone (Tzone), which forms at the junction between the central and peripheral domains, where anti-parallel bundles of F-actin are found, and the base of some filopodia, where parallel actin-filament bundles occur (Geraldo et al., 2008; Mizui et al., 2009; Worth et al., 2013). Although wide-field and confocal immunofluorescence microscopy suggest that drebrin A is present in both the neck and the head of dendritic spines (Ivanov et al., 2009a) the precise localisation of the protein, particularly in relation to F-actin in spines, is unclear. Although super-resolution microscopy is increasingly being used to determine the distribution of F-actin in dendritic spines it has not yet been used to determine the distribution of drebrin (MacGillavry and Hoogenraad, 2015).

The organisation of F-actin in spines is not completely understood. In spine heads, F-actin is branched with the barbed ends of the filaments directed distally, whereas in the spine neck, F-actin can also be bundled, although whether these are parallel or anti-parallel bundles is unresolved (Fifkova and Delay, 1982; Korobova and Svitkina, 2010). Dendritic spines are thought

to develop from dendritic filopodia but these have several structural and compositional features that are different from the filopodia of growth cones and migrating cells. Although the major cytoskeletal component of dendritic filopodia is F-actin, as it is in non-dendritic filopodia, the organisation of the filaments is different. F-actin in dendritic filopodia is mainly branched and the occasional bundles are not always parallel whereas in non-dendritic filopodia the F-actin is highly bundled and parallel (Korobova and Svitkina, 2010). Consistent with these differences in F-actin organisation, dendritic filopodia contain Arp2/3, capping proteins and myosin II, and no fascin, unlike non-dendritic filopodia (Korobova and Svitkina, 2010). Arp2/3 and capping protein are necessary for F-actin branching and myosin II forms anti-parallel bundles of F-actin whereas fascin forms parallel bundles. Drebrin clearly binds to parallel F-actin bundles, which are the only ones found in growth cone filopodia, but to what extent it binds to anti-parallel bundles is unclear, although these are found in the Tzone of the growth cone, where drebrin localises (Geraldo et al., 2008; Mizui et al., 2009) and possibly in dendritic filopodia where drebrin is also present. Myosin IIB is also concentrated in the Tzone where it organises anti-parallel F-actin bundles (Rochlin et al., 1995; Medeiros et al., 2006) and, since drebrin probably binds myosin IIB (Table 1), this interaction might contribute to its Tzone localisation, as well as direct F-actin binding.

Drebrin is a multi-domain protein of which the best characterised is the N-terminal actin-depolymerising factor homology (ADFH) domain (Fig. 1, Larbolette et al., 1999; Kessels et al., 2000; Xu and Stamnes, 2006). The ADFH domain is structurally conserved among five families of actin-binding proteins including actin depolymerising factor (ADF)/cofilin, Abp1 and twinfillin (Poukkula et al., 2011). In these other families, the ADFH domain binds globular (G)- or F-actin but there is no evidence of actin binding by the drebrin ADFH domain (Hayashi et al., 1999; Chew et al., 2005; Xu and Stamnes, 2006; Grintsevich et al., 2010; Worth et al., 2013). Instead, F-actin-binding activity in drebrin resides in two adjacent domains downstream of the ADFH domain: a coiled-coil (CC) and a helical (Hel) domain (Fig. 1) that independently or co-operatively bind or bundle F-actin (Worth et al., 2013). There then follows a polyproline-rich region (PP) and a large C-terminal region called the blue box (BB, Worth et al., 2013) that harbours binding sites for the PSD scaffolding protein cupidin/homer 2 (Shiraishi-Yamaguchi et al., 2009) and motifs for Siah-2 binding (Trivedi et al., 2016) (Table 1). Since profilin is a candidate drebrin interactor (Table 1) and

interacts with polyproline regions of many proteins, it is tempting to suggest that the PP domain of drebrin might bind profilin. The 46 amino acid insert that distinguishes the A form of drebrin from drebrin E is located at the C-terminal end of the Hel domain and has no known function (Fig. 1). The insert in drebrin A might confer a novel function on the molecule or modulate an existing one, such as F-actin binding. Although there has been no comparative characterization of the F-actin-binding properties of drebrin E and A, heterologous expression of either isoform in cells has similar effects on F-actin organization and stability (Dun and Chilton, 2010).

There is cell biological and *in vitro* structural evidence that drebrin stabilises F-actin. Over-expression of drebrin E or A in heterologous cells and neurons increases the levels of F-actin (Ikeda et al., 1996; Geraldo et al., 2008; Mizui et al., 2009; Worth et al., 2013) whereas, conversely, siRNA-mediated knockdown of drebrin decreases F-actin levels in growth cones of cultured cortical neurons (Mizui et al., 2009) In vascular smooth muscle cells isolated from the heterozygotes of drebrin knockout mice, where drebrin is reduced by a half, there is a reduction of F-actin (Stiber et al., 2016). Over-expressing high levels of drebrin in heterologous cells induces the formation of prominent cell processes containing thick bundles of F-actin (Shirao et al., 1992, 1994; Keon et al., 2000; Chew et al., 2005; Geraldo et al., 2008; Worth et al., 2013). Drebrin binding to F-actin *in vitro* produces a twist in the filament that leads to filament stiffening and stabilisation (Sharma et al., 2011; Mikati et al., 2013). Drebrin protects F-actin against depolymerising agents such as cytochalasins (Ikeda et al., 1996; Ivanov et al., 2009b). There is biochemical evidence that drebrin competes with many other actin-binding proteins for actin filaments including ADF/cofilin, α-actinin, tropomyosins and fascin and this might indirectly affect F-actin stability (Ishikawa et al., 1994; Sasaki et al., 1996; Zhao et al., 2006; Grintsevich et al., 2010, 2014; Sharma et al., 2011; Mikati et al., 2013). Whether these are examples of direct competition for a common binding site or indirect competition resulting from allosteric effects is not clear. There is *in vitro* evidence that drebrin, binding to F-actin, can inhibit the F-actin severing activity of ADF/cofilin (Grintsevich et al., 2014). The competition between drebrin and ADF/cofilin for F-actin binding might be an example of an allosteric effect because ADF/cofilin produces an opposing twist in F-actin to that produced by drebrin (Sharma et al., 2011). As pointed out by Cammarata et al

(2016), the potential inhibition of the F-actin severing activity of ADF/cofilin by drebrin competition might impact on the progression of microtubules into filopodia during drebrin/EB3 coupling.

Several binding partners for drebrin have been identified including: afadin, EB3, cupidin-2/homer, CXCR4, myosin II, profilin and Shia-2 (Table 1). The microtubule-binding protein EB3 is a member of a family of so-called +TIP proteins that bind to the plus-ends of microtubules and regulate their dynamics (reviewed in: van de Willige et al., 2016). Mammalian cells express three EB proteins: EB1 and EB3 are microtubule plus-end trackers whereas EB2 is at best a weak plus-end tracker and is the least studied member of the family. The EB proteins are positioned at the core of a cluster of proteins that localise to microtubule plus-ends by virtue of the fact that they harbour binding sites for other +TIP proteins including APC and CLASP (Cammarata et al., 2016). EB1 and EB3 have both common and distinct binding partners and drebrin is one of a growing number of proteins that bind preferentially to EB3 (Table 2). In neurons undergoing neuritogenesis and in growth cones, drebrin, bound to parallel F-actin at the base of filopodia, interacts with EB3 located at the plus-ends of dynamic microtubules entering filopodia (Geraldo et al., 2008; Worth et al., 2013). The evidence for this includes the demonstration of direct binding between drebrin and EB3 in filopodia using FRET/FLIM (Geraldo et al., 2008) and the observation that a dominant negative construct, EB3M, containing the drebrin binding domain of EB3 blocks neuritogenesis (Geraldo et al., 2008) and neuronal cell migration (Trivedi et al., 2016). Thus, the drebrin/EB3 interaction cross-links dynamic microtubules to F-actin in filopodia and this likely forms part of the cellular mechanism underlying the response to axon growth and growth cone guidance cues (Cammarata et al., 2016). The drebrin/EB3/Cdk5 pathway has been independently confirmed in other cellular contexts where dynamic microtubules interacting with F-actin underlies cell shape changes, suggesting that the pathway is canonical (Bazelliéres et al., 2012; Merriam et al., 2013; Ketschek et al., 2016).

The functions of drebrin and EB3 are regulated by direct phosphorylation (Gordon-Weeks, 2016). Drebrin exists in two structural conformations, a closed one in which the BB auto-inhibits the F-actin-binding activity in the CC domain by binding to a region centred on S142 near the junction between the ADFH domain and the CC domain (Fig. 1) and an open conformation in which binding of the BB is relieved by cyclin-dependent kinase 5 (Cdk5) phosphorylation of S142

(Worth et al., 2013). In the closed conformation, drebrin can only bind to single actin filaments whereas in the open conformation both F-actin-binding activities are available and drebrin can bundle F-actin or straddle pre-existing F-actin filaments (Fig. 1). As well as regulating drebrin F-actin binding, Cdk5 phosphorylation, by converting drebrin to the open conformation, exposes the binding site for EB3 (Worth et al., 2013). EB3 is a substrate of the mitotic kinases Aurora A and B (Ban et al., 2009) and the phosphatase calcineurin and dephosphorylation activates EB3 (Komarova et al., 2012).

2. Drebrin function in dendritic spines

The dendritic spines of excitatory (glutamatergic) synapses on cortical and hippocampal neurons undergo a series of morphological and molecular changes associated with changes in synaptic strength and memory formation (reviewed in: Rochefort and Konnerth, 2012; Hlushchenko et al., 2016). Synapse strength can either be enhanced, by short bursts of high-frequency input, leading to long-term potentiation (LTP), or reduced, by prolonged low frequency input, causing long-term depression (LTD). LTP is induced by activation of the N-methyl-D-aspartate receptor (NMDA) which leads to long-lasting incorporation of α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptors in the post-synaptic density (PSD) of dendritic spines and to changes in spine morphology. Despite spine shape being a continuum, three main spine shape categories have been recognized: thin, stubby and mushroom, in increasing order of size (reviewed in: Rochefort & Konnerth 2012; Hlushchenko et al., 2016). An increase in synaptic strength accompanying LTP is associated with spine enlargement, whereas LTD, where synaptic strength declines, is associated with a decrease in spine size and morphologically simpler spines. Changes in dendritic spine morphology are achieved by the rapid and reversible re-organization of the cytoskeleton within the spine. The dynamic behaviour of two cytoskeletal filaments, F-actin and microtubules, dominate dendritic spine changes in response to synaptic activity. Increased synaptic activity and strength is accompanied by an increase in spine F-actin and F-actin stability, which is responsible for spine enlargement, whereas a reduction in synaptic activity reduces F-actin in spines and increases F-actin dynamics (Okamoto et al., 2004; Honkura et al., 2008). Spine head volume correlates closely with the area of the PSD (Harris and Stevens, 1989) and the number of AMPA receptors (Matsuzaki et al., 2001).

2.1. Structural role of drebrin A in spine morphogenesis

Drebrin A influences dendritic spine morphology by regulating F-actin, plays a role in activity-dependent accumulation of PSD-95, the major scaffolding protein at the PSD, and in redistribution of the NMDA type of glutamate receptor (Ivanov et al*.,* 2009b). These effects of drebrin on spine activity are dependent on F-actin binding (Ivanov et al., 2009b). Over-expression of drebrin in mature neurons in culture produces dendritic spines with abnormally long necks (Hayashi and Shirao, 1999; Ivanov et al., 2009b; Merriam et al., 2013) consistent with the idea that drebrin stabilises F-actin in the spine neck.

2.2. Activity-driven microtubule capture and insertion into dendritic spines

Dynamic microtubules in dendrite shafts can transiently enter dendritic spines by polymerizing from their plus-ends, the end at which tubulin assembly occurs preferentially (Gu et al*.* 2008; Hu et al*.* 2008; Jaworski et al*.* 2009; Wagner et al., 2011; Merriam et al., 2013). The frequency of invasion is low, with approximately 1% of dendritic protrusions containing a microtubule at any one time (Hu et al. 2008) and the dwell time is in the order of minutes but, importantly, dwell time and the frequency of invasion are enhanced by pharmacological interventions that mimic LTP (Gu et al. 2008, Hu et al. 2008; Mitsuyama et al*.* 2008; Jaworski et al. 2009; Merriam et al*.* 2011) and supressed by eliciting LTD (Kapitein et al*.* 2011). Depolymerisation of microtubules in dendrites using drugs such as nocodazole reduces spine density along the dendrite without affecting dendrite numbers (Jaworski et al., 2009). Dendritic microtubules are oriented with their plus-ends directed either towards or away from the cell body and can therefore polymerise antero- or retrogradely, and can be nucleated acentrosomally within the dendrite (Delandre et al., 2016). Microtubules invading spines are oriented with their plus-ends distal, i.e. directed toward the spine head. This behavior is reminiscent of microtubules invading the filopodia of growth cones (Geraldo & Gordon-Weeks 2009; Gordon-Weeks & Fournier, 2013) and axonal branches (Ketschek et al., 2016). In all of these cases, the microtubule-binding +TIP protein EB3, is located at the tip of the growing microtubule. There is now considerable evidence that microtubule invasion of dendritic spines underlies morphological and physiological changes

associated with memory formation including F-actin stabilization, spine maturation and enlargement of the PSD (Dent et al. 2011). Consistent with these findings, shRNA-mediated knock-down of EB3 reduces spine enlargement (Gu et al. 2008; Jaworski et al. 2009). An early event in dendritic spine enlargement associated with increased synaptic strength is an enlargement of the PSD and a concomitant increase in PSD-95, the major scaffolding protein of the PSD. Brain-derived neurotrophic factor (BDNF) induces spine formation and maturation, an effect that is blocked by microtubule depolymerisation using nocodazole and enhanced by stabilizing microtubules with substoichiometric concentrations of taxol (Gu et al., 2008). Exposure of dendritic spines to BDNF induces an increase of the post-synaptic density protein PSD-95 in the PSD along with spine maturation and this has also been shown to depend on invasion of dendritic spines by microtubules (Hu et al. 2011). Importantly, the frequency of microtubule invasion into dendritic spines and the number of spines invaded is enhanced by over-expression of drebrin and, conversely, reduced by siRNA-mediated knock-down of drebrin (Merriam et al., 2013). Whether drebrin and EB3 are acting independently of each other or are interacting in the drebrin/EB3 pathway in spine maturation has yet to be determined.

3. Synaptotoxicity and dendritic spine loss in Alzheimer's disease

Alzheimer's disease is primarily a disease of synapse dysfunction caused by Amyloid β (Aβ) oligomers derived from Alzheimer's precursor protein and loss of synapses is the tightest pathological correlate of cognitive decline in Alzheimer's disease (Klein, 2013).

3.1. Drebrin loss from dendritic spines precedes synapse loss

One of the earliest molecular changes that precede synapse loss and neurodegeneration in Alzheimer's disease and related cognitive disorders is a dramatic loss of drebrin from dendritic spines (Ivanov et al., 2009a; Dun and Chilton, 2010). This occurs in mild cognitive impairment (Counts *et al.,* 2012), Alzheimer's disease (Harigaya et al., 1996; Hatanpaa et al., 1999; Shim and Lubec, 2002; Calon et al., 2004; Zhao et al., 2006) and Down's syndrome, where individuals inevitably develop Alzheimer's (Weitzdoerfer et al., 2001; Shim and Lubec, 2002). Whether or not drebrin loss from dendritic spines is causal to cognitive decline in Alzheimer's disease and related cognitive disorders is not fully resolved. There is a correlation between the degree of drebrin

reduction in dendritic spines and the severity of cognitive impairment (Kojima and Shirao, 2007). Transgenic mouse models of familial Alzheimer's disease also show a loss of drebrin from dendritic spines (Lee and Aoki 2012; Calon et al., 2004; Calon et al*.,* 2005; Mahadomrongkul et al., 2005; Aoki et al., 2007; Lacor et al., 2007). Drebrin loss occurs before loss of pre-synaptic markers, such as synaptophysin, suggesting it precedes synapse loss (Counts et al., 2012).

Reduction of drebrin A by intracerebral administration of antisense oligonucleotides in rats causes cognitive deficits (Kobayashi et al*.,* 2004) and transgenic mice in which the exon coding for the spliced insert of drebrin A has been deleted by homologous recombination, so that only the E form can be expressed, show deficits in homeostatic dendritic spine plasticity (Aoki et al*.,* 2009) and subtle deficits in cognition (Kojima et al*.,* 2010). However, there is a compensatory up-regulation of drebrin E in these mice which probably limits the severity of the synaptic impairment (Kojima et al., 2010). Nonetheless, in the drebrin A knockout mouse, LTP is impaired in the adult, but not, curiously, in the adolescent, suggesting that drebrin A, and therefore presumably the insert, has a unique functional role in dendritic spine plasticity (Kojima et al., 2016).

Drebrin loss from dendritic spines can be replicated *in vitro* in mature embryonic hippocampal cultures within hours by exposure to Aβ oligomers (Zhao et al., 2006; Lacor et al*.,* 2007). Recently, proteolysis of drebrin has been proposed as the mechanism underlying the pathological loss of drebrin from dendritic spines (Chimura et al., 2015). The activity of several proteases, including calpain and caspases, are increased in Alzheimer's disease. Drebrin is a caspase-6 target (LeBlanc, 2013), but it is not known if it is cleaved by caspase-6 in Alzheimer's disease. Prolonged over-activation of NMDA-type glutamate receptors in cultured hippocampal neurons, which mimics some features of the effects of Aβ oligomers on neurons, causes an increase in intracellular calcium which activates calpain, a calcium-dependent protease, resulting in drebrin proteolysis and, at the same time, a decrease in F-actin (Chimura et al., 2015). Calpain activity is also responsible for the proteolytic conversion of p35, a regulatory subunit of Cdk5, to p25, a truncated and constitutively active form which enhances Cdk5 activity in Alzheimer's disease (reviewed in: Shah and Lahiri, 2014). This might abnormally increase the levels of S142 phosphorylated drebrin impacting F-actin organization. If the open conformation of drebrin, i.e. the S142 phosphorylated form, is more susceptible to proteolysis than the closed form this might lead

to a positive feed-forward loop with consequent catastrophic drebrin degradation. Although proteolysis at synapses increases in Alzheimer's disease, drebrin breakdown products have not been seen. Proteolytic fragments might have been missed because only one antibody had been used previously to detect drebrin by immunoblotting in Alzheimer's disease brain tissue and transgenic mouse models of Alzheimer's disease and the epitope that it recognises, which is in the C-terminal BB, might be lost from proteolytic fragments (Hatanpaa et al., 1999; Calon et al., 2004; Zhao et al., 2006; Counts et al. 2012). It is unclear whether the loss of drebrin through proteolysis causes a loss of drebrin function or a dominant negative effect through one of the proteolytic fragments. Future studies will also have to address the question of whether or not Aβ can also induce drebrin proteolysis. In contrast, brief and transient activation of NMDA receptors *in vitro* has been reported to accompany a transient shift of drebrin A from dendritic spines to dendritic shafts followed by a re-entry into spines without changes in overall drebrin levels (Sekino et al., 2006; Bosch and Hayashi, 2012; Merriam et al., 2013; Mizui et al., 2014). LTP *in vivo* is also associated with an influx of drebrin into the spine (Fukazawa et al., 2003; Bosch et al., 2014).

Changes in other actin-binding proteins that occur in neurodegenerative disease might displace drebrin from F-actin in the dendritic spine into the dendritic shaft where proteolytic degradation could occur. This could occur through direct competition with drebrin binding to F-actin or indirectly through changes to F-actin such as depolymerisation brought about by severing or structural changes in the filament. In Alzheimer's disease there is an inverse relationship between the loss of drebrin and an increase in ADF/cofilin (Zhao et al., 2006). ADF/cofilin activity, including F-actin severing, is negatively regulated by LIM kinase phosphorylation and the activity and levels of the kinase that activates LIM kinase, PAK, is reduced in Alzheimer's disease (Zhao et al., 2006). Significantly, PAK can protect against Aβ oligomer-induced drebrin loss (Zhao et al., 2006).

4. Conclusions and future perspectives

The drebrin/EB3/Cdk5 molecular pathway co-ordinates dynamic microtubules and F-actin interactions that underlie various cell shape changes, such as neuritogenesis, neuronal migration, growth cone turning and dendritic spine plasticity, in response to guidance cues. It is unlikely that this pathway is the only one that has evolved to couple dynamic microtubules to F-actin and indeed

other candidate pathways have been identified (Coles and Bradke, 2015). How cells utilise different pathways coupling dynamic microtubules to F-actin and under what circumstances are areas of future research. How this pathway is regulated is only just beginning to be understood. There is evidence for regulation of drebrin and EB3 by phosphorylation and additional interactors of both proteins have also been identified, although the mechanistic details of these regulatory processes have yet to be worked out. Unravelling the distinct roles played by drebrin E and A, and in particular the function of the A insert, are also important goals.

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Figure legends

Figure 1. **Model of a molecular mechanism for coupling of F-actin to microtubules by the drebrin/EB3/Cdk5 pathway in filopodia.**

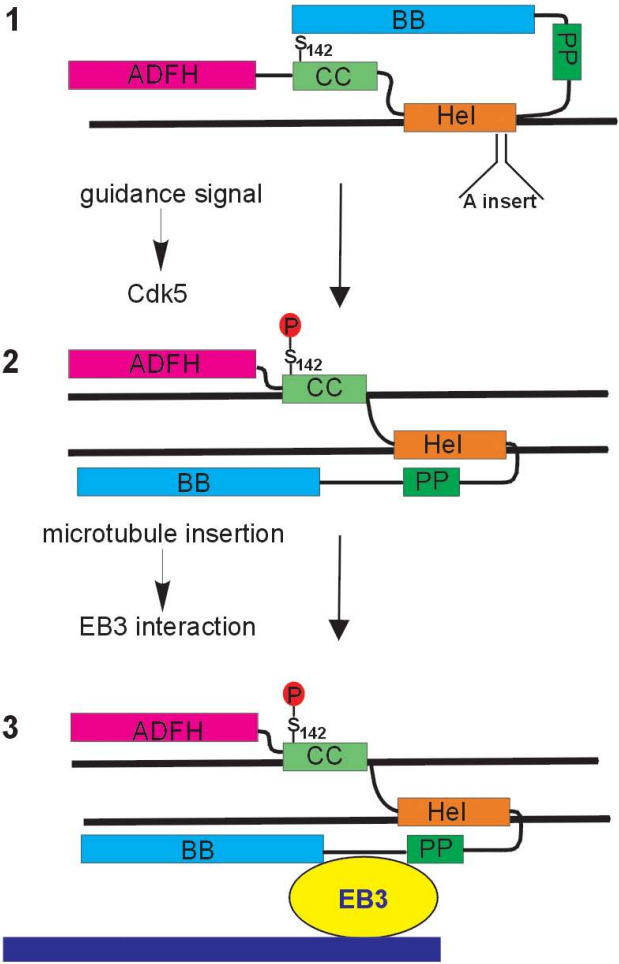
The diagram shows the domain structure of drebrin in the "closed" conformation when the BB domain binds at the border between the ADFH and CC domains where S142 is located (1). In this conformation drebrin can bind to actin filaments but does not bundle or straddle pre-existing actin filament bundles. One filopodial actin filament, represented by a black line, is also shown. The location of the 46 amino acid insert that distinguishes the drebrin A isoform from drebrin E is also shown (A insert). Activation of Cdk5 by intracellular signalling pathways driven by extrinsic guidance cues leads to S142 phosphorylation (2) and the consequent relief of the intramolecular occlusion of one actin filament binding domain by the BB domain. This produces an "open" conformation and drebrin now bundles actin filaments, or straddles pre-existing actin filament bundles, by co-operative binding of the CC and Hel domains. Drebrin in the "open" conformation and bound to actin filaments at the base of filopodia is now in an appropriate state to interact with the +TIP protein EB3 located at the plus end of a microtubule (purple line) entering the filopodium, and thereby couple actin filaments to a microtubule (3).

Table 1
Drebrin interactors

Interactor	**Function**	**Reference**
afadin	Adherens junction protein	Rehm et al., 2013
Arp3	Junction dynamics during spermatogenesis. Component of the F-actin nucleating and branching Arp2/3 complex	Li *et al*., 2011
cupidin/homer 2	Post-synaptic density scaffolding protein	Shiraishi-Yamaguchi et al., 2009
connexin-43	Gap junction protein	Butkevich et al., 2004
CXCR4	Chemokine receptor for the chemokine CXCL12	Pérez-Martínez et al., 2010; Gordón-Alonso et al., 2013
EB3	+TIP protein regulating microtubule dynamics	Geraldo et al., 2008; Bazelliéres et al., 2012
myosin IIB	Actin-binding motor protein	Bazelliéres et al., 2012
profilin	Actin monomer-binding protein	Mammoto et al., 1998
PTEN	Lipid phosphatase, negative regulator of PI3K signalling pathway, tumour suppressor	Kreis et al., 2013
Siah-2	E3 ubiquitin ligase	Trivedi et al., 2016
spikar	transcriptional co-activator, dendritic spine density	Yamazaki et al., 2013

Table 2
EB3 interactors not shared with EB1

Interactor	**Function**	**Reference**
drebrin	Stabilises F-actin, couples F-actin to microtubules	Geraldo et al., 2008
Aurora A and B	Mitotic kinases, phosphorylate EB2 and EB3 but not EB1	Ban et al., 2009
calcineurin	Ca^{2+}-dependent phosphatase	Komarova et al., 2012
DDA3	microtubule-associated protein, activator of β-catenin pathway	Hsieh et al., 2007
inositol 1,4,5-trisphosphate receptor	inositol trisphosphate activated Ca^{2+} channel in endoplasmic reticulum	Geyer et al., 2015
PSD-95	Post-synaptic density scaffolding protein	Sweet et al., 2011
Siah-1	E3 ubiquitin ligase	Ban et al., 2009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