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ADF/cofilin-actin rods in neurodegenerative diseases

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Abstract

Dephosphorylation (activation) of cofilin, an actin binding protein, is stimulated by initiators of neuronal dysfunction and degeneration including oxidative stress, excitotoxic glutamate, ischemia, and soluble forms of β -amyloid peptide (A β). Hyperactive cofilin forms rod-shaped cofilin-saturated actin filament bundles (rods). Other proteins are recruited to rods but are not necessary for rod formation. Neuronal cytoplasmic rods accumulate within neurites where they disrupt synaptic function and are a likely cause of synaptic loss without neuronal loss, as occurs early in dementias. Different rod-inducing stimuli target distinct neuronal populations within the hippocampus. Rods form rapidly, often in tandem arrays, in response to stress. They accumulate phosphorylated tau that immunostains for epitopes present in “striated neuropil threads,” characteristic of tau pathology in Alzheimer disease (AD) brain. Thus, rods might aid in further tau modifications or assembly into paired helical filaments, the major component of neurofibrillary tangles (NFTs). Rods can occlude neurites and block vesicle transport. Some rod-inducing treatments cause an increase in secreted A β . Thus rods may mediate the loss of synapses, production of excess A β , and formation of NFTs, all of the pathological hallmarks of AD. Cofilin-actin rods also form within the nucleus of heat-shocked neurons and are cleared from cells expressing wild type huntingtin protein but not in cells expressing mutant or silenced huntingtin, suggesting a role for nuclear rods in Huntington disease (HD). As an early event in the neurodegenerative cascade, rod formation is an ideal target for therapeutic intervention that might be useful in treatment of many different neurological diseases.

Keywords

Cytoskeleton; Alzheimer disease; hippocampus; vesicle transport; amyloid beta; oxidative stress; Huntington disease; peptidomimetics; phosphorylated tau

Alzheimer disease pathology and presumptive initiators

Alzheimer Disease (AD) is diagnosed post mortem where the classical pathological hallmarks are extracellular plaques containing amyloid beta (A β) peptides [1] and intra-neuronal neurofibrillary tangles of the hyperphosphorylated microtubule-associated protein tau [2]. One of the major challenges in AD research is to identify the mechanisms that generate the same common pathologies in sporadic and familial AD brains. Because AD is an age-related disorder, one early focus for its cause was mitochondrial dysfunction due to aging [3]. Mitochondrial poisons (e.g. herbicides and insecticides) have been implicated in Parkinson disease [4] and also could contribute to sporadic AD. Mitochondrial dysfunction produces reactive oxygen species, initiating oxidative stress, or reciprocally, other initiators of oxidative stress could lead to mitochondrial dysfunction. Regardless of the initiator, mitochondrial insufficiency decreases cellular ATP. Another potential AD initiator is excessive extracellular glutamate, the major CNS excitatory neurotransmitter [5,6]. Glutamate excitotoxicity likely occurs as a result of decreased glial uptake and neuronal ATP decline. Any of these mechanisms for AD initiation could be exacerbated by a failing cerebrovasculature that impacts clearance and exchange mechanisms.

Cofilin Pathology in AD

A seldom examined molecular pathology of AD brains, named cofilin pathology because of the abnormal immunostaining of cofilin [7,8], may help explain the development of sporadic AD and link its pathology to familial AD. Actin depolymerizing factor (ADF) and cofilin are two members of an essential protein family that enhances the rapid assembly and/or disassembly (dynamics) of actin, which is critical to many cell behaviors. The two proteins share 70% sequence identity, have the same single phospho-regulatory site and cryptic nuclear localization sequence, and have qualitatively similar but quantitatively different effects on actin *in vitro* and *in vivo* [9,10,11]. The proteins travel together with actin in the same component of slow axonal transport [12,13], suggesting a role for ADF/cofilin in the transport and exchange of actin into cytoskeletal structures during neuronal development. Rodent hippocampal neurons and human brain contain 5–12 fold more cofilin than ADF [7,14]; hence we will hereafter just refer to cofilin unless studies have been done specifically to express or examine ADF.

At low ratios (<1:100) to actin subunits in filamentous actin (F-actin), cofilin severs filaments forming more filament ends that can either nucleate filament growth or more rapidly depolymerize filaments, depending on the amount of available actin monomers [15]. At higher ratios, cofilin severing is rapid but transient as its binding to F-actin stabilizes a twisted form of actin, eliminating the phalloidin binding site and allowing filaments to bundle [15,16] (reviewed in [17]). Cofilin's ability to bind to actin and dynamize filament assembly/disassembly is inhibited by phosphorylation of its ser3 by LIM kinase and other

kinases (reviewed in [11]). Active cofilin, generated by specific and highly regulated phosphatases in the slingshot [18] and chronophin [19] families, binds ADP-actin with higher affinity than ATP-actin [20,21]. In stressed cells, ATP declines, oxidative potential increases, and these effects activate cofilin phosphatases [22,23]. ATP-actin levels decrease [24], and cofilin binds ADP-actin generating cofilin-saturated ADP-actin filaments, which aggregate *in vivo* [7] or *in vitro* [25] to form rods. Many rod-inducing stresses decrease the primary cellular reducing agent reduced glutathione, and the four free sulfhydryl groups of cofilin are prime targets for oxidation [26,27], which can enhance actin bundling into rods *in vitro*.

Cofilin immunostaining (IHC and IF) was observed in ovoid aggregates and in rod-like tandem arrays in AD brain [7]. Recently, Sandra Siedlak and George Perry found electron micrographs that showed a rod-like bundle of actin-sized filaments in a human AD brain section [17]. The similarities between the ultrastructure of this bundle and that of cofilin-actin rods induced experimentally in organotypic slices of rodent brain (see below) are striking.

Isolation and characterization of rods

Cofilin-actin rods induced by ATP-depletion have been isolated from non-neuronal cell lines expressing a human cofilin-GFP or *Xenopus* ADF/cofilin-GFP, and from non-neuronal cells and cultured rat cortical neurons in which rods are composed only of endogenously expressed proteins [25]. Rods are stable in DTT, EGTA and ATP but not in non-ionic detergents. Rods formed from endogenous proteins contain cytoplasmic actins (β - and γ -actin) and cofilin-1 identified by proteomic analysis. Immunoblot and silver staining analysis of neuronal rod proteins show a 1:1 ADF/cofilin:actin ratio based upon standard curves of purified proteins. Several other proteins were identified in rods, some of which are contaminants (*e.g.* tubulins and keratins) and others whose roles in rod formation have been investigated by immunostaining. Only actin and cofilin are in rods during all stages of rod formation. Thus, core rod components are ADF/cofilin and β - and γ -actins, confirmed by *in vitro* rod assembly from these purified proteins [25].

Signaling to ADF/cofilin-actin rods formation

Several pathways leading to cofilin activation from externally applied stress agents have been characterized (Figure 1). Disruption of mitochondrial electron transport causes ATP-depletion and release of chronophin from inhibition by its intracellular complex with Hsp90 [22]. Because silencing of chronophin by siRNA significantly, but only partially, delays rod formation and cofilin dephosphorylation, slingshot phosphatase may play a secondary role [22]. Since most neuronal stress agents induce a decline in ATP, chronophin activation may often be involved.

Externally applied ATP induces rods in some neurons through activation of the P2X receptor and calcium influx [28]. Elevated calcium binds to calmodulin and the complex stimulates calcineurin, which dephosphorylates slingshot, releasing it from its inhibitory binding partner 14-3-3 ζ [23, 29]. 14-3-3 ζ also is involved in peroxide induced rods, but in

this case it is through sulfhydryl oxidation of 14-3-3 ζ and its release of active slingshot [23]. Full activation of slingshot after release from 14-3-3 ζ requires F-actin binding [29,30].

Because glutamate activates several different receptor types in neurons, one of which is the calcium permeable ionotropic NMDA receptor, we anticipated glutamate would activate slingshot via the calcium/calmodulin/calcineurin-mediated pathway. Surprisingly, glutamate-induced rod formation in cultured rat embryonic hippocampal neurons (5–7 days *in vitro*) is mediated entirely by AMPA receptors and is calcium-independent¹; how the AMPA-induced depolarization mediates cofilin dephosphorylation has not been determined. Whether AMPA receptors alone mediate rod formation in older neurons is currently being investigated.

A β -induced rod formation is inhibited in ~50% of the neurons in which cdc42 is missing or a dominant negative cdc42 is expressed [31], suggesting that about half the hippocampal neurons that form rods in response to A β utilize a cdc42-dependent pathway. This pathway may be similar or identical to the one downstream of brain derived neurotrophic factor, in which cdc42 activation inhibits rho and decreases rho kinase and LIM kinase activities, and consequently phospho-cofilin levels [32,33]. The cofilin phosphatase slingshot also inhibits LIM kinase by direct dephosphorylation [30], suggesting that decreased LIM kinase activity will occur when slingshot is activated. Together these studies show that different stress agents effect rod formation by a plethora of pathways that activate cofilin phosphatases, inhibit cofilin kinases, or do both.

About 1 μ M of the oligomeric synthetic A β ₁₋₄₂ gives maximal rod response in which only about 20% of the hippocampal neurons (localized to the dentate gyrus and mossy fiber tract) form rods [34]. A cell culture secreted A β fraction, containing mainly A β dimer and trimer (A β d/t), obtained from Dennis Selkoe [35,36], gave a greater maximal rod response (~30% of neurons localized to similar regions), but at concentrations of ~200 pM, 5,000 fold more potent than synthetic A β mixtures². Furthermore, the concentration of A β d/t that induces rod formation in organotypic hippocampal slices of juvenile or adult rat is the same that causes memory and learning deficits when introduced into adult rat brain by a single intraventricular infusion [36]. Of interest, cognitive deficits in adult rats occur between 1–4 h after infusion and completely disappear by 24 h [36]. Rod formation induced in organotypic slices by A β d/t is twice control levels within 1 h and becomes highly significant ($p < 0.001$) by 2 h. Rods are fully reversible, disappearing 24 h after washout of the A β [31].

These findings led to our hypothesis that in familial AD, caused by mutations in pathways that lead to excessive A β d/t production, rods form primarily from the effects of A β d/t, whereas in sporadic AD other stress stimuli, e.g. cardiovascular insufficiency, oxidative stress, or excitotoxic glutamate, initiate rod formation [37,38]. The rods then block transport and enhance the formation and release of A β , some of which forms plaques and some of which, presumably as A β dimer or A β d/t [39], spreads the degenerative zone. This model explains the similar pathologies found in familial and sporadic AD.

¹Pak CW, Shaw AE, Minamide LS, Davis RC, Bamburg JR. Glutamate-induced cofilin-actin rod formation requires AMPA receptors and is associated with a disruption in APP-YFP transport. Mol Biol Cell Suppl. (abstract in press) (2009)

²Davis RC, Maloney MT, Minamide LS, Podlisny M, Selkoe DJ and Bamburg JR. Manuscript in preparation.

Cofilin-actin rods form in neurites and block vesicular transport and distal synaptic activity

Transport inhibition is one of the earliest defects in a human mutant APP transgenic mouse model for AD and in human AD brain [40]. Defects consist of axonal swellings containing organelles, vesicles and microtubule-associated proteins including motor molecules. Cofilin-actin rods form in axons and dendrites of cultured neurons treated with A β , where they inhibit vesicular transport and accumulate vesicles containing APP and A β peptides [34,38]. Although some rod-inducing agents block vesicle transport throughout the neurites of cells that form rods, probably due to a more global drop in ATP, other agents cause vesicle accumulation at sites of rod formation and vesicles remain dispersed in neurites without rods, demonstrating that rods can serve as sites of vesicle transport inhibition [34]. Synaptic dysfunction distal to rods has been characterized electrophysiologically in *Aplysia* neurons [41].

Rods and APP accumulation and A β secretion

Since much of the BACE cleavage of APP occurs in endosomes [42,43], endosomal APP undergoing retrograde transport may be prevented by rods from reaching lysosomes for degradation. Thus we hypothesized that the A β produced within them is not degraded and that stalled vesicles will then accumulate more A β and secrete it when they fuse with the cell membrane. Testing this model has not been easy because a sensitive assay for total rodent A β was not available and cofilin has functions in the secretory process separate from its rod-forming ability. Using a sensitive ELISA for total rodent A β developed in collaboration with Covance³, we demonstrated a 2.2 fold increase in secreted A β (normalized to cell number) in rat embryonic cortical neuron cultures with peroxide-induced rods compared to untreated cultures. No significant change in intracellular A β level was found between rod-containing and untreated neurons and the extracellular A β gradually increased each day suggesting that enhanced secretion of the contents of stalled vesicles takes place. These results lend support to our feed-forward hypothesis that A β induction of rods will potentiate secretion of A β in a positive loop [34]. A nearly identical increase in A β secretion (2.4 fold) from chicken tectal neurons treated with peroxide was quantified using immunoprecipitation and western blotting [44]. Some rod-inducing stimuli (ATP-depletion, glutamate stimulation) do not increase A β -secretion but freeze vesicle transport blocking accumulation at rods³. This finding suggests that interactions between different vesicle populations at rods may be required to bring about enhanced A β formation and/or secretion.

Different stimuli targeting different populations in hippocampus

We developed a mapping method for hippocampal organotypic slices with rod formation as a measured end point; our data demonstrate that discrete neuronal populations are sensitive to different stress-inducing agents [31]. Glutamate-induced rods are prevalent in the hippocampal CA3 region, whereas rods induced by synthetic amyloid beta (A β ₁₋₄₂) peptide

³Marsden IT, Pak CW, Maloney MT, Davis RC, Minamide LS, Bamburg JR. Feed-forward mechanisms of A β production and secretion in cortical neurons. Mol Biol Cell Suppl. (abstract in press) (2009)

oligomers and especially the A β /t² occur mostly in the dentate gyrus and mossy fiber tract [31]. This location suggests a major impact on cognitive function.

The dentate gyrus, with input from the perforant pathway axons and cholinergic neurons from the basal forebrain, plays a central role in associative memory [45], where dentate granule cells play a crucial role in pattern separation [46,47]. The CA3 pyramidal cells receive the granule cell output via the mossy fibers and aid in pattern completion [48] required for rapid one-trial contextual learning and pattern completion-based memory recall [49]. Because of its central role in associative memory, the dentate gyrus has been extensively studied in AD brain [47]. There is an early loss of synapses (48% decrease) before a measurable loss of neurons is detected [50,51]. Synaptic loss correlates with cognitive dysfunction during AD progression with early, mild and severe AD cases showing a decline in staining of the synaptic marker synaptophysin of about 25, 45 and 65%, respectively [52]. Because they block neurite transport and cause atrophy distal to them without killing neurons [7,41], A β -induced rods provide a plausible mechanism for synaptic loss in these regions. Thus they represent a novel target for therapeutic intervention.

Cofilin-actin rods as mediators of ischemic injury

Time-lapse imaging in cofilin-GFP expressing cells shows that rod formation begins within 4–8 min of anoxia in hippocampal slices [31] and plateaus by 10 min, suggesting a role in synaptic dysfunction during hypoxic/ischemic injury or traumatic brain injury. During early phases of rod formation induced by overexpression of cofilin-GFP, rods form transiently and may move both anterogradely and retrogradely within processes before undergoing disassembly. Transient rods can fuse to form larger structures that still move, and sometimes larger rods give rise to smaller ones that eventually disappear. When rods reach a certain size, as determined by the intensity of the cofilin-GFP, their motility ceases and they inhibit transport of other materials within the neurite⁴.

Role of cofilin-actin rods in formation of striated neuropil threads

In Alzheimer's disease, cofilin-actin rods and thread-like inclusions containing phosphorylated microtubule-associated protein tau form in the neuropil of the brain. The tau-containing structures have been referred to as striated neuropil threads [53], which comprise >85% of cortical tau pathology [54] and are directly correlated to cognitive decline and disease progression [55–57]. However, the mechanism leading to their assembly has remained elusive.

The 12E8 monoclonal antibody, which recognizes human tau phosphorylated on ser262 and ser356, immunostains tau in a pattern that co-localizes with cofilin-actin rods in chicken, rat and human neurons (Figure 2) [58]. The rod-like staining for tau is induced by several treatments that cause cofilin-actin rod formation, including cofilin overexpression, but does not occur in neurons similarly treated but in which cofilin expression is silenced. Furthermore, cofilin-actin rods precede tau immunostaining, suggesting cofilin-actin rods form a tau-binding matrix. FRET experiments show that cofilin and tau are in close

⁴Munsie L, Atwal RS, Marsden I, Wild EJ, Bamburg JR, Tabrizi SJ and Truant R. Manuscript submitted.

proximity. Bundling and accumulation of F-actin was previously observed to mediate tau induced degeneration in mice expressing mutant forms of tau associated with Frontal Temporal Dementia [59]. These results combined with our observations suggest a common pathway for phospho-tau and cofilin accumulation in neurites that can be initiated by oxidative stress, mitochondrial dysfunction or A β , all suspected initiators of synaptic loss in AD. We hypothesize that tau binding to cofilin-actin rods aids in its assembly into paired helical filaments, the main component of neurofibrillary tangles.

Nuclear cofilin-actin rods

Nuclear inclusion bodies, in the form of rods and sheets, were observed as long ago as the late 19th century [60; reviewed in 61]. Although their function is unclear, their frequent appearance in neurons from normal aged brain (as well as other tissues) suggests that they serve some physiological purpose. There is evidence that the formation of nuclear inclusion bodies *in vivo* can be a response to aging or stress. For example, none of the neurons of the cochlear nucleus in 12-day postnatal rats contained nuclear inclusions, but in adult and aged rats up to 23% of these neurons contained these structures [61]. Nuclear rods also appear spontaneously in sympathetic neuron explants after 2–3 weeks and increase in size with increasing time in culture [62]. The percentage of neurons with nuclear inclusions can be increased *in vivo* by intense electrical stimulation or local perfusion of agents that increase concentrations of intracellular cAMP [63,64].

In 1978, Fukui demonstrated that intranuclear rods, composed of actin, could be induced in *Dictyostelium* by treatment of the cells with 7–15% dimethylsulfoxide (DMSO) [65]. Nuclear actin rods have been induced in cultured mammalian cells with DMSO [66,67], heat shock [68,69] cytochalasin D [70], trifluoperazine, a calmodulin inhibitor, [71], forskolin, a cAMP inducing agent [72], and the Ca²⁺/Mg²⁺ ionophore A23187 in the presence of 100 mM Mg²⁺ [73]. The rods are formed from actin derived from the disassembly of cytoplasmic F-actin and the subsequent movement of actin into the nucleus [67]. Neuronal nuclear rods can be immunostained with antibodies against actin and cofilin (Figure 3), but not typically with fluorescent derivatives of phalloidin [74], suggesting they have a similar structure to cytoplasmic rods.

Other than actin, the only components that have been positively identified in these rods are ADF and cofilin. Ohta et al. [75] showed that dephosphorylation of phospho-cofilin occurred in fibroblasts prior to nuclear translocation in response to DMSO, suggesting that the phosphorylation state may regulate nuclear uptake. Dephosphorylation of ADF/cofilins also has been observed prior to nuclear translocation in T-cells in response to co-stimulatory signals [76]. However, Saito et al. [77] showed that both ADF and cofilin were totally dephosphorylated in thyroid cells in response to thyrotropin, but formed nuclear rods in these cells only after exposure to DMSO. Taken together, these data suggest that dephosphorylation of ADF/cofilin is necessary but not sufficient for nuclear uptake.

Both ADF and cofilin contain an identical nuclear localization sequence (NLS) P-X-X-X-K-K-R-K-K [78, 79], which is necessary for nuclear translocation resulting from heat shock [80]. A synthetic peptide containing this NLS rapidly accumulates in nuclei when injected

into the cytoplasm of myotubes [81], suggesting that the exposure of the NLS in ADF and cofilin is regulated. Since actin lacks a NLS, ADF and cofilin may serve as chaperones to transport actin into the nucleus under conditions that induce rod formation.

Although no specific role for nuclear rods is known, actin has roles in chromatin remodeling, formation of heterogeneous nuclear ribonucleoprotein complexes, and in transcription from all three RNA polymerases (reviewed in [82]). Thus, sequestering actin into nuclear rods with cofilin could have major effects on gene expression.

Nuclear rods in Huntington disease

Huntington disease (HD) is caused by a CAG triplet-repeat expansion in the gene encoding huntingtin, which results in an expanded polyglutamine tract in the protein [83]. Although the biological function of the entire 350 kDa huntingtin protein has remained elusive, it has been implicated in vesicular trafficking, nucleocytoplasmic transport, and transcription modulation [84–87]. Huntingtin is normally found associated with the endoplasmic reticulum (ER) surface, but under cell stress, including temperature shock and reduced ATP, huntingtin is released from the ER and undergoes nuclear entry. Huntingtin associates with nuclear cofilin-actin rods in cultured heat-shocked cells from a striatal neuron-derived cell line⁴. Upon removal of the stress, rods normally disappear. However, silencing huntingtin expression with siRNA or expressing a human HD mutant form of huntingtin with a large (111 amino acid) polyglutamine tract in place of wild type huntingtin inhibits clearance of nuclear cofilin-actin rods, suggesting that one role of normal huntingtin is clearing cofilin-actin nuclear rods.

Rods as a target for therapeutic intervention

Because cofilin modulates actin in many different cellular processes [11,88], it has been difficult to determine if the sequestering of cofilin into rods has detrimental effects in cells due to the rod formation *per se*, or whether it is the alteration of cofilin activity at some other place that is having the detrimental effect. Thus, for example, when we treat neurons with a rod inducing stimulus and see a decline in synaptic activity, we could be observing effects on delivery of synaptic material required for maintenance of activity or we could be observing a direct effect on local cofilin-actin dynamics within the synapse (or both). To get around this problem, we have taken two approaches. The first approach is to identify surface mutations on cofilin that do not alter normal cofilin-dependent actin dynamics but do prevent the mutant cofilin from incorporating into rods, especially in cells in which endogenous cofilin expression is silenced. Such a mutant cofilin could be used to make a knock-in mouse which should be resistant to forming rods and thus useful for testing directly the importance of rods in the degenerative response and synaptic loss. However, we also have shown that the ability of cells to form rods is transiently neuroprotective [89]. By tying up virtually all the cofilin but only a small fraction of the actin in rods, cells spare ATP that normally is consumed in actin filament dynamics [90], which can help maintain ionic homeostasis for several minutes giving the cell a chance to recover from its stress. Thus, there is no guarantee that a viable mouse which never forms rods can be obtained. The second approach is to identify peptides that will disrupt the surface interface between

adjacent cofilin-actin filaments in the rod but not normal cofilin-actin binding. These peptides could then be made cell permeable and used to inhibit rod formation in acute experiments in cultured neurons or organotypic slices.

Studies on mutant cofilins

We examined several mutations of cofilin on the non-actin binding surface. Mutant cofilin-RFP chimeras were expressed from plasmids transfected into different cell lines that express only cofilin and not ADF. Cofilin-actin rods were induced in the cytoplasm upon ATP depletion and in the nucleus upon 10% DMSO treatment. Many of the mutant cofilin-RFPs incorporated into cytoplasmic and nuclear rods nearly identically to wild type (wt) cofilin-RFP. However, several mutants showed > 50% decrease in cells forming both nuclear and cytoplasmic rods. Since residual rods could be due to mutant incorporation into rods initiated by endogenous proteins, we retested their ability to form rods in cells in which endogenous cofilin was silenced. One mutant did not form any rods and a second gave significantly reduced rod formation. Both mutant cofilins were phosphorylated to the same extent as wt cofilin in cells expressing an active form of LIM kinase and were dephosphorylated in cells expressing wt slingshot, suggesting the proteins folded correctly and served as LIM kinase and slingshot substrates. Bacterial expression and isolation of each of these mutant proteins allowed us to characterize their actin binding and dynamizing activities. One of the mutants (R21Q) had about a 10 fold weaker binding to F-actin, explaining its inability to incorporate into rods. The other mutant behaved similarly to wt cofilin in actin binding assays suggesting it might be useful in generating a knock-in mouse if it rescued normal actin dynamics in cofilin silenced cells.

We initially used a variety of functional assays to see if the mutants could rescue some cofilin-dependent activity in cofilin-silenced cells. Major assays used include: 1) cell division (cytokinesis) in which cofilin-silenced cells accumulate as a multinucleated phenotype; 2) Golgi organization in which cofilin-silenced cells exhibit a dispersed Golgi vesicle array; and 3) cell polarization/migration in which cofilin-silenced cells have a poor ability to undergo polarized migration either in wound healing (time to wound closure) or in spontaneously polarized migrating cells (chick cardiac fibroblasts [91]). Each of these assays has its strengths in trying to identify a non-rod forming mutant cofilin that maintains its essential actin dynamizing capability. Unfortunately, none of the assays performed as expected when we rescued with vectors expressing wt cofilin. For example the multinucleated cell type decreased from 24% in the cofilin-silenced cells to about 11% in cells expressing wt cofilin-RFP, significantly above the 3% average multinucleated phenotype in the parental cell cultures. We reasoned that the RFP might be inhibiting full cofilin activity so we expressed untagged wt cofilin from vectors expressing a fluorescent protein from a separate promoter (to identify expressing cells) but obtained about the same level of rescue. Another possibility that could explain the limited rescue, even with wt cofilin, is that cofilin overexpression from a strong CMV promoter might not create an adequate time window during which optimal cofilin activity required for rescue is expressed. Too much cofilin may be just as inhibitory to some actin functions as too little. To circumvent this problem we are remaking our expression plasmids using the cofilin promoter. This work is in progress.

Testing peptidomimetics to block rod formation

Although the structure of the cofilin-actin complex has not been directly determined, both NMR and X-ray crystal structures have been determined for several ADF/cofilin family members from across phylogeny. All have a very similar core structure, called the ADF-homology domain, also found in some other actin binding proteins where it may be repeated more than once [92]. Models of the complex between cofilin and F-actin (based on the F-actin model of Holmes et al. [93]) have been built using molecular dynamics simulations, structural homology considerations, and synchrotron radiolytic footprinting data [94–97]. In addition, the crystal structure of one ADF-H domain of twinfilin in complex with actin was recently determined [98]. We considered residues exposed on the surface of the cofilin bound to F-actin to be likely candidates for interaction between adjacent cofilin-saturated filaments in forming rods. Thus we prepared synthetic peptides corresponding to the entire exposed surface regions (Figure 4) and tested their ability to block rod formation when injected into cells expressing human cofilin-GFP. Peptides were injected with rhodamine-dextran so that injected cells could be scored for rods following ATP-depletion. Initial injections were performed with peptides made at 2 mg/ml. Assuming injection volumes of about 10% of the cell volume the final intracellular concentrations of most of the peptides should be approximately 100 μ M, or about 5 times the estimated 20 μ M concentration of cofilin. At this concentration, one peptide gave ~20% decrease in rod formation.

While these studies were in progress we completed the characterization of rods isolated from cells expressing cofilin-GFP and cells where rods formed from endogenous proteins. Rods formed from endogenous proteins were more unstable in 0.5 M salt than were rods formed from cofilin-GFP [25], suggesting that the interface between the adjacent cofilin-actin filaments will differ in the two cases. Thus, we are now retesting all of the same peptides used above for their abilities to inhibit endogenous rod formation in A431 cells subjected to ATP-depletion.

Conclusions

Cofilin's ability to enhance F-actin severing and subunit turnover when at low stoichiometry to F-actin subunits and to bind and stabilize F-actin when at high stoichiometry is a form of self-regulation, which when coupled to its phospho-regulation by a number of upstream regulators, provides an exquisite mechanism for spatial and temporal control of actin filament dynamics. The reversible stress-induced bundling of the cofilin-saturated F-actin into rods, which occurs in all cells expressing adequate levels of ADF/cofilin proteins, is possibly just an additional regulatory layer to further conserve ATP for other processes. However, in neurons, where ADF/cofilin is distributed within the neurites and forms rods that inhibit transport and thus inhibit their own ability to recover, additional complications arise that can compromise synaptic function. The transport of actin into the nucleus may also be a normal cellular function of cofilin that gets compromised under certain types of stress leading to formation of nuclear rods. These might also have a transient protective effect through changes in the transcriptome but might be detrimental to long-term neuronal function. Developing new tools to test the role of rods in different aspects of

neurodegenerative diseases is critical to further our understanding of rods and how their manipulation might be of therapeutic significance.

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References

1. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. *Nat Rev Mol Cell Biol.* 2007; 8:101–112. [PubMed: 17245412]
2. Goedert M, Spillantini MG. A century of Alzheimer's disease. *Science.* 2006; 314:777–781. [PubMed: 17082447]
3. Fukui H, Moraes CT. The mitochondrial impairment, oxidative stress and neurodegeneration connection: reality or just an attractive hypothesis. *Trends Neurosci.* 2008; 31:251–256. [PubMed: 18403030]
4. Hatcher JM, Pennell KD, Miller GW. Parkinson's disease and pesticides: a toxicological perspective. *Trends Pharmacol.* 2008; 29:322–329.
5. Arias C, Becerra-Garcia F, Tapia R. Glutamic acid and Alzheimer's disease. *Neurobiol.* 1998; 6:33–43.
6. Lancelot E, Beal MF. Glutamate toxicity in chronic neurodegenerative disease. *Prog Brain Res.* 1998; 116:331–347. [PubMed: 9932386]
7. Minamide LS, Striegl AM, Boyle JA, Meberg PJ, Bamburg JR. Neurodegenerative stimuli induce persistent ADF/cofilin-actin rods that disrupt distal neurite function. *Nat Cell Biol.* 2000; 2:628–636. [PubMed: 10980704]
8. Zhao L, Ma QL, Calon F, Harris-White ME, Yang F, Lim GP, et al. Role of p21-activated kinase pathway defects in the cognitive deficits of Alzheimer disease. *Nat Neurosci.* 2006; 9:234–242. [PubMed: 16415866]
9. Bamburg JR. Proteins of the ADF/cofilin family: essential regulators of actin dynamics. *Annu Rev Cell Dev Biol.* 1999; 15:185–230. [PubMed: 10611961]
10. Chen H, Bernstein BW, Sneider J, Boyle JA, Minamide LS, Bamburg JR. In vitro activity differences between proteins of the ADF/cofilin family define two distinct subgroups. *Biochemistry.* 2004; 43:7127–7142. [PubMed: 15170350]
11. Van Troys M, Huyck L, Leyman S, Dhaese S, Vandekerckhove J, Ampe C. Ins and outs of ADF/cofilin activity and regulation. *Eur J Cell Biol.* 2008; 87:649–667. [PubMed: 18499298]
12. Mills RG, Minamide LS, Yuan A, Bamburg JR, Bray JJ. Slow axonal transport of soluble actin with actin depolymerizing factor, cofilin, and profilin suggests actin moves in an unassembled form. *J Neurochem.* 1996; 67:1225–1234. [PubMed: 8752130]
13. Flynn KC, Pak CW, Shaw AE, Bradke F, Bamburg JR. Growth cone-like waves transport actin and promote axonogenesis and neurite branching. *Dev Neurobiol.* 2009; 69:761–779. [PubMed: 19513994]
14. Garvalov BK, Flynn KC, Neukirchen D, Meyn L, Teusch N, Wu X, et al. Cdc42 regulates cofilin during the establishment of neuronal polarity. *J Neurosci.* 2007; 27:13117–13129. [PubMed: 18045906]
15. Andrianantoandro E, Pollard TD. Mechanism of actin filament turnover by severing and nucleation at different concentrations of ADF/cofilin. *Molecular Cell.* 2006; 24:13–23. [PubMed: 17018289]
16. McGough A, Pope B, Chiu W, Weeds A. Cofilin changes the twist of F-actin: implications for actin filament dynamics and cellular function. *J Cell Biol.* 1997; 138:771–781. [PubMed: 9265645]

17. Bamburg JR, Bloom GS. Cytoskeletal pathologies of Alzheimer disease. *Cell Motil Cytoskel.* 2009; 66:635–649.
18. Niwa R, Nagata-Ohashi K, Takeichi M, Mizuno K, Uemura T. Control of actin reorganization by Slingshot, a family of phosphatases that dephosphorylate ADF/cofilin. *Cell.* 2002; 108:233–246. [PubMed: 11832213]
19. Gohla A, Birkenfeld J, Bokoch GM. Chronophin, a novel HAD-type serine protein phosphatase, regulates cofilin-dependent actin dynamics. *Nat Cell Biol.* 2005; 7:21–29. [PubMed: 15580268]
20. Maciver SK, Weeds AG. Actophorin preferentially binds monomeric ADP-actin over ATP-bound actin: consequences for cell locomotion. *FEBS Lett.* 1994; 347:251–256. [PubMed: 8034013]
21. Ressad F, Didry D, Egile C, Pantaloni D, Carlier MF. Control of actin filament length and turnover by actin depolymerizing factor (ADF/cofilin) in the presence of capping proteins and ARP2/3 complex. *J Biol Chem.* 1999; 274:20970–20976. [PubMed: 10409644]
22. Huang TY, Minamide LS, Bamburg JR, Bokoch GM. Chronophin serves as an ATP-sensing mechanism for cofilin dephosphorylation and neuronal cofilin-actin rod formation. *Dev Cell.* 2008; 15:691–703. [PubMed: 19000834]
23. Kim JS, Huang TY, Bokoch GM. Reactive oxygen species regulate a slingshot-cofilin activation pathway. *Mol Biol Cell.* 2009; 20:2650–2660. [PubMed: 19339277]
24. Atkinson SJ, Hosford MA, Molitoris BA. Mechanism of actin polymerization in cellular ATP-depletion. *J Biol Chem.* 2004; 279:5194–5199. [PubMed: 14623892]
25. Minamide LS, Maiti S, Boyle JA, Davis RC, Coppinger JA, Bao Y, Huang TY, Yates J, Bokoch GM, Bamburg JR. Isolation and characterization of cofilin-actin rods from stressed cells. *J Biol Chem.* Dec 17. 2009 Epub ahead of print.
26. Pfannstiel J, Cyrklaff M, Habermann A, Stoeva S, Griffiths G, Shoeman R, et al. Human cofilin forms oligomers exhibiting actin bundling activity. *J Biol Chem.* 2001; 276:49476–49484. [PubMed: 11679578]
27. Fratelli M, Demol H, Puype M, Casagrande S, Eberini I, Salmons M, et al. Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. *Proc Nat Acad Sci USA.* 2002; 99:3505–3510. [PubMed: 11904414]
28. Homma K, Niino Y, Hotta K, Oka K. Ca(2+) influx through P2X receptors induces actin cytoskeleton reorganization by the formation of cofilin rods in neurites. *Mol Cell Neurosci.* 2008; 37:261–270. [PubMed: 17993279]
29. Wang Y, Shibasaki F, Mizuno K. Calcium signal-induced cofilin dephosphorylation is mediated by Slingshot via calcineurin. *J Biol Chem.* 2005; 280:12683–12689. [PubMed: 15671020]
30. Soosairajah J, Maiti S, Wiggan O, Sarmiere P, Moussi N, Sarcevic R, et al. Interplay between components of a novel LIM kinase1-slitshot phosphatase complex regulate ADF/cofilin activity. *EMBO J.* 2005; 24:473–486. [PubMed: 15660133]
31. Davis RC, Maloney MT, Minamide LS, Flynn KC, Stonebraker MA, Bamburg JR. Mapping cofilin-actin rods in stressed hippocampal slices and the role of cdc42 in amyloid β -induced rods. *J Alzheimers Dis.* 2009; 18:35–50. [PubMed: 19542631]
32. Gehler S, Shaw AE, Sarmiere PD, Bamburg JR, Letourneau PC. Brain derived neurotrophic factor regulation of growth cone filopodial dynamics is mediated through actin depolymerizing factor/cofilin. *J Neurosci.* 2004; 24:10741–10749. [PubMed: 15564592]
33. Chen, T-j; Gehler, S.; Shaw, AE.; Bamburg, JR.; Letourneau, PC. Cdc42 participates in the regulation of ADF/cofilin and retinal growth cone filopodial dynamics by Brain Derived Neurotrophic Factor. *J Neurobiol.* 2006; 66:103–114. [PubMed: 16215999]
34. Maloney MT, Minamide LS, Kinley AW, Boyle JA, Bamburg JR. Beta-secretase-cleaved amyloid precursor protein accumulates at actin inclusions induced in neurons by stress or amyloid beta: a feedforward mechanism for Alzheimer's disease. *J Neurosci.* 2005; 25:11313–11321. [PubMed: 16339026]
35. Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, et al. Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation in vivo. *Nature.* 2002; 416:535–539. [PubMed: 11932745]

36. Cleary JP, Walsh DM, Hofmeister JJ, Shankar GM, Kuskowski MA, Selkoe DJ, et al. Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci.* 2005; 8:79–84. [PubMed: 15608634]
37. Maloney MT, Bamburg JR. Cofilin-mediated neurodegeneration in Alzheimer's disease and other amyloidopathies. *Mol Neurobiol.* 2007; 35:21–44. [PubMed: 17519504]
38. Maloney MT, Kinley A, Pak C, Bamburg JR. ADF/cofilin, actin dynamics and disease. *Protein Reviews.* 2008; 8:83–187.
39. Shankar GM, Li S, Mehta TH, Garcia-Munoz A, Shepardson NE, Smith I, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brain impair synaptic plasticity and memory. *Nat Med.* 2008; 14:837–842. [PubMed: 18568035]
40. Stokin GB, Lillo C, Falzone TL, Brusch RG, Rockenstein E, Mount SL, et al. Axonopathy and transport defects early in the pathogenesis of Alzheimer's disease. *Science.* 2005; 307:1282–1288. [PubMed: 15731448]
41. Jang DH, Han JH, Lee SH, Lee YS, Park H, Lee SH, et al. Cofilin expression induces cofilin-actin rod formation and disrupts synaptic structure and function in Aplysia synapses. *Proc Natl Acad Sci U S A.* 2005; 102:16072–16077. [PubMed: 16247020]
42. Ehehalt R, Keller P, Haass C, Thiele C, Simons K. Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. *J Cell Biol.* 2003; 160:113–123. [PubMed: 12515826]
43. Thomas RS, Liddell JE, Murphy LS, Pache DM, Kidd EJ. An antibody to the beta-secretase cleavage site on amyloid-beta-protein precursor inhibits amyloid-beta production. *J Alzheimers Dis.* 2006; 10:379–390. [PubMed: 17183149]
44. Goldsbury C, Whiteman IT, Jeong EV, Lim Y-A. Oxidative stress increases levels of endogenous amyloid-beta peptides secreted from primary chick brain neurons. *Aging Cell.* 2008; 7:771–775. [PubMed: 18691184]
45. Morris RG. Elements of a neurobiological theory of hippocampal function: the role of synaptic plasticity, synaptic tagging and schemas. *Eur J Neurosci.* 2006; 23:2829–2846. [PubMed: 16819972]
46. McHugh TJ, Jones MW, Quinn JJ, Balthasar N, Coppari R, Elmquist JK, et al. Dentate gyrus NMDA receptors mediate rapid pattern separation in the hippocampus. *Science.* 2007; 317:94–99. [PubMed: 17556551]
47. Ohm TG. The dentate gyrus in Alzheimer's disease. *Prog Brain Res.* 2007; 163:723–740. [PubMed: 17765747]
48. Nakazawa K, Quirk MC, Chitwood RA, Watanabe M, Yeckel MF, Sun LD, et al. Requirement for hippocampal CA3 NMDA receptors in associative memory recall. *Science.* 2002; 297:211–218. [PubMed: 12040087]
49. Nakashiba T, Young JZ, McHugh TJ, Buhl DL, Tonegawa S. Transgenic inhibition of synaptic transmission reveals role of CA3 output in hippocampal learning. *Science.* 2008; 319:1260–1264. [PubMed: 18218862]
50. Bertoni-Freddari C, Fattoretti P, Casoli T, Caselli U, Meier-Ruge W. Deterioration threshold of synaptic morphology in aging and senile dementia of Alzheimer's type. *Anal Quant Cytol Histol.* 1996; 18:209–213. [PubMed: 8790834]
51. Scheff SW, Price DA. Synaptic pathology in Alzheimer's disease: a review of ultrastructural studies. *Neurobiol Aging.* 2003; 24:1029–1046. [PubMed: 14643375]
52. Masliah E, Mallory M, Hansen L, De Teresa R, Alford M, Terry R. Synaptic and neuritic alterations during the progression of Alzheimer's disease. *Neurosci Lett.* 1994; 174:67–72. [PubMed: 7970158]
53. Velasco ME, Smith MA, Siedlak SL, Nunomura A, Perry G. Striation is the characteristic neuritic abnormality in Alzheimer disease. *Brain Res.* 1998; 813:329–333. [PubMed: 9838181]
54. Mitchell TW, Nissanol J, Han LY, Mufson EJ, Schneider JA, Cochran EJ, et al. Novel method to quantify neuropil threads in brains from elders with or without cognitive impairment. *J Histochem Cytochem.* 2000; 48:1627–1638. [PubMed: 11101631]

55. Braak H, Alafuzoff I, Arzberger T, Kretschmar H, Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol.* 2006; 112:389–404. [PubMed: 16906426]
56. Augustinack J, Schneider A, Mandelkow E, Hyman B. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. *Acta Neuropathol.* 2002; 103:26–35. [PubMed: 11837744]
57. Giannakopoulos P, von Gunten A, Kovari E, Gold G, Herrman FR, Hof PR, et al. Stereological analysis of neuropil threads in the hippocampal formation: relationships with Alzheimer's disease neuronal pathology and cognition. *Neuropath Appl Neurobiol.* 2007; 33:334–343.
58. Whiteman IT, Gervasio OL, Cullen KM, Guillemin GJ, Jeong EV, Wittig PK, et al. Activated ADF/cofilin sequesters phosphorylated microtubule-associated protein during the assembly of Alzheimer-like neuronal cytoskeletal striations. *J Neurosci.* 2009; 29:12994–13005. [PubMed: 19828813]
59. Fulga T, Elson-Schwab I, Khurana V, Steinhilb M, Spires T, Hyman B, et al. Abnormal bundling and accumulation of F-actin mediates tau-induced neuronal degeneration in vivo. *Nat Cell Biol.* 2006; 9:139–148. [PubMed: 17187063]
60. Mann G. Histological changes induced in sympathetic, motor, and sensory nerve cells by functional activity. *J Anat Physiol.* 1894; 29:100–108.
61. Feldman ML, Peters A. Intranuclear rods and sheets in rat cochlear nucleus. *J Neurocytol.* 1972; 1:109–127. [PubMed: 4358834]
62. Masurovsky EB, Benitez HH, Kim SU, Murray MR. Origin, development, and nature of intranuclear rodlets and associated bodies in chicken sympathetic neurons. *J Cell Biol.* 1970; 44:172–191. [PubMed: 4901373]
63. Seite R, Mei N, Vuillet-Luciani J. Effect of electrical stimulation on nuclear microfilaments and microtubules of sympathetic neurons submitted to cycloheximide. *Brain Res.* 1973; 50:419–423. [PubMed: 4705509]
64. Seite R, Leonetti J, Luciani-Vuillet J, Vio M. Cyclic AMP and ultrastructural organization of the nerve cell nucleus: stimulation of nuclear microtubules and microfilaments assembly in sympathetic neurons. *Brain Res.* 1977; 124:41–51. [PubMed: 191148]
65. Fukui Y. Intranuclear actin bundles induced by dimethyl sulfoxide in interphase nucleus of *Dictyostelium*. *J Cell Biol.* 1978; 76:146–157. [PubMed: 338616]
66. Fukui Y, Katsumaru H. Dynamics of nuclear actin bundle induction by dimethyl sulfoxide and factors affecting its development. *J Cell Biol.* 1980; 84:131–140. [PubMed: 7188610]
67. Sanger JW, Sanger JM, Kreis TE, Jockusch BM. Reversible translocation of cytoplasmic actin into the nucleus caused by dimethyl sulfoxide. *Proc Natl Acad Sci USA.* 1980; 77:5268–5272. [PubMed: 7001475]
68. Welch WJ, Suhan JP. Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. *J Cell Biol.* 1985; 101:1198–1211. [PubMed: 3900086]
69. Iida K, Iida H, Yahara I. Heat shock induction of intranuclear actin rods in cultured mammalian cells. *Exp Cell Res.* 1986; 165:207–215. [PubMed: 3519257]
70. Yahara I, Harada F, Sekita S, Yoshihira K, Natori S. Correlation between effects of 24 different cytochalasins on cellular structure and cellular events and those on actin in vitro. *J Cell Biol.* 1982; 92:69–78. [PubMed: 7199054]
71. Osborn M, Weber K. Damage of cellular functions by trifluoperazine, a calmodulin-specific drug. *Exp Cell Res.* 1980; 130:484–488. [PubMed: 7192637]
72. Osborn M, Weber K. Actin paracrystal induction by forskolin and by db-cAMP in CHO cells. *Exp Cell Res.* 1984; 150:408–418. [PubMed: 6319166]
73. Osborn M, Weber K. Dimethylsulfoxide and the ionophore A23187 affect the arrangement of actin and induce nuclear actin paracrystals in PtK2 cells. *Exp Cell Res.* 1980; 129:103–114. [PubMed: 6775963]

74. Nishida E, Iida K, Yonezawa N, Koyasu S, Yahara I, Sakai H. Cofilin is a component of intranuclear and cytoplasmic actin rods induced in cultured cells. *Proc Natl Acad Sci USA*. 1987; 84:5262–5266. [PubMed: 3474653]
75. Ohta Y, Nishida E, Sakai H, Miyamoto E. Dephosphorylation of cofilin accompanies heat shock-induced nuclear accumulation of cofilin. *J Biol Chem*. 1989; 264:16143–16148. [PubMed: 2777782]
76. Samstag Y, Eckerskorn C, Wesselborg S, Henning S, Wallich R, Meuer SC. Costimulatory signals for human T-cell activation induce nuclear translocation of pp19/cofilin. *Proc Natl Acad Sci USA*. 1994; 91:4494–4498. [PubMed: 8183936]
77. Saito T, Lamy F, Roger PP, Lecocq R, Dumont JE. Characterization and identification as cofilin and destrin of two thyrotropin- and phorbol ester-regulated phosphoproteins in thyroid cells. *Exp Cell Res*. 1994; 212:49–61. [PubMed: 8174642]
78. Matsuzaki F, Matsumoto S, Yahara I, Yonezawa N, Nishida E, Sakai H. Cloning and characterization of porcine brain cofilin cDNA. *J Biol Chem*. 1988; 263:11564–11568. [PubMed: 3403546]
79. Abe H, Endo T, Yamamoto K, Obinata T. Sequence of cDNAs encoding actin depolymerizing factor and cofilin of embryonic chicken skeletal muscle: two functionally distinct actin regulatory proteins exhibit high structural homology. *Biochemistry*. 1990; 29:7420–7425. [PubMed: 1699599]
80. Iida K, Matsumoto S, Yahara I. The KKRRK sequence is involved in heat shock-induced nuclear translocation of the 18-kDa actin-binding protein, cofilin. *Cell Struct Funct*. 1992; 17:39–46. [PubMed: 1586966]
81. Abe H, Nagaoka R, Obinata T. Cytoplasmic localization and nuclear transport of cofilin in cultured myotubes. *Exp Cell Res*. 1993; 206:1–10. [PubMed: 8482351]
82. Zheng B, Han M, Bernier M, Wen J-k. Nuclear actin and actin-binding proteins in the regulation of transcription and gene expression. *FEBS J*. 2009; 276:2669–2685. [PubMed: 19459931]
83. MacDonald ME, Ambrose CM, Duyao MP, Myers RH, Lin C, Srinidhi L, et al. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*. 1993; 72:971–983. [PubMed: 8458085]
84. Truant R, Atwal R, Burtnik A. Hypothesis: Huntingtin may function in membrane association and vesicular trafficking. *Biochem Cell Biol*. 2006; 84:912–917. [PubMed: 17215878]
85. Truant R, Atwal RS, Burtnik A. Nucleocytoplasmic trafficking and transcription effects of huntingtin in Huntington's disease. *Prog Neurobiol*. 2007; 83:211–227. [PubMed: 17240517]
86. Trushina E, Dyer RB, Badger JD 2nd, Ure D, Eide L, Tran DD, et al. Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. *Mol Cell Biol*. 2004; 24:8195–8209. [PubMed: 15340079]
87. Xia J, Lee DH, Taylor J, Vandelft M, Truant R. Huntingtin contains a highly conserved nuclear export signal. *Hum Mol Genet*. 2003; 12:1393–1403. [PubMed: 12783847]
88. Bamburg JR, Wiggan O. ADF/cofilin and actin dynamics in disease. *Trends Cell Biol*. 2002; 12:598–605. [PubMed: 12495849]
89. Bernstein BW, Chen H, Boyle JA, Bamburg JR. Formation of actin-ADF/cofilin rods transiently retards decline of mitochondrial potential and ATP in stressed neurons. *Am J Physiol Cell Physiol*. 2006; 291:C828–C839. [PubMed: 16738008]
90. Bernstein BW, Bamburg JR. Actin-ATP hydrolysis is a major energy drain for neurons. *J Neurosci*. 2003; 23:1–6. [PubMed: 12514193]
91. Dawe HR, Minamide LS, Bamburg JR, Cramer LP. ADF/cofilin controls cell polarity during fibroblast migration. *Curr Biol*. 2003; 13:252–257. [PubMed: 12573223]
92. Lappalainen P, Kessels MM, Cope MJTV, Drubin DG. The ADF homology (ADF-H) domain: a highly exploited actin-binding module. *Mol Biol Cell*. 1998; 9:1951–1959. [PubMed: 9693358]
93. Holmes KC, Popp D, Gebhard W, Kabsch W. Atomic model of the actin filament. *Nature*. 1990; 347:44–49. [PubMed: 2395461]
94. Lappalainen P, Fedorov EV, Fedorov AA, Almo SC, Drubin DG. Essential functions and actin-binding surfaces of yeast cofilin revealed by systematic mutagenesis. *EMBO J*. 1997; 16:5520–5530. [PubMed: 9312011]

95. Pope BJ, Gonsior SM, Yeoh S, McGough A, Weeds AG. Uncoupling actin filament fragmentation by cofilin from increased subunit turnover. *J Mol Biol.* 2000; 298:649–661. [PubMed: 10788327]
96. Kamal JK, Benchaar SA, Takamoto K, Reisler E, Chance MR. Three-dimensional structure of cofilin bound to monomeric actin derived by structural mass spectrometry data. *Proc Natl Acad Sci U S A.* 2007; 104:7910–7915. [PubMed: 17470807]
97. Grintsevich EE, Benchaar SA, Warshaviak D, Boontheung P, Halgand F, Whitelegge JP, et al. Mapping the cofilin binding site on yeast G-actin by chemical cross-linking. *J Mol Biol.* 2008; 377:395–409. [PubMed: 18258262]
98. Paavilainen VO, Oksanen E, Goldman A, Lappalainen P. Structure of the actin-depolymerizing factor homology domain in complex with actin. *J Cell Biol.* 2009; 182:51–59. [PubMed: 18625842]
99. Shaw AE, Minamide LS, Bill CL, Funk JD, Maiti S, Bamburg JR. Cross-reactivity of antibodies to actin-depolymerizing factor/cofilin family proteins and identification of the major epitope recognized by a mammalian actin-depolymerizing factor/cofilin antibody. *Electrophoresis.* 2004; 25:2611–2620. [PubMed: 15300782]

Figure 1.
Summary of signaling pathways discussed in text for stress-induced cofilin-activation leading to rod formation.

Figure 2.

Accumulation of (B) phosphorylated tau (12E8 antibody staining) at (A) cofilin-actin rods in antimycin A-treated hippocampal neurons. (C) Overlay of images. E18 rat hippocampal neurons were cultured 5 days and then treated with antimycin A for 30 min. Cells were fixed with 4% formaldehyde for 45 min and permeabilized for 90 s in 0.05% Triton X-100. Higher levels of Triton X-100 or treatment for longer periods reduces immunostaining of cofilin in rods. Methanol permeabilization, which is ideal for cofilin-actin rod immunostaining, destroys the 12E8 epitope. Bar=10 μ m.

Figure 3.

Nuclear ADF/cofilin-actin rods induced in neurons by 10% DMSO. (A–D) Time-course of rod formation after addition of 10% DMSO. A=0 time; B=15 min; C=45 min; D=90 min. Cells were fixed in 4% paraformaldehyde for 30 min, permeabilized with -20° methanol for 3 min and stained for ADF/cofilin with affinity purified rabbit 1439 antibody [99]. Bar for A–D= 10 μ m. Transmission electron micrographs of sections through the cell body of hippocampal neurons that were untreated (E) or treated 90 min with 10% DMSO (F). Rods in longitudinal section and cross-section are present around the nucleus but are also within white box. Bar for E and F= 1 μ m. (G) Nuclear rod from another cell that was stained with immunogold for ADF/cofilin. Virtually all of the ADF/cofilin immunogold staining lies over rods. Bar=0.1 μ m.

Figure 4.

Ribbon structures of human cofilin with space filling models of the peptides of the non F-actin-binding face used for studies on disruption of rod formation. (A) View of molecule from the normal actin binding surface. (B) View of molecule showing exposed peptides on non-actin binding interface. Peptides shown are: S8-V20 (red), R21-K30 (yellow), C39-N46 (green), N46-D59 (cyan) and K73-R81 (magenta).