Communicative & Integrative Biology 8:6, e1114194; November/December 2015; Published with license by Taylor & Francis Group, LLC

Novel functions for ADF/cofilin in excitatory synapses - lessons from gene-targeted mice

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Keywords: actin, actin dynamics, ADF, ADHD, cofilin, dendritic spine, excitatory synapse, neurotransmitter release, synaptic plasticity, vesicle exocytosis

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Submitted: 09/29/2015

Revised: 10/23/2015

Accepted: 10/23/2015

http://dx.doi.org/10.1080/19420889.2015.1114194

Addendum to: 1. Wolf M, Zimmermann AM, Görlich A, Gurniak CB, Sassoè-Pognetto M, Friauf E, Witke W, Rust MB. ADF/cofilin controls synaptic actin dynamics and regulates synaptic vesicle mobilization and exocytosis. Cereb Cortex 2015; 25:2863–75. 2. Zimmermann AM, Jene T, Wolf M, Görlich A, Gurniak CB, Sassoè-Pognetto M, Witke W, Friauf E, Rust MB. ADHD-like phenotype in a mouse model with impaired synaptic actin dynamics. Biol Psychiatry 2015; 78:95–106.

Actin filaments (F-actin) are the major structural component of excitatory synapses. In excitatory synapses, F-actin is enriched in presynaptic terminals and in postsynaptic dendritic spines, and actin dynamics – the spatiotemporally controlled assembly and disassembly of F-actin – have been implicated in pre- and postsynaptic physiology, additionally to their function in synapse morphology. Hence, actin binding proteins that control actin dynamics have moved into the focus as regulators of synapse morphology and physiology. Actin depolymerizing proteins of the ADF/cofilin family are important regulators of actin dynamics, and several recent studies highlighted the relevance of cofilin 1 for dendritic spine morphology, trafficking of postsynaptic glutamate receptors, and synaptic plasticity. Conversely, almost nothing was known about the synaptic function of ADF, a second ADF/cofilin family member present at excitatory synapses, and it remained unknown whether ADF/cofilin is relevant for presynaptic physiology. To comprehensively characterize the synaptic function of ADF/cofilin we made use of mutant mice lacking either ADF or cofilin 1 or both proteins. Our analysis revealed presynaptic defects (altered distribution and enhanced exocytosis of synaptic vesicles) and behavioral abnormalities reminiscent of attention deficit-hyperactivity disorder in double mutants that were not present in single mutants. Hence, by exploiting gene-targeted mice, we demonstrated the relevance of ADF for excitatory synapses, and we unraveled novel functions for ADF/cofilin in presynaptic physiology and behavior.

Actin-binding proteins (ABPs) of the ADF/cofilin family are crucial regulators of actin dynamics (for review see[1]), which can speed up actin dynamics by accelerating the dissociation of actin monomers (G-actin) from the minus end of actin filaments (F-actin) and by severing F-actin.[2,3] Depending on the local G-actin concentration and on the activity of ABPs that promote actin polymerization, ADF/cofilin-mediated filament severing can either result in net assembly or disassembly of F-actin.[4] Moreover, at high concentrations, ADF/cofilin can promote F-actin assembly by nucleating new and by stabilizing preexisting filaments.[5] Actin binding of ADF/cofilin is inhibited upon phosphorylation of a conserved serine residue at position 3 (Ser3), and LIM kinases (LIMK) have been recognized as important regulators of ADF/cofilin activity.[6] Indeed, analysis of LIMK1 mutant mice initially implicated ADF/cofilin in synapse physiology and suggested a role in dendritic spine morphology and synaptic plasticity.[7] The mammalian ADF/cofilin family consist of 3 proteins, namely cofilin 1 (non-muscle cofilin, n-cofilin), cofilin 2 (muscle-cofilin, m-cofilin), and ADF (actin-depolymerizing factor, destrin). While cofilin 1 and ADF show a broad tissue distribution, are both broadly expressed in the brain and present at excitatory synapses,[8-11] cofilin 2 is the major ADF/cofilin form in muscle cells and mutations in the human cofilin 2 gene *CFL2* have been associated with nemaline myopathies.[12-15]

In agreement with the postsynaptic defects described for LIMK1 mutant mice,[7] several studies demonstrated a crucial role of cofilin 1 in postsynaptic mechanisms (for review see[16]). These studies

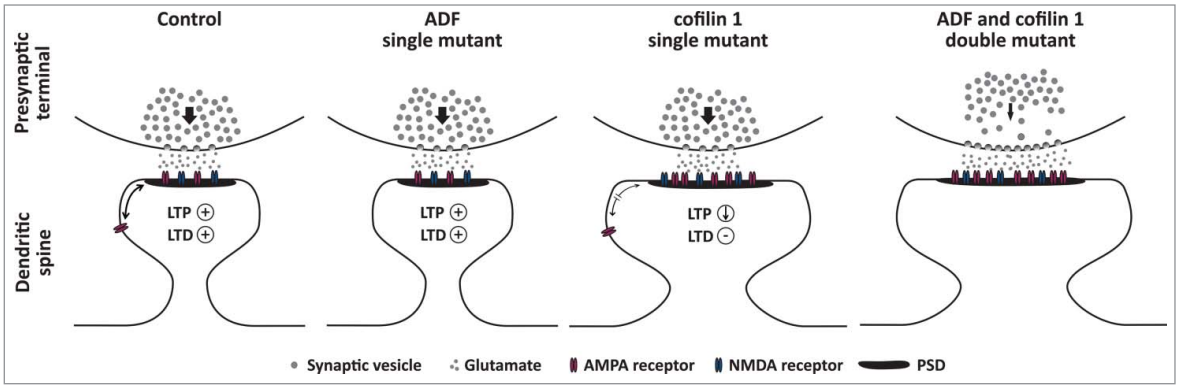

Figure 1. Schematic diagram illustrating the functions of ADF and cofilin 1 at excitatory synapses as deduced from the analyses of single and double mutant mice.[10,11,25,26] In ADF mutant mice, the morphology of excitatory synapses is unchanged and these mutants do not display any defects in pre- (distribution, recruitment or exocytosis of synaptic vesicles) or postsynaptic mechanisms (LTP, LTD). Conversely, dendritic spine profiles and the postsynaptic density (PSD) are both enlarged in cofilin 1 mutants, and these morphological changes are associated with reduced LTP, absence of LTD, and impaired extra-synaptic mobility of AMPA receptors (indicated by a thinner double arrow compared to the control synapse). Like in ADF mutants, presynaptic mechanisms are unchanged in cofilin 1 mutants. Compared to single mutants, dendritic spine size is further increased in double mutants lacking both ADF and cofilin 1, and double mutants display presynaptic defects that are not present in single mutants including an altered distribution, a reduced recruitment (indicated by the thinner arrow compared to control and single mutant synapses) and elevated exocytosis of synaptic vesicles. Whether AMPA receptor mobility is affected in ADF and/or double mutants, or whether LTP and LTD are impaired in double mutants has not been tested experimentally yet.

revealed that cofilin 1 controls i) actin dynamics in dendritic spines, ii) dendritic spine density and morphology, iii) trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and ultimately iv) long-term potentiation (LTP) and long-term depression (LTD),[10,17-22] 2 forms of synaptic plasticity that are widely considered as major cellular mechanisms underlying learning and memory.[23] Together, these studies let cofilin 1 emerge as an important regulatory factor that controls structural und functional aspects of synaptic plasticity via synaptic actin dynamics (**Fig. 1**). Moreover, they suggest a role in learning and memory, which indeed has been demonstrated by behavioral analysis of cofilin 1 mutant mice that performed worse in paradigms of associative learning or by accelerated extinction of aversive memory upon increased activity of ADF/cofilin in the rat infralimbic cortex.[10,21]

ADF shares very similar biochemical properties with cofilin 1 (for review, see[24]), and, like cofilin 1, it was found broadly expressed in the adult brain and present in presynaptiC-terminals and in dendritic spines.[9,11] However, while the relevance of cofilin 1 for synapse physiology and behavior is well established,[16] relatively little is known about the role of ADF, because *in vitro* studies almost exclusively focused on cofilin 1, and ADF mutant mice did not display synaptic or behavioral defects.[11] However, we found elevated cofilin 1 levels in synaptic structures from ADF mutants,[11] and we therefore hypothesized i) functional redundancy of ADF and cofilin 1 in the mouse brain and ii) that cofilin 1 can compensate for the loss of ADF in excitatory synapses. To test these hypotheses and to comprehensively characterize the synaptic function of ADF/cofilin, we generated double mutant mice lacking both ADF and cofilin 1. Indeed, these mutants displayed synaptic defects that were not present in ADF or cofilin 1 single mutants, thereby validating our aforementioned working hypotheses.

Compared to cofilin 1 single mutants, dendritic spine size was strongly increased in the hippocampus and striatum of double mutants,[25,26] thus demonstrating that ADF is relevant for dendritic spine morphology, too. Moreover, our data let us suggest that cofilin 1 can fully compensate for the loss of ADF at excitatory synapses, while ADF can only partially rescue cofilin 1s inactivation. Notably, a role of ADF in spine morphology and a redundant function of ADF and cofilin 1 in dendritic spine morphology has just recently been confirmed in rat hippocampal slices.[22] While our initial characterization of double mutant mice revealed a role of ADF in spine morphology, its function in dendritic spine actin dynamics, AMPA receptor trafficking or synaptic plasticity has not been examined yet, but will be tackled in future studies.

The analysis of double mutants not only revealed a redundant postsynaptic function for ADF and cofilin 1, but also demonstrated their relevance for presynaptic physiology.[25,26] In hippocampal synapses of double mutants, we found an altered distribution of synaptic vesicles, including an elevated number of synaptic vesicles attached to the active zone.[25] Consequently, synaptic vesicle exocytosis was increased in hippocampal synapses from these mice, and, similarly, we found increased glutamate release in the striatum. Such defects were not present in ADF or cofilin 1 single mutants.[10,11,25,26] Hence, cofilin 1 emerged as the predominant ADF/cofilin form in dendritic spines, while ADF and cofilin 1 are apparently equally important for presynaptic physiology. Together, our analysis of double mutant mice unraveled a novel function for ADF/cofilin in neurotransmitter release, which is in line with presynaptic defects upon inactivation of upstream regulators such as RhoB, ROCK2, PAK, LIMK1 or slingshot.[7,27-30] Based on its

relevance for actin dynamics, it is very likely that ADF/cofilin controls neurotransmitter release via regulating the presynaptic actin cytoskeleton, although this has not been demonstrated experimentally yet. However, in line with this idea, a pivotal role of actin dynamics in vesicle exocytosis has been demonstrated by exploiting actin drugs (for review see[31]) or by manipulating the activity of actin dynamics regulators such as profilin2 or DRR1.[32-34]

Interestingly, double mutant mice displayed behavioral alterations that were not present in single mutants, again providing compelling evidences for overlapping functions of ADF or cofilin 1 in the mouse brain.[26] These alterations included strongly increased locomotor activity, working memory deficits, impulsivity and a paradoxical calming effect of pharmacological treatment with psychostimulants such as methylphenidate, thus closely modeling typical clinical symptoms of attention deficit-hyperactivity disorder (ADHD) for which methylphenidate is widely prescribed in clinical practice.[35] Hence, our analysis of double mutant mice revealed that defects in neuronal actin dynamics can cause behavioral abnormalities reminiscent of human psychiatric diseases, thereby suggesting defective neuronal actin dynamics as a pathomechanism in mental disorders. Indeed, several genes that have been associated with human psychiatric diseases such as schizophrenia (*DISC1, SHANK2*), autism spectrum disorders (*SHANK2, SHANK3, FMRP*) or ADHD (*GIT1*) have just recently been linked to neuronal actin dynamics.[36-42] Future studies will show whether defective neuronal actin dynamics caused by mutations in and/or dysregulation of ABPs can be causative for mental disorders in humans, and whether modulating the activity of ABPs will allow novel treatment strategies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

This work was supported by a Research grant (24/2014 MR) of the University Medical Center Giessen and Marburg (UKGM).

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