COMMENTARY

Filopodia formation and Disabled degradation downstream of Reelin

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During *Drosophila* embryogenesis, Abl (Abelson tyrosine kinase) is localized in the axons of the CNS (central nervous system). Mutations in Abl have a subtle effect on the morphology of the embryonic CNS, and the mutant animals survive to the pupal and adult stages. However, genetic screens have identified several genes that, when mutated along with the *Abl* gene, modified the phenotypes. Two prominent genes that arose from these screens were *enabled* (*Ena*) and *disabled* (*Dab*). It has been known for some time that Enabled and its mammalian homologues are involved in the regulation of actin dynamics, and promote actin polymerization at the leading edge of motile cells. It was a defect in actin polymerization in migrating neurons in particular that resulted in the identification of Enabled as an important regulator of neuronal migration. Defects in Disabled, in both *Drosophila* and mammals, also gave rise to neuronal defects which, in mice, were indistinguishable from phenotypes observed in the *reeler*

mouse. These observations suggested that mDab1 (mammalian Disabled homologue 1) acted in a pathway downstream of Reelin, the product of the *reelin* gene found to be defective in *reeler* mice. Now, in this issue of the *Biochemical Journal*, Takenawa and colleagues have demonstrated that Disabled also acts in a pathway to regulate actin dynamics through the direct activation of N-WASP (neuronal Wiskott–Aldrich syndrome protein). Furthermore, they were also able to link several lines of investigation from other groups to show that the ability of mDab1 to regulate actin dynamics during cell motility was under the negative control of tyrosine phosphorylation, leading to ubiquitin-mediated degradation of mDab1.

Key words: actin polymerization, cell migration, filopodium, neuronal Wiskott–Aldrich syndrome protein (N-WASP), phosphorylation, ubiquitination.

Abl (Abelson), along with Src, were two of the first non-receptor tyrosine kinases to be identified. Ever since the pioneering studies by Baltimore, Hunter, Sefton and others [1,2] in the late 1970s and early 1980s, work has continued apace to define the precise cellular pathways and roles of Abl activation. One of the major advances in our understanding of Abl function was as a result of the genetic screens in *Drosophila* carried out by Frank Gertler while in Michael Hoffmann's laboratory [3,4]. Mutations in *Abl*, which is expressed at high levels in the *Drosophila* nervous system, are relatively mild; Disabled was identified in a screen for proteins that enhanced the phenotype, i.e. made it more severe, and Enabled was identified in a screen that suppressed the phenotype, i.e. made it less severe. As is the custom with *Drosophila* mutations, they were given quirky names that resembled the effects observed in *Drosophila*; however, these relate to the actual gene or its function. In this case, the names have proven quite apt and were subsequently adopted for the mammalian homologues, as we shall see below. Screens in *Drosophila* have identified several genes that interact with Abl and are involved in regulating neuronal morphogenesis (reviewed in [5]). In other systems, approx. 50 different proteins have been demonstrated to interact with Abl and related kinases [6], and it turns out that many of these proteins have roles in regulating the actin cytoskeleton. One of the pervading themes that has arisen from the screens in *Drosophila* is a critical role for Abl in the control of neuronal cytoskeletal structures. Perhaps the best understood example of this is Enabled. Through initial studies principally in non-neuronal cells, but also confirmed later in neurons, Enabled acts to suppress the activity of capping protein at the barbed end of actin filaments at the leading edge of motile cells. The suppression of capping activity leads to an increase in structures called filopodia: long thin finger-like actin-containing

projections at the front of the cell that explore the environment as the cell moves forward, rather like the tentacles on a slug or snail, and which are crucial to cell motility. The growth cones of migrating neurons are typically full of these dynamic probing structures, hence the effect on neuronal migration when *Ena* (the Enabled gene) is mutated. In mammals, the situation gets slightly more complex: there is not just one Abl activity, but two, Abl and Arg (Abl-related gene) that probably share redundant functions in neuronal development if knockout studies in mice are anything to go by [7]. While there is only one *Ena* gene in *Drosophila*, there are three related proteins in mammals, Mena (mammalian *Ena*), VASP (vasodilator-stimulated phosphoprotein), together referred to as Ena/VASP family, which also includes EVL (Ena/VASP-like). There are likewise multiple *Dab* (Disabled) genes in mammals, but it is mDab1 (mammalian Disabled homologue 1) that is expressed most prominently in neuronal tissues. Disabled was originally identified as an enhancer of Abl phenotypes in *Drosophila* [3], and mutant embryos fail to develop any axon bundles in the CNS (central nervous system), but this gave no direct clue to a role in the regulation of the actin cytoskeleton.

Although nearly a decade of studies into Ena/VASP function have provided huge advances in our understanding of fundamental mechanisms underlying the control of the actin cytoskeleton and cell migration [8], and, more recently, it has been discovered that Abl itself has a more direct role in the organization of the actin cytoskeleton by binding and bundling actin filaments reviewed in [9], the function of Disabled has remained elusive. In mice, genetic studies have revealed that mutations in the mDab1 gene, *scrambler* and *yotari*, yielded phenotypes indistinguishable from that of the *reeler* mouse. The *reeler* mouse has a sort of inside-out layering of cells in the brain, due to defects in the migration of the outermost layer of cells from inside to outside. These studies

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suggest that both mDab1 and Reelin play essential roles in the cell migration or guidance necessary for correct brain organization. The *reeler* gene encodes a protein called Reelin, which is secreted by specialized neurons in the outer layers of the brain, and acts as a guidance cue by binding to the Reelin receptors VLDLR (very-low-density lipoprotein receptor) and ApoER2 (apolipoprotein E receptor 2) in the migrating neurons. Reelin receptor binding also leads to mDab1 phosphorylation on tyrosine, which has important consequences for mDab1. Because mice with mutations in *Reelin*, *mDab1* or both *VLDLR* and *ApoER2* have virtually the same phenotype, it was thought that these genes act in a linear genetic pathway (see [10] for a recent review).

Takenawa and colleagues now draw all these elements together in a single study, published in this issue of the *Biochemical Journal* [11], and demonstrate that, like Abl and Ena, mDab1 also has a role in neuronal migration through regulation of the actin cytoskeleton, and, furthermore, that mDab1 activity is regulated by tyrosine-phosphorylation-stimulated degradation of mDab1 via a ubiquitin-mediated degradation pathway. mDab1 serves to increase actin polymerization, but, unlike Enabled, it has a direct effect on the actin-polymerization machinery itself rather than a negative regulatory effect on a capping protein. Rather surprisingly, mDab1 is able to directly bind and activate N-WASP. WASP, the neuronal homologue N-WASP and related Scar proteins are all key activators of the Arp2/3 (actin-related protein 2/3) complex that initiates new actin polymerization at the leading edge of cells which is essential for cell motility. WASP proteins are normally activated by the Rho family GTPase Cdc42 (cell-division control 42) by binding to a specific sequence known as a CRIB (Cdc42/Rac-interactive binding) motif. The binding of Cdc42 to the CRIB motif induces a conformational change relieving an autoinhibitory interaction between the CRIB motif and the C-terminus of N-WASP, leading to activation of N-WASP and Arp2/3 binding. mDab1 appears to act independently of Cdc42, however, and via its PTB (phosphotyrosine binding) domain can directly activate N-WASP by binding to a short amino acid sequence NRFY (Asn-Arg-Phe-Tyr), an analogue of the PTB binding NPXY (Asn-Pro-Xaa-Tyr) sequence adjacent to the CRIB motif, presumably resulting in an activating conformational change similar to that induced by Cdc42. The mDab1–N-WASP complex can then directly initiate new actin polymerization mediated by the Arp2/3 complex independently of other known activators of N-WASP, such as Cdc42. Indeed, the PTB domain of mDab1 was shown to be necessary and sufficient to induce actin polymerization. When overexpressed in non-neuronal cells, the result of this new actin polymerization was the induction of numerous filopodia, important for cell migration, and this activity required N-WASP activity, but was largely independent of Cdc42. Similar findings were also obtained in direct biochemical assays examining the activation of actin polymerization, and the interactions between regions of mDab1 and N-WASP; importantly, they were also able to demonstrate an interaction between mDab1 and N-WASP in embryonic mouse brain, suggesting that the two proteins can interact in the right place and at the right time to be involved in brain development. But, if this were not enough, Takenawa and colleagues were also able to complete another piece of the Reelin signalling pathway. mDab1 was known to be phosphorylated by Src family kinases in response to Reelin stimulation, and this in turn led

to the ubiquitin–proteasome-dependent degradation of mDab1, a key part of the mechanism of Reelin-mediated inhibition of cell motility (see [12] and references therein). Interestingly, once phosphorylated on tyrosine, mDab1 also acts positively to increase further tyrosine phosphorylation of itself in a sort of negative-feedback self-destruct cycle. Reports of these sort of tyrosine-phosphorylation-mediated feedback loops modulating actin regulatory proteins are becoming more prevalent (see [9,13]). Takenawa and colleagues went on to demonstrate that an increase in mDab1 tyrosine phosphorylation by the Src family kinase Fyn not only prevented mDab1-induced filopodia formation, but also resulted in the loss of mDab1 immunostaining from cells. Loss of mDab1 staining was a result of the activity of the ubiquitin ligase Cbl, predominantly expressed in brain and able to recognize phosphorylated tyrosine residues, which is responsible for ubiquitination of mDab1 in response to phosphorylation of mDab1 by Fyn, leading to subsequent degradation through the ubiquitin–proteasome pathway.

Although much of the work described in the present study [11] was conducted in non-neuronal cells for ease of manipulation, a successful strategy employed to elucidate Enabled function [8], it will nevertheless be important to establish that the same pathways exist in neuronal cells. The gaps in the Reelin signalling pathway that have been filled now provide the basis for further dissection and studies into the roles of mDab1 in signalling to the actin cytoskeleton and promoting cell motility in neurons *in vitro* and *in vivo*.

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