Molecular Neurobiology

ISSN 0893–7648/07/35(1): 21–43/$30.00
ISSN (Online) 1559–1182

Cofilin-Mediated Neurodegeneration in Alzheimer's Disease and Other Amyloidopathies

Michael T. Maloney and James R. Bamburg*

Department of Biochemistry and Molecular Biology and Molecular, Cellular and Integrative Neurosciences Program, Colorado State University, Fort Collins, CO

Abstract

Transport defects may arise in various neurodegenerative diseases from failures in molecular motors, microtubule abnormalities, and the chaperone/proteasomal degradation pathway leading to aggresomal-lysosomal accumulations. These defects represent important steps in the neurodegenerative cascade, although in many cases, a clear consensus has yet to be reached regarding their causal relationship to the disease. A growing body of evidence lends support to a link between neurite transport defects in the very early stages of many neurodegenerative diseases and alterations in the organization and dynamics of the actin cytoskeleton initiated by filament dynamizing proteins in the ADF/cofilin family. This article focuses on cofilin, which in neurons under stress, including stress induced by the amyloid-β (Aβ) 1-42 peptide, undergoes dephosphorylation (activation) and forms rod-shaped actin bundles (rods). Rods inhibit transport, are sites of amyloid precursor protein accumulation, and contribute to the pathology of Alzheimer's disease. Because rods form rapidly in response to anoxia, they could also contribute to synaptic deficits associated with ischemic brain injury (e.g., stroke). Surprisingly, cofilin undergoes phosphorylation (inactivation) in hippocampal neurons treated with $A\beta_{1\text{-}40}$ at high concentrations, and these neurons undergo dystrophic morphological changes, including accumulation of *pretangle phosphorylated-τ*. Therefore, extremes in phosphoregulation of cofilin by different forms of Aβ may explain much of the Alzheimer's disease pathology and provide mechanisms for synaptic loss and plaque expansion.

Index Entries: Alzheimer's disease; Down syndrome; stroke; ADF/cofilin; actin; amyloid-β; inclusions; transport; neurodegeneration.

Received July 17, 2006; Accepted September 5, 2006.
*Author to whom correspondence and reprint requests should be addressed. E-mail: James.Bamburg@ColoState. edu

Introduction

Ischemia, oxidative stress, and excitotoxic insults are key factors driving neurodegeneration. These can arise from stroke, trauma, or mitochondrial dysfunction or may be initiated or enhanced by genetic risk factors *(1)*. With these upstream initiating factors and final stage pathology well-defined, the focus of current research lies in deciphering the biochemical pathways involved in the disease process and to what extent each contributes to it. These investigations are defined partly by the necessity and, in some cases, an inability to connect cellular and biochemical aspects of neurodegeneration with the behavioral consequences observed in the associated human diseases. One example of this disparity is a failure to define the molecular mechanisms responsible for the cognitive decline associated with early stages of Alzheimer's disease (AD). Lower scores on the mini-mental state examination and other neuropsychological tests directly correlate with reduced synapse number in AD *(2,3)*. During this period of synapse loss, overt neuronal loss is minimal *(4,5)*. Transport defects arising from abnormal regulation of the neuronal cytoskeleton present a promising model not only for explaining early cognitive decline but also for bringing together key features driving progressive neurodegeneration.

The cytoskeleton is a dynamic array of proteins creating a critical framework upon which many cellular functions rely. Abnormal regulation of the neuronal cytoskeleton can lead to improper location of growth cone paths, dendritic spine abnormalities, transport defects, protein aggregation and cell death. Actin aggregates in the form of cofilin-actin rods have been identified in postmortem brains of patients with AD *(6)*. The abnormal regulation of actin and cofilin proteins can be linked to a wide range of human neurodegenerative disorders, including corticobasal degeneration, William's syndrome, fragile X syndrome, AD, dystonia with dementia, and spinal muscular atrophy. Although these diseases ultimately result from a range of defects, including dendritic spine abnormalities, loss of long-term potentiation (LTP) and long-term depression, and alterations in specific messenger RNA (mRNA) transport and translation, they are all related to processes involving the actin cytoskeleton *(7)*. This article reviews the role and regulation of cofilin in cellular actin dynamics and explains how its abnormal regulation could be at the root of many neurodegenerative diseases.

Amyloidopathies: Down Syndrome and Alzheimer's Disease

Amyloidopathies are a class of degenerative diseases arising from the accumulation of amyloid-β protein aggregates. Many different proteins or proteolytic fragments of proteins are capable of forming amyloid-β aggregates. Some common neuronal amyloidoses include AD, Down syndrome (DS), spongiform encephalopathies (a.k.a. prion diseases), Parkinson's disease, and Huntington's disease. In DS and AD, the major peptide components of the extracellular amyloid aggregates are derived from proteolysis of a large transmembrane protein, the amyloid precursor protein (APP).

APP can be divided into three domains: a large extracellular N-terminal domain, a single transmembrane domain, and a short cytoplasmic C-terminal tail. Multiple isoforms of APP are expressed, ranging in size from 695 to 770 amino acids, with APP695 being the most abundant in brain. The *APP* gene is found on chromosome 21 in humans and on chromosome 16 in mice. Proteolytic processing of full-length APP occurs via one of two pathways. The nonamyloidogenic pathway, which dominates APP processing in most cell types, begins with cleavage by α-secretase (ref. *8*; Fig. 1A). α-Secretase is a protein that resembles the tumor necrosis factor (TNF)-α converting enzyme and belongs to the disintegrin/metalloproteinase family. This cleavage releases the soluble APP N-terminus (sAPPα) extracellularly and leaves the membrane-bound 83-amino acid C-terminus (C83). C83 undergoes subsequent cleavage

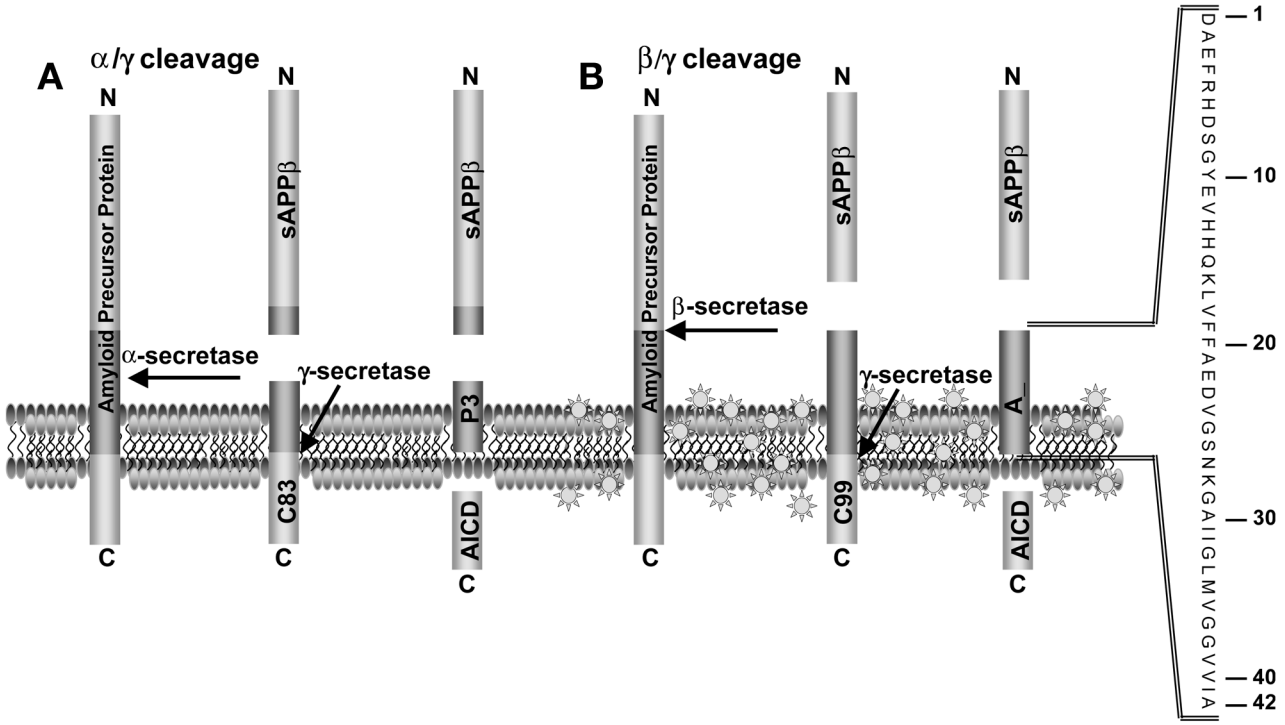

Fig. 1. Proteolytic process of the amyloid precursor protein (APP). **(A)** Sequential processing of APP by α- and γ-secretases yields a soluble extracellular fragment (sAPPα) and an 83-amino acid transmembrane peptide that is cleaved to yield a soluble P3 fragment and an APP-intracellular domain (AICD), which is targeted to the nucleus. **(B)** Sequential APP processing by β- and γ-secretases occurs mainly in membrane lipid raft domains and gives rise to a soluble extracellular domain (sAPPβ) and a 99-amino acid transmembrane fragment that is cleaved to yield AICD and the amyloidogenic peptides (Aβ). Variability in the C-terminal cleavage site by γ-secretase gives rise to peptides most commonly either 40 or 42 amino acids in length.

by γ-secretase to release the transcriptionally active APP intracellular domain and the soluble P3 fragment. γ-Secretase is multisubunit complex composed of nicastrin, Pen2, Aph-1, and presenilin. Presenilin, an aspartyl protease, is the enzymatically active component of the γ-secretase complex. In Chinese hamster ovary cells stably expressing γ-secretase, approx 6% of total γ-secretase resides at plasma membrane, where it maintains full functional activity *(9)*.

APP can alternatively undergo amyloidogenic processing initiated by β-site APP cleavage enzyme (BACE), an aspartic acid protease commonly referred to as β-secretase. Similarly to the α-cleavage of APP, β-cleavage releases a soluble extracellular N-terminal fragment (sAPPβ) and leaves behind a membrane-bound 99-amino acid C-terminal peptide (C99). BACE is enriched within cholesterol and glycolipid-rich membrane raft domains. The β-cleavage event occurs primarily during endocytosis or within early endosomal compartments *(10)*. C99 subsequently undergoes cleavage by γ-secretase to produce the 40- to 42-amino acid amyloid-β peptides ($A\beta_{1\text{-}40}$; $A\beta_{1\text{-}42;}$ Fig. 1B). The endosomal Aβ will either become liberated into the extracellular space upon endosomal fusion with the plasma membrane or may be trafficked back to the soma, where lysosomal fusion can help degrade the peptide produced. Aβ peptides are about 4 kDa and are dominated by β-sheet secondary structures that predispose their polymerization

into soluble oligomers and eventually into fibrils and insoluble aggregates. Fibrils of the extracellularly released Aβ peptides eventually aggregate to form deposits known as senile plaques, the presence of which is a hallmark for the diagnosis of AD.

DS (also known as trisomy 21) is a genetic disorder arising from the triplication of human chromosome 21 (equivalent genes are on chromosome 16 of mouse). Among the genes on chromosome 21 are those encoding *APP* and *BACE-2*. Children affected with DS suffer from impaired learning and cognitive development and are recognizable by stereotypic craniofacial deformities. Average life expectancy is reduced as a result of a multiplicity of complications. One common feature associated with DS is the development of the pathological hallmarks of AD in all patients living to age 40 yr or beyond *(11)*.

AD is a neurodegenerative disorder currently diagnosed postmortem by the presence of senile plaques and neurofibrillary tangles in the brain. AD presents clinically as a steady decline in cognitive ability, beginning with the loss of short-term memory and progressing through stages of increasing dementia. Cognitive decline continues as a precipitous loss of the ability to recall common names, dates, and places and inability to perform increasingly simple tasks that require planning. Late stages of AD are characterized by hallucinations, paranoia, loss of verbal communication, loss of fine and later gross motor control often resembling Parkinson's disease, and incontinence; finally, the patient falls into a vegetative state, in which they remain until death. Progression through these stages may take anywhere from months to years, and symptoms can vary greatly from case to case; however, the pathology found in postmortem brains is largely stereotypical and includes the extracellular senile plaque (composed primarily of insoluble aggregates of amyloid peptides) and intracellular neurofibrillary tangles (composed of the microtubule binding protein tau). The vast majority of AD is sporadic, whereas familial AD (FAD) accounts for fewer than 5% of all cases *(12,13)*. FAD mutations have been identified in genes encoding *APP* and in *presenilins 1* and *2 (13)*. Additionally, expression of a common polymorphism (ε4) found in the apolipoprotein-E (ApoE) is correlated with increased risk for a later onset AD *(14,15)*. These mutations all lead to increased cerebral Aβ levels either via increased production and aggregation or via decreased clearance or degradation.

The common features of neuropathology shared by late-onset AD, early onset FAD, and DS (including senile plaques and cerebral atrophy) have shed light on the mechanisms at work in these amyloidopathies. The "amyloid hypothesis" states that increasing cerebral accumulation of Aβ over years to decades exacerbates cognitive decline, neurodegeneration, and senile plaque deposition associated with AD. This hypothesis found its roots in the characterization of the Aβ peptide as the major component of senile plaques *(16)* and has since been strengthened by genetic and biochemical data. The identification of inherited genetic mutations with high penetrance in early onset FAD has led to the development of transgenic mouse models that display neurodegeneration with similarities to human AD. In vitro studies of the Aβ peptide have demonstrated acute toxicity against cultured neurons as well as depressive effects on LTP, transient cognitive impairment, loss of cellular homeostasis, and impaired transport.

Actin and Cofilin in Neurodegenerative Disease

Actin is a 42-kDa cytoskeletal protein expressed ubiquitously in eukaryotes. Monomeric, globular actin (G-actin) readily polymerizes into filaments (F-actin). Actin filaments are dynamic biopolymers that continually undergo cycles of assembly and disassembly. Monomers of actin can be added to or removed from either end of a filament, but a polarity exits that influences the on/off rate of G-actin. Filament assembly dynamics are

also influenced by the nature of the actin-bound adenine nucleotide, adenosine triphosphate (ATP) or adenosine disphosphate (ADP). ATP-actin has a higher affinity for filament assembly than ADP-actin. Following the addition of an ATP-actin monomer to the plus end of a growing filament, the γ-phosphate is rapidly hydrolyzed followed by the slower release of inorganic phosphate (P*i*). Loss of P*i* induces a conformational change in the ADP-actin subunit within the filament. Actin filaments are nonequilibrium polymers and, under steady state conditions, undergo treadmilling, during which ATP-actin monomers polymerize on the filament plus end and ADP-actin depolymerizes from the minus end. Interestingly, regarding neuronal stress, ATP hydrolysis associated with actin dynamics can consume up to 50% of the total ATP used by growing neurons *(17)*.

Actin depolymerizing factor (ADF) and cofilin are members of a 13- to 19-kDa family of actin binding proteins. Members of this family share high sequence homology, and in metazoans they are partly regulated by phosphorylation of a conserved Ser3 residue. Because in human brain the cofilin: ADF expression ratio is greater than 10:1 *(6)*, we only refer to cofilin hereafter. Phosphorylation by LIM kinases (LIMK) or TES kinases inactivates cofilin and dephosphorylation by slingshot (SSH; ref. *18)*, or chronophin *(19,20)* phosphatases restore cofilin's actin-dynamizing activity. Dephosphorylated cofilin binds cooperatively *(21,22)* along regions of ADP-actin *(23,24)*, resulting in enhanced filament severing *(25)* and minus end depolymerization (Fig. 2A). Cofilin enhances the dynamics of actin filaments within the cell and, along with profilin and/or Srv2/Cap1 (which enhance nucleotide exchange on actin monomers), increases actin subunit cycling through filaments by 100- to 150-fold (Fig. 2A). Therefore, cofilin contributes enormously to the ATP utilization by the actin cytoskeleton.

p21-Activated kinases (PAKs) are important modulators of cofilin activity. PAKs 1 and 4 phosphorylate and activate LIMK, which then phosphorylates and inactivates cofilin (p-cofilin) *(26–29)*. PAK4 also phosphorylates (inactivates) SSH-1 *(30)*, enhancing cofilin inactivation. However, activated SSH-1 can reverse the effects of PAK by dephosphorylating both LIMK and cofilin, thereby increasing cofilin activity *(30)*. The PAK-cofilin signaling axis is important for dendritic spine morphogenesis, and consequently, PAK mutations have been linked to several human developmental mental retardation syndromes characterized by dendritic spine abnormalities *(31–34)*.

Cofilin aggregates and actin bundles have been observed in AD brain *(6)*. Transient cellular stress can cause a sharp decline in local ATP concentrations, especially within domains of neurites where mitochondria are not uniformly distributed. The loss of ATP produces a rapid rise in the concentrations of both ADP-actin and activated (dephosphorylated) cofilin (Fig. 2B). Under these conditions, the cofilin-actin complex readily assembles to form filaments that associate into cylindrical aggregates with tapered ends (rods). Rods, first observed in nuclei of cells *(35)*, form in various cell lines in response to ATP depletion or treatment with 10% DMSO *(36–44)* and form in neurons in response to these same factors, as well as oxidative stress (peroxide and nitrous oxide), excitotoxic glutamate *(6)*, and treatment with soluble and fibrillar forms of $A\beta_{1\text{-}42}$ *(45)*. Therefore, rod formation is a stereotypical cellular response to a wide range of stress factors that model common mediators of neurodegeneration.

Rods that form initially in response to ATP depletion are transient and disappear upon ATP recovery; however, rods reappear in neurites in which irreversible mitochondrial damage has occurred, and these rods become persistent *(6)*. Persistent rods have been observed for several days without loss of the neuron, although neurites distal to the rod eventually undergo withering. Rod formation represents a promising mechanism to explain the loss of synaptic connections and cognitive decline without associated loss of neurons, as is often reported in early stages

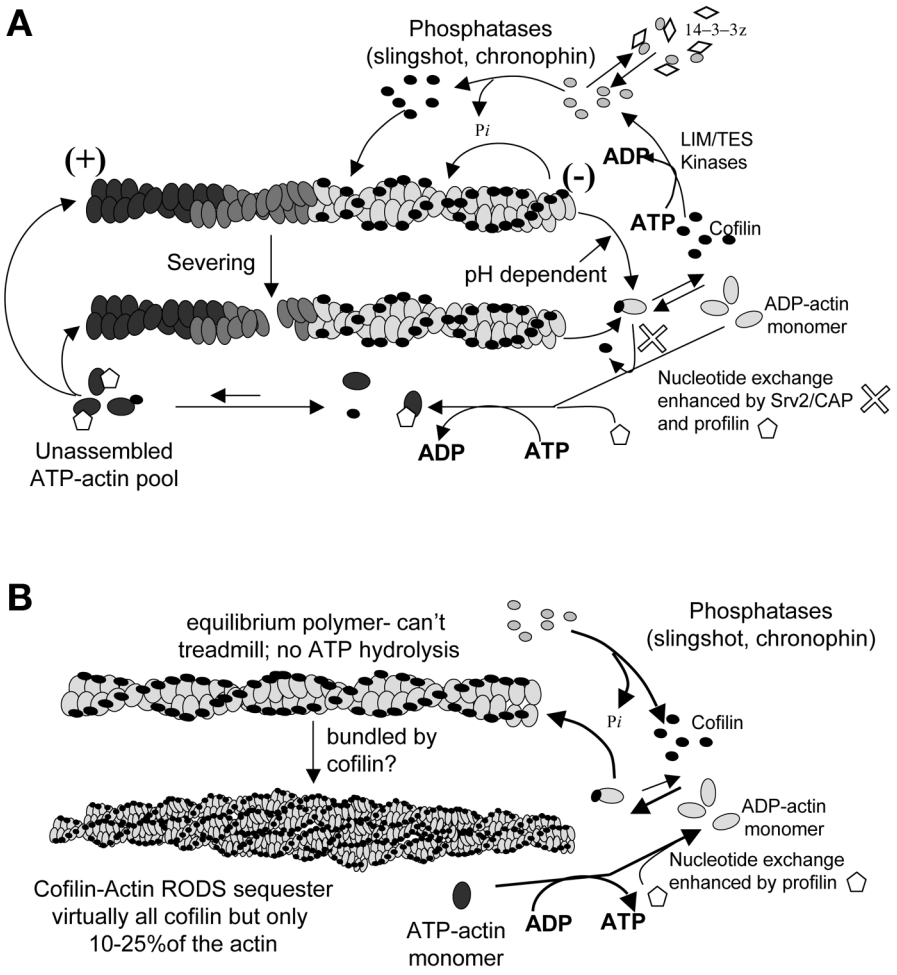

Fig. 2. Schematic diagram showing how cofilin enhances actin treadmilling in normal cells and how cofilin-actin rods form in cells under stress. **(A)** Phosphocycling of cofilin by LIM/TES kinases and slingshot or chronophin phosphatases enhances actin filament turnover and treadmilling. The phosphorylated form of cofilin may be sequestered by 14-3-3 family scaffolding proteins. Active cofilin binds along ADP-actin subunits to promote severing and increased rate of subunit loss from filament (-) ends. The dissociated ADP-actin undergoes nucleotide exchange enhanced by Srv2/Cap1 or profilin and the ATP-actin monomer has low affinity for cofilin, allowing it to recycle back onto the plus end of a growing filament. A rapid hydrolysis of the actin bound ATP to ADP-P*i* is followed by the loss of inorganic phosphate. **(B)** When cells are under stress and ATP levels fall, cofilin undergoes enhanced dephosphorylation and actin exchanges bound ATP for ADP. Cofilin has higher affinity for ADP-actin subunits and binds to them and assembles them into cofilin-saturated ADP-actin filaments. These filaments can bundle to form rods, presumably because of the ability of cofilin to neutralize the negative surface charge on F-actin. Within these rods there is little if any turnover of subunits until ATP levels recover.

of neurodegenerative diseases. Because microtubules are often disrupted in neurites distal to rods, especially when rods occlude the entire neurite, rod formation also can explain disruption of microtubule-based transport.

Rods can be uniquely characterized by their immunoreactivity to antibodies against both actin and cofilin, and when properly fixed are refractory to phalloidin, a mushroom toxin with F-actin binding properties

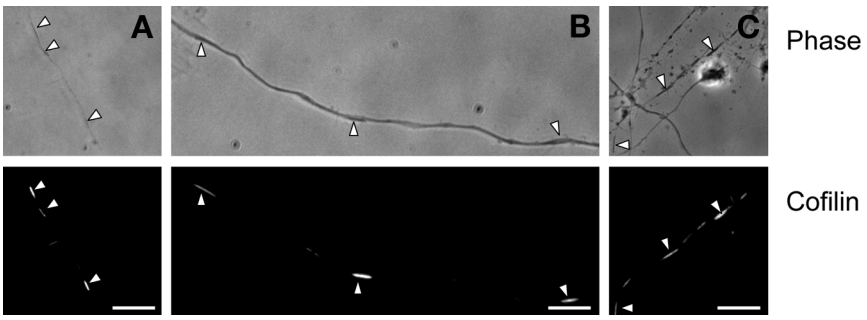

Fig. 3. Phase and fluorescence images of cultured hippocampal neurons containing rods. Rods are preserved in cultured neurons by fixation in 4% paraformaldehyde for more than 30 min. Upon extraction with cold methanol, rods are often visible in phase micrographs as dark rigid structures within neurites (**A–C**, top row). Rods label strongly with antibodies to cofilin (A–C, bottom row). Arrows in top and bottom panels mark identical positions. Scale bars = 10 μm.

(6). The absence of phalloidin binding suggests that the actin in rods is saturated with cofilin; subunits in cofilin-saturated actin filaments are slightly rotated with respect to normal F-actin, eliminating the phalloidin binding site *(24)*. In cultured neurons, rods typically appear either as single structures or in linear arrays within neurites and can enlarge to completely occlude the neurite, sometimes observed by phase microscopy as a swelling of the neurite caliber (Fig. 3).

Rod-like cofilin immunostaining is found in close proximity to amyloid plaques in both human AD brain *(6)* and in brain of transgenic AD mice *(34,45)*. Significantly, these aggregates are also present in perfusion-fixed transgenic mice (Tg2576) that express a human mutant APP, suggesting that their presence in human AD brain is not a postmortem artifact *(45)*. In AD brains, greater than 97% of dense core amyloid plaques are associated with rod-like cofilin aggregates, whereas approx 45% of cofilin aggregates are found isolated from amyloid plaques *(6)*. Rod-like cofilin staining associated with diffuse cofilin pathology often is remarkably similar in size, spacing, and appearance to rods observed in cultured neurons. The high spatial correlation between amyloid plaques and cofilin pathology has sparked closer investigations into the relationship between Aβ and cofilin regulation.

Amyloid-$\beta_{1\text{-}42}$ Induces Formation of Cofilin-Actin Rods

Treatment of cultured rat hippocampal neurons with $A\beta_{1\text{-}42}$ induces rod formation in approx 18% of the total population *(45)*. Rods form rapidly in neurons treated with $A\beta_{1\text{-}42}$, reaching 50% of the maximum response within 6 h in neurons treated with 1 μ*M* of soluble $A\beta_{1\text{-}42}$ oligomers ($sA\beta_{1\text{-}42}$) and by 24 h when treated with $sA\beta_{1\text{-}42}$ at concentrations as low as 10 n*M*. ATP levels decline in human cortical neurons within hours to days of exposure to sAβ at concentrations as low as 100 n*M* *(46)*. $A\beta_{1\text{-}42}$ treatment causes the dephosphorylation (activation) of cofilin within the soma and neurites of only those neurons that form rods *(45)*. Labeling of a subpopulation (30–50%) of neurons with extracellular $sA\beta_{1\text{-}42}$ oligomers also has been reported *(47)*. Together, these studies suggest that $sA\beta_{1\text{-}42}$ can induce rod formation by activating cofilin in a neuronal subpopulation that has high specificity/affinity for sAβ-binding.

Synaptic dysfunction is the most established correlate of cognitive decline in AD *(48,49)*. Recent studies using *Aplysia kurodai* neurons found that cofilin overexpression led to rod formation, synapse loss, and, distal to the rod, impairment of synaptic plasticity measured by electrophysiological methods

(50). Although cofilin overexpression did not affect the gross morphology of the neuron, a decrease in the number of presynaptic varicosities was observed. Additionally, cofilin overexpression impaired both basal synaptic transmission and LTP. Neither cell death nor induction of an apoptotic cascade was found to be responsible for these effects.

Cofilin pathology in both AD and DS is spatially and temporally associated with marked reduction of PAK protein levels and activity *(34)*. In postmortem human and Tg2576 mouse brains, activated phospho-PAK (pPAK) immunostaining resembles staining of intraneuronal $A\beta_{1\text{-}42}$ that accumulates along with APP-carboxy-terminal fragments within enlarged endosomal and lysosomal structures *(34,51)*. Application of a PAK inhibitory peptide to cultured neurons induced the formation of cofilin rods; intracerebroventricular injection of this peptide into wild-type mice induced rod-like cofilin pathology and social recognition memory deficits *(34)*. These findings suggest that a loss of pPAK may lead to local pathology related to the formation of cofilin aggregates similar to those observed in human AD and Tg2576 mouse brain and may contribute to impaired cognitive functioning.

Synapse failure has been attributed to soluble Aβ oligomers *(52)* partly because of their ability to disrupt LTP *(53–55)*. Following treatment of cultured hippocampal neurons with sAβ oligomers, greater than 90% of the punctate oligomer binding colocalized with the synaptic marker PSD-95 *(52)*. A similar punctate staining of human cortical neurons showed 43 ± 13% sAβ oligomer colocalization with synaptophysin and PSD-95 within 1 h after treatment with 5 μ*M* of sAβ *(46)*. Memory deficits have been observed in young rats when administered with a 56-kDa sAβ species (Aβ*56) purified from the brains of Tg2576 mice *(56)*. Chinese hamster ovary (CHO) cells that express a mutant form of APP associated with human AD secrete sAβ peptides *(57–59)*. Rats injected intracerebroventricularly with the CHO-cell-derived sAβ consistently showed significant but transient disruption of a complex learned behavior *(60)*. The Aβ-induced block of LTP was reversed by injection of monoclonal antibodies to Aβ *(61)*. Fractionation by size exclusion chromatography determined that the LTP disruption and memory impairments were caused by low-*n* oligomers of Aβ but not by monomers *(59,60)*. Together with the ability of Aβ to induce rods, these findings support a molecular mechanism in which rods play a pivotal role in producing the underlying memory and cognitive dysfunction of neurodegenerative disease.

Different Signaling Pathways of Fibrillar Amyloid-β and Soluble Amyloid-β Affect Cofilin

The concept that different isoforms and/or conformations of Aβ deliver independent signals to neurons is widely supported. It is particularly interesting that soluble Aβ and fibrillar Aβ (fAβ) may be differentially affecting ADF/cofilin regulation. Such opposing effects could explain the presence of dystrophic neurites, often found to be associated with senile plaques, and cognitive impairment associated with sAβ. Although the term Aβ is used to describe a spectrum of peptide species, the neurotoxic effects of different Aβ peptides are not the same. Investigations are beginning to elucidate differences in the biological activity of Aβ species, especially on the phosphorylation (activity) of cofilin (Fig. 4).

Amyloid $\beta_{1\text{-}40}$ fibrils ($fA\beta_{1\text{-}40}$) induce dystrophy in cultured cortical neurons via activation of focal adhesion proteins *(62–64)*. Treatment of human cortical neurons with 20 μ*M* of $fA\beta_{1\text{-}40}$ for 10 d produced progressive dystrophy and minimal neurotoxicity *(46)*. Focal adhesions provide a link to the actin cytoskeleton whereby integrin receptors can activate intracellular signals that regulate actin cytoskeletal dynamics *(65,66)*. During focal adhesion activation, a multiprotein complex containing LIMK1 and PAK becomes recruited via P95PKL-mediated binding to paxillin *(67,68)*. Activated paxillin and

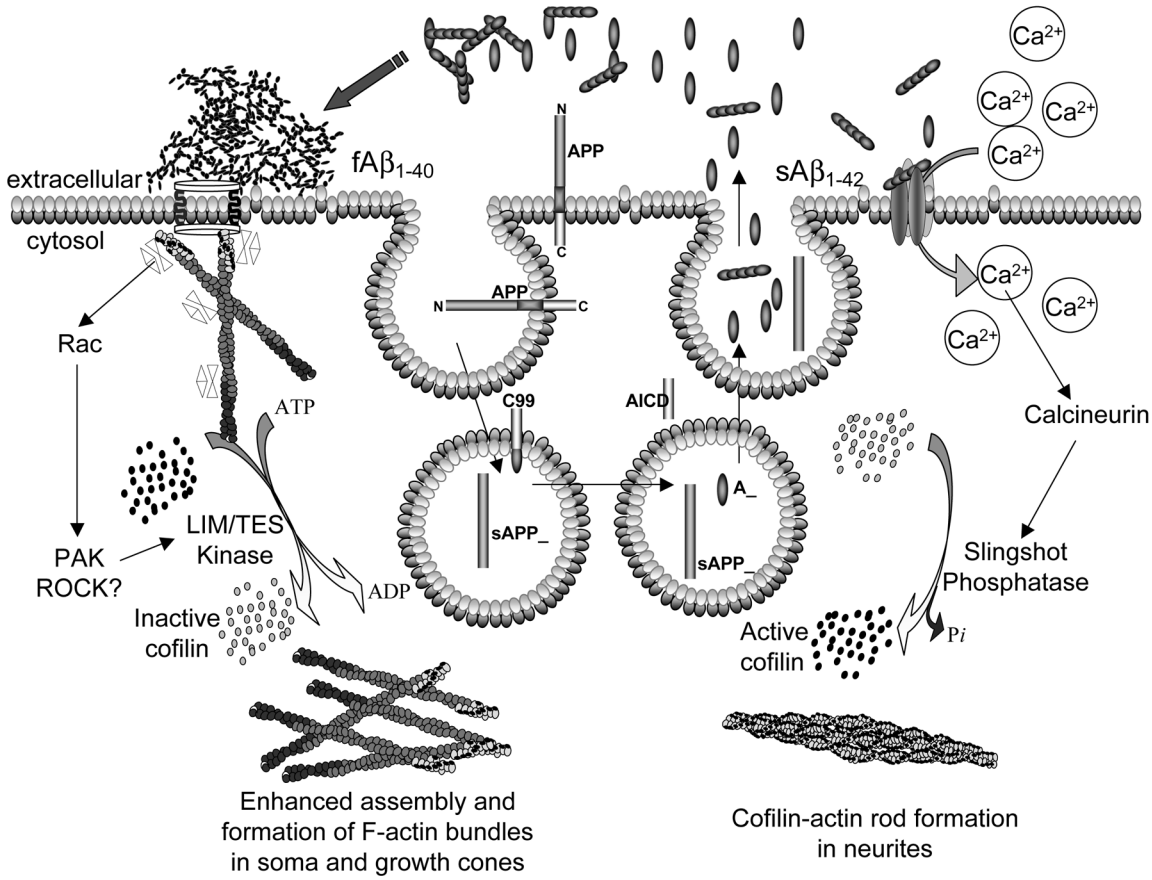

Fig. 4. Hypothetical model showing possible signaling pathways of $fA\beta_{1\text{-}40}$ and $sA\beta_{1\text{-}42}$ that lead to opposing effects on cofilin activity. APP is cleaved by β- and γ-secretases to produce Aβ within endosomes. Some Aβ assembly may occur before membrane fusion releases soluble species, including monomers, dimers, trimers and other oligomers into the extracellular space. The binding of sAβ to as yet unidentified receptors may occur at synaptic locations and leads to increased intracellular Ca^{2+}, presumably from extracellular sources. Elevated Ca^{2+} can lead to a multiplicity of cellular effects including calcineurin activation, which in turn activates slingshot. Active slingshot can dephosphorylate cofilin, leading to its hyperactivity and, if coupled with ATP reduction, rod formation. Extracellular Aβ also can polymerize into fibrils (fAβ), some of which precipitates to form senile plaques. The activation of integrin receptors by $fA\beta_{1\text{-}40}$ causes recruitment of adhesion proteins and can lead to activation of Rho family GTPases, whose downstream kinases activate LIMK, which inactivates cofilin. Enhanced polymerization and bundling of F-actin in the cell body, growth cones and other lamella ensues, possibly leading to neuritic dystrophy.

focal adhesion proteins are found with high frequency associated with senile plaques in human AD brain *(64)*. *Drosophila paxillin* positively regulates Rac and negatively regulates Rho, thereby paxillin is a modulator of the Rho family of GTPases and affects the LIMK pathway (ref. *68*; Fig. 4).

$fA\beta_{1\text{-}40}$-mediated neurite dystrophy occurs via LIMK1 activation upstream of the actin cytoskeletal rearrangements *(69)*. Activated LIMK1 phosphorylates (inactivates) cofilin. Cofilin inactivation leads to decreased actin dynamics and increased formation of phalloidin-stained actin bundles. These bundles are

generally not localized within the neurites, where rods are found, but tend to be in the cell soma, growth cones, and other lamella. $A\beta_{1\text{-}40}$-induced neuronal dystrophy is reduced when cultures are transfected with a mutant paxillin missing the LIM binding domain *(64)*. Treatment of cultured hippocampal neurons with 20 μM of $fA\beta_{1\text{-}40}$ induces activation of LIMK, increased actin bundling, pretangle phosphorylated-τ in neurons that develop dystrophic and tortuous neurites, and inactivation of cofilin *(69)*. These effects were blocked by the addition of a peptide (S3) that competes with cofilin for phosphorylation by LIMK *(69)*. Treatment with the same concentration of $sA\beta_{1\text{-}40}$ did not reproduce the effects of $fA\beta_{1\text{-}40}$, indicating that the solubility/presence of fibrils is the major factor driving the cellular effects.

Soluble species of Aβ (also called Aβ-derived diffusible ligands [ADDLs]) bind specifically to synaptic sites on cultured hippocampal neurons *(47,70)*. Additionally, ADDLs are toxic to cultured neurons at nanomolar concentrations *(53)*, and at 500 n*M* they prevent high-frequency stimulation-induced LTP measured from the dentate gyrus in acute hippocampal slices *(55)*. sAβ has been linked to hippocampus-dependent temporal memory deficits in mice *(71)*. Transgenic mice expressing mutant forms of human APP and presenilin-1 (Tg6799) or human mutant APP alone (Tg2675) both displayed elevated levels of sAβ and temporal memory deficits *(71)*. Deletion of the *BACE1* gene lowered the concentration of sAβ to wild-type levels and rescued temporal memory deficits in Tg6799 mice, demonstrating a direct role of Aβ formation in memory loss.

Cofilin pathology and abnormalities in PAK activity have been observed in human AD brain and in brains from Tg2576 mice *(6,34,45)*. Immunization of Tg2576 mice against Aβ decreased sAβ without affecting insoluble Aβ and the levels of pPAK inversely correlated with the remaining sAβ *(34)*. Persistent reduction in pPAK was observed as early as 2 h following treatment of dissociated rat hippocampal neurons with soluble $A\beta_{1\text{-}42}$ oligomers and occurred with oligomer concentrations as low as 10 n*M*. Although these reports demonstrate a link between increased sAβ, decreased active PAK, and increased active cofilin and rod formation, the mechanisms by which the sAβ initiates these changes is currently unknown, but cofilin activation arises from a decline in active LIMK1.

A more direct pathway for activation of cofilin could also play an important role in rod formation. The cofilin phosphatase SSH-1L is activated by dephosphorylation of inhibitory phosphorylation sites by the calcium-activated phosphatase calcineurin (also known as PP2B) *(72)*. Elevated intracellular calcium occurs in neuroblastoma cells treated with sAβ oligomers, but not with monomers or fAβ *(73)*, and in rat cortical neurons treated with $sA\beta_{1\text{-}40}$ *(74)*, suggesting that SSH activation may be an alternative mechanism by which sAβ peptides activate cofilin. Calcineurin also has been implicated in Aβ-mediated inhibition of LTP *(75)*. However, calcineurin activity is decreased in AD brain *(76)*, suggesting that overactivation of cofilin might be differentially regulated both spatially and temporally by a decline in PAK/LIMK activities and increased calcineurin/SSH-1 activities (Fig. 4).

Transport Defects in Neurodegenerative Diseases

Axonopathy and transport defects have been reported in brains of mice expressing human mutant APP behind a prion promoter (Tg-swAPP^{prp}) *(77)*. The formation of blockages, resembling axonal swellings and dystrophic neurites, is one of the earliest reported degenerative phenotypes, occurring as early as 1 yr prior to the formation of senile plaques in these mice. These swellings are often found in areas lacking amyloid deposition in early stage human AD, suggesting that they precede, and may play a role in, amyloid deposition. Swellings in the neurites of cultured hippocampal neurons harvested from Tg2576 mice (same mutant APP but driven by a differ-

ent promoter) contain aberrant accumulation of oligomeric Aβ *(78)*. Swellings have also been reported to form within cholinergic axons of the nucleus basalis magnocellularis in early stage human AD brain *(77)*. Reduced kinesin expression enhances the frequency of axonal defects and increases Aβ and amyloid deposition in Tg-swAPP^{prp} mice *(77)*.

Enlarged early endosomes develop in neurons before birth in DS brains *(79)* and appear years before significant formation of Aβ deposits and the neurofibrillary pathology associated with AD and DS *(80–82)*. During the progression of AD and DS, both the number and size of basal forebrain cholinergic (BFC) neurons decrease *(83–86)*. Aβ has been proposed to act specifically on BFC neurons via activation of whole-cell voltage-activated currents in these neurons but not in GABAergic neurons *(87)*. BFC neuronal loss, a classic feature of both AD and DS, results at least partly from defective retrograde transport of nerve growth factor (NGF) from the hippocampus *(88)*. BFC neurons depend on NGF for the normal function and development. NGF produced in the hippocampus binds to receptors on BFC axons and is retrogradely transported as an active signaling endosome to the cell body *(89)*.

Reduced BFC neuronal size and number as well as regression of their hippocampal terminal fields occur in the partial trisomy 16 (Ts65Dn) mouse model of DS *(88)*. Increased levels of NGF in the hippocampus of Ts65Dn mice occur as a result of failed retrograde transport to the soma of BFC neurons *(88)*. Although the exact cause for the defect remains undetermined, NGF binding and internalization in synaptosomes prepared from Ts65Dn mice showed no impairment compared to wild-type (2N) mice, suggesting that the failure rests in an axonal abnormality that affects retrograde transport. In Ts65Dn mice, intracerebroventricular NGF infusion improved BFC neuronal size and restored some cholinergic innervation of the hippocampus *(88)*.

Treatment of hippocampal neurons in culture with glutamate or Aβ disrupts anterograde and retrograde fast axonal transport by formation of actin bundles *(90)*. Although these actin aggregates were observed by phalloidin staining, they were most likely cofilin-actin rods that form upon glutamate treatment *(6)* but from which cofilin was lost during permeabilization because of the short (3-min) fixation in 4% paraformaldehyde. Fixation of rods requires 30 to 45 min in paraformaldehyde *(45)*. Excessive glutamate in extracellular space is recognized as an excitotoxin playing a role in the development of AD and other neurodegenerative diseases *(91,92)*. A decline in the ability of glia to take up and process glutamate to glutamine may be a contributing factor to age-related cognitive decline and could mediate rod formation.

Application to cultured rat hippocampal neurons of $A\beta_{25\text{-}35}$, a sequence containing much of the neurotoxic activity of the Aβ peptides *(93)*, slowed axonal transport within a few minutes and did so in a dose-dependent manner from 200 n*M* to 20 μ*M*. Although transport was restored within 30 min following washout of 2 μ*M* of $A\beta_{25\text{-}35}$ after a 26-min exposure *(90)*, washout of 20 μ*M* $A\beta_{25\text{-}35}$ was not reversible and resulted in a continuous decline in transport. Interestingly, treatment with 20 μ*M* $A\beta_{1\text{-}42}$ preincubated under aggregating conditions, reproduced the effects observed with $A\beta_{25\text{-}35}$; however, treatments with the same concentration of freshly prepared $A\beta_{1\text{-}42}$ (probably monomer) or with pre-incubated (probably fibrillar) $A\beta_{1\text{-}40}$ failed to impair transport *(90)*. These findings highlight the importance of Aβ peptide composition (sequence and length), species (monomers, oligomers, fibrils), and solubility (soluble vs insoluble) to its biological activity. Furthermore, these results suggest that the inhibitory effects of $A\beta_{25\text{-}35}$ and preincubated $A\beta_{1\text{-}42}$ on transport and the subsequent aggregation of actin are intimately linked.

Several lines of evidence place APP at the center of transport defects in both AD and DS *(94)*. Increased expression of APP is found in DS and increased levels of APP-carboxy-terminal-fragments accumulate in AD pathology. Ts65Dn mice display enlarged early endosomes in BFC neurons *(82)*. Selective deletion of one copy of

APP from Ts65Dn mice eliminated the enlarged endosomal phenotype *(79)*; however, the overexpression of APP alone did not produce endosomal phenotypes. The magnitude of NGF transport defects associated with BFC neuron degeneration correlates with APP expression levels *(95)*. This suggests that although *APP* gene dosage is critical to the development of endosomal abnormalities and transport defects, additional genes found on the triplicated region of mouse chromosome 16 are also required *(79)*. Some initially identified candidate genes located on the triplicated region of human chromosome 21 encode *BACE2* and *superoxide dismutase-1 (79)*. The *BACE2* gene has recently been excluded as a contributor to the pathogenesis of Alzheimer's disease in patients with DS, but surprisingly, an increase in BACE1 activity through its enhanced maturation in the Golgi does contribute *(96,97)*. Furthermore, two other genes, named *DSCR1* and *DYRK1A*, which lie within the Down syndrome critical region of chromosome 21, act synergistically to prevent nuclear occupancy of NFATc transcription factors *(98)*, providing a possible explanation of how BACE1 activity could be altered.

Vesicles containing APP, presenilin-1, and BACE all undergo kinesin-I-mediated fast anterograde axonal transport *(99–102)*. Evidence has been presented for both direct interactions between APP and kinesin *(99,103)* and indirect coupling via c-Jun NH2-terminal kinase-interacting protein *(104)*. APP, BACE, and presenilin-1 all accumulate in axonal swellings, associated with increased levels of Aβ that form during normal brain aging or after head trauma *(45,105–109)*. $A\beta_{1\text{-}42}$ accumulates within endosomal vesicles in neurons in the brains of Tg2576 mice, in primary neuronal cultures from these mice, and in human AD brain *(78,110)*. Accumulations are associated with pathological alteration within the neurites and synaptic compartments of mouse and human AD brains. Vesicles containing APP, BACE, presenilin-1 and β-cleaved APP all accumulate at rods in cultured neurons *(45)*.

Transport defects may stimulate proteolytic processing of APP or allow the Aβ peptides to assemble into more toxic oligomers, thereby exacerbating the formation of senile plaques in AD *(45,77,111)*. Following its synthesis, APP is trafficked from the endoplasmic reticulum though the constitutive secretory pathway to the plasma membrane *(8)*. APP remains at the plasma membrane briefly before being internalized into endosomes *(8)*. In addition to APP, early endosomes contain many key players of AD and DS, including BACE and γ-secretases, Aβ peptides, ApoE, low-density lipoprotein (LDL), the LDL receptor, and NGF *(82,113)*. Following endocytosis, APP is first delivered to early endosomes and is then delivered to the late endosomal/trans Golgi network (TGN) *(114)*. The γ-secretase complex is found localized within several compartments, including late endosomes, Golgi, and TGN *(115)*. Intracellular Aβ is found in Rab5-positive early endosomes and is prominent in enlarged endosomes from DS and AD brain *(79)*. These findings suggest that Aβ overproduction and accumulation in AD and DS may signify earlier disease-related disturbances of endosomal signaling or transport *(82,116)*. Indeed, transport defects may initiate a cycle of locally increased APP processing into Aβ, causing additional vesicle stalling, which further stimulates additional APP processing. Such a mechanism constitutes a positive feedback spiral, in which axonal blockages and Aβ production become mutually stimulatory and contribute to early synaptic loss *(6,45,77)*.

Cofilin-Regulated Actin Dynamics and Golgi-Derived Vesicle Delivery

In addition to affecting actin dynamics in neurites and growth cones, cofilin is also required for maintaining dynamic sorting tubules of the Golgi *(117)* from which the vesicular cargo, new neuronal plasma membrane, and secretory material for the axonal and dendritic compartments are derived. The two LIM domains of LIMK1 target this kinase to the Golgi, where it can downregulate cofilin activ-

ity and alter the growth rates of the axon and dendrites by changing the targeting of Golgi-derived material to these different compartments *(117)*. Overexpression of the active cofilin S3A mutant stimulates formation and elongation of sorting tubules that contain dendritic targeted material, whereas inactivating cofilin, either through expressing a Golgi-targeted LIMK1 or expressing the mutant cofilin S3E, enhances formation of axon-targeted vesicles. Therefore, even in the absence of cofilin-actin rods, which block transport directly, abnormal cofilin activities within the cell body also influence the sorting and transport of new membrane (and specific receptors therein) as well as secretory proteins (e.g., NGF), whose delivery and uptake by neighboring neurons might be essential for their long-term viability.

Cholesterol, Amyloid Precursor Protein Processing, and Alzheimer's Disease

Epidemiological studies have indicated that high-serum cholesterol levels are associated with increased risk of AD and that cholesterol regulation and metabolism may be altered in AD *(118,119)*. This correlation is strengthened by the inheritance of the ε4 allele of ApoE as a high-risk factor with familial late-onset AD. There is also evidence that amyloidogenic processing of APP is altered by cellular cholesterol levels in vitro. Subcellular distribution of cholesterol may influence APP cleavage because mutations and pharmacological inhibitors of the Niemann-Pick complex 1 cholesterol transport pathway alter the localization of presenilin/γ-secretase and lead to Aβ aggregate production *(120–123)*. The form and distribution of cholesterol in cells may modulate APP processing in a complex fashion *(124,125)*.

Cholesterol depletion in cultured cells by lovastatin and methyl-β-cyclodextrin extraction inhibits APP processing by BACE1 and lowers Aβ production *(10,126–129)*. Moderate, but not complete, depletion of cholesterol results in increased Aβ production *(130)*, and cholesterol enrichment leads to elevated amyloidogenic APP processing *(131–133)*. Therefore, activation of the BACE-APP processing pathway appears to be sensitive to membrane cholesterol levels.

Lipid rafts or detergent-insoluble membrane regions are areas rich in cholesterol and sphingolipids and are sites for endocytosis. BACE undergoes palmitoylation, targeting it to lipid rafts *(8)*. Expression of a glycosylphosphatidylinositol-linked BACE chimera, which targets BACE exclusively to rafts, increased APP processing at the β-cleavage site *(134,135)*. Endocytic vesicles containing the raft microdomains also have been implicated in amyloidogenic processing of APP in other studies *(10)*. The entire γ-secretase complex, which includes presenilin-1, mature nicastrin, Aph-1, and Pen2, also associates with rafts *(115)*. Early endocytic vesicles undergo retrograde transport via microtubule networks before being merged into the lysosomal or secretory pathways. Defective transport in this system may be at the heart of cholesterol- and ApoE-related effects in AD.

Statins inhibit HMG-CoA reductase, an enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis *(124)*. Statins enhance sAPPα ectodomain cleavage, thereby reducing amyloidogenic APP processing and $A\beta_{1\text{-}42}$ production. Although this effect is believed to result partly from reduced cholesterol, recent evidence also supports a cholesterol-independent effect *(124,136)*. Statins can modulate the association of the Rho family GTPases and other proteins with the inner leaflet of the plasma membrane *(137,138)* via effects on the isoprenoid pathway *(124,136)*. Furthermore, the Rho-ROCK pathway has been implicated in regulating γ-secretase activity toward APP proteolysis *(139)*.

Statin treatment of cultured N2a neuroblastoma cells elevated intracellular levels of APP and β-cleaved fragments because of inhibited trafficking of APP through the secretory pathway resulting from low cellular isoprenoid levels *(124)*. Expression of dominant-negative ROCK increased sAPPα shedding, whereas

expression of constitutively active ROCK inhibited the statin-stimulated shedding *(136)*. Although cholesterol depletion does not inhibit APP cell surface trafficking, it slows endocytosis, thereby reducing the levels of intracellular Aβ via a different pathway than statins. These data suggest that intracellular Aβ is largely affected by isoprenoids, whereas secreted Aβ is affected more by cholesterol. Inhibition of isoprenylation of key G proteins in the Rho and Rab families is associated with cytoskeletal alterations and decreased efficiency of vesicular transport *(140,141)*. These findings provide a direct link between upstream effectors of cofilin and APP processing into Aβ.

Mitochondrial Function, Apoptosis, and Rod Formation

Mitochondria produce much of the ATP required to power nearly every cellular function. Over a lifetime, mitochondria in postmitotic cells become less efficient partly because of damage caused by increased levels of reactive oxygen species (ROS). Increased production of ROS also arises because lowered mitochondrial efficiency provides a feed-forward model for ROS generation. Rundown of mitochondria contributes to normal aging and is a probable contributor to cellular dysfunction in neurodegenerative disease *(142,143)*. Rod formation occurs within minutes in greater than 80% of cultured neurons treated with sodium azide to shut down the electron transport chain and 2-deoxyglucose to poison glycolysis *(6)*. Under these conditions, ATP levels run down, rapidly leading to large pools of ADP-actin and dephosphorylated (active) cofilin.

In cultured embryonic neurons, actin treadmilling consumes nearly 50% of the available ATP *(17)*. For a short period in neurons undergoing stress, the loss of both mitochondrial membrane potential and ATP in neurites that form rods is slower than in neurites without them *(144)*. Therefore, the formation of rods transiently protects neurites by slowing filament turnover and its associated ATP hydrolysis. Because actin dynamics is such a major energy drain for neurons, the ability to turn off the actin treadmill during times of transient stress would provide the cell with additional time and ATP required for surviving transient insults. Rods provide this mechanism by sequestering nearly all available cofilin into nondynamic structures (ref. *144*; Fig. 5).

Altered mitochondrial function has been implicated in the pathogenesis of AD *(145–147)*. Treatment of cultured human cortical neurons with sAβ oligomers produced a rapid decline in mitochondrial membrane potential *(46)*. Quantitative proteomic analysis of mitochondria isolated from rat cortical neurons treated with Aβ peptides showed increased association of cofilin among nine other proteins with statistically elevated levels *(148)*. Cofilin is among a handful of proteins upregulated in human AD brain when screening was performed using a complementary DNA library from embryonic rat brains to identify neuron specific genes *(149)*.

Intrinsic apoptosis, a process initiated when cytochrome *c* becomes released from mitochondria via the opening of voltage-dependent anion channels (VDACs), plays a role in neuronal degeneration associated with AD *(150)*. The release of cytochrome *c* triggers formation of the apoptosome, a committed step in mitochondrial-dependent apoptosis. Cofilin plays an important role in the initiation of mitochondrial-dependent apoptosis *(151)*. Treatment of neuroblastoma cells with pro-apoptotic staurosporine or etoposide induced a translocation of cofilin to the outer mitochondrial membrane within 30 min, peaking 2 h after treatment *(151)*. Suppression of cofilin expression by small interfering RNA resulted in protection from apoptosis induced by these agents. Dephosphorylation of cofilin Ser3 is required for its translocation to mitochondria. Additionally, the pseudophosphorylated form of cofilin (S3D) does not localize to mitochondria, and its overexpression inhibits staurosporine-induced apoptosis. In PC12 cells, overexpression of LIMK1 protects cells from serum-induced apoptosis by inactivating cofilin and inhibiting both caspase 3 and

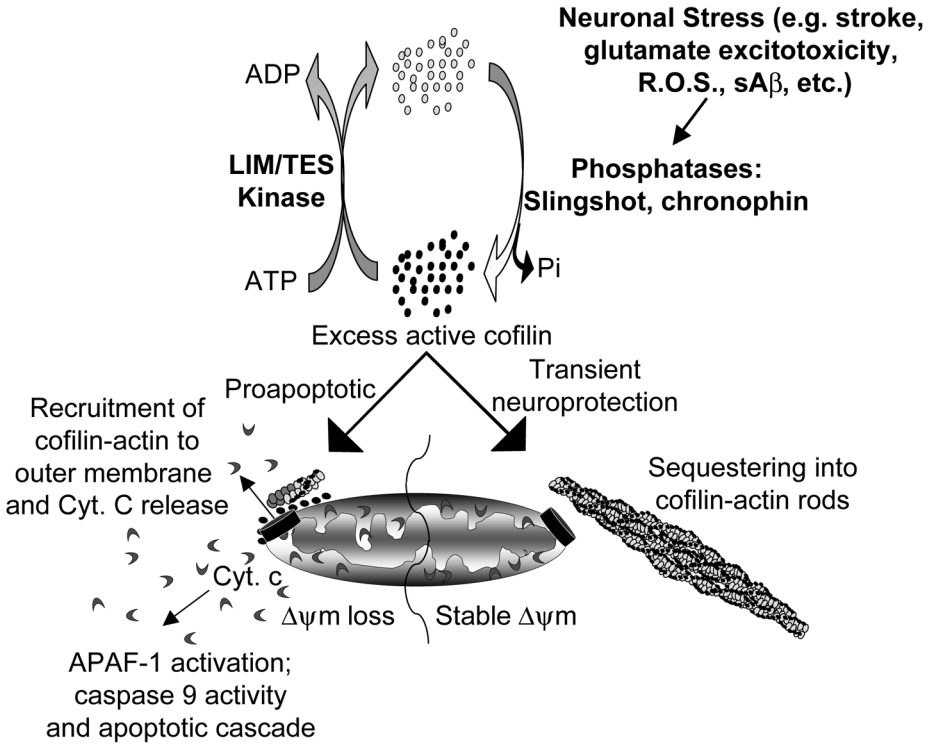

Fig. 5. Schematic showing postulated role of cofilin-actin rods in neuroprotective signaling. Intrinsic apoptosis occurs when cytochrome *c* escapes from the mitochondrial intermembrane space via the opening of voltage-gated anion channels (VDACs), a process that requires dephosphorylated cofilin with actin binding capability. The cytochrome *c* binds to APAF-1 and the complex activates caspase 9, triggering an apoptotic cascade. Accompanying this process is the loss of mitochondrial membrane potential. Postmitotic cells would benefit from a strategy designed to delay the initiation of apoptosis during transient insult. The sequestration of active cofilin into rods inhibits or delays its mitochondrial translocation and the subsequent decline in mitochondrial membrane potential and increased cytochrome *c* release.

JNK activation *(152)*. Because the translocation of activated cofilin to mitochondria is both upstream of and necessary for cytochrome *c* release *(151)*, sequestering cofilin into rods may abate its translocation to mitochondria and enhance survival of stressed neurons *(144)*.

A key role for the actin cytoskeleton in aging and apoptosis has come from studies in yeast *(153)*. Decreased actin turnover during normal aging or that induced by the application of actin-stabilizing drugs leads to accumulation of F-actin aggregates and triggers a rise in ROS *(153,154)*. Actin has been proposed to play a direct role in apoptosis *(155,156)* by regulating the opening of mitochondrial VDACs *(157,158)*. Gelsolin, an F-actin severing protein, protects cells from apoptosis by blocking loss of both mitochondrial membrane potential and cytochrome *c* release via the closing of VDACs *(159,160)*. The rapidity of rod formation during stress and their rapid disappearance after recovery, as well as the ability of rods to preserve ATP and retard the loss of mitochondrial membrane potential, suggest rods function as part of a regulated neuroprotective mechanism (Fig. 5).

Conclusion

Understanding the molecular mechanisms involved in the early stages of AD is critical for

the development of preventative strategies. This article presents evidence for the involvement of abnormal regulation of cofilin in transport defects that may lie at the heart of a neurodegenerative cascade. Cofilin-actin rods provide a link between seemingly unrelated aspects of AD, such as synaptic loss leading to cognitive decline and increased production and deposition of Aβ. Furthermore, numerous reports highlighted herein demonstrate direct and indirect effects of various neurodegenerative stimuli on cofilin regulation.

The opposing effects of $fA\beta_{1\text{-}40}$ and $sA\beta_{1\text{-}42}$ on cofilin activity could arise from: (a) their interactions with different neuronal populations; (b) interactions with different receptors on the same neuronal population; or (c) concentration effects of ligand on the same receptor that alters its intracellular signaling via clustering or conformational effects. Regardless of which of these mechanisms gives rise to the differences observed, it is significant that the oligomeric state and solubility of the different Aβ peptides mediate opposing effects on cofilin in vitro.

Acknowledgments

The authors thank Barbara Bernstein, Laurie Minamide, Alisa Shaw, Chi Pak, Kevin Flynn, and Richard Davis for valuable discussions. We gratefully acknowledge support from the National Institutes of Health through grants NS40371 (J. R. Bamburg) and NS43115 (J. R. Bamburg and M. T. Maloney), a grant-in-aid from Sigma Xi (M. T. Maloney) and grant IIRG-01-2730 from the Alzheimer's Association (J. R. Bamburg).

References

1. Bazan, N. G., Palacios-Pelaez, R., and Lukiw, W. J. (2002). Hypoxia signaling to genes: significance in Alzheimer's disease. *Mol Neurobiol.* **26(2-3),** 283–298.
2. DeKosky, S. T. and Scheff, S. W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Ann Neurol*. **27(5),** 457–464.
3. Terry, R. D., Masliah, E., Salmon, D. P., et al. (1991). Physical basis of cognitive alterations in Alzheimer's disease; synapse loss is the major correlate of cognitive impairment. *Ann Neurol*. **30(4),** 572–580.
4. Coleman, P. D. and Yao, P. J. (2003). Synaptic slaughter in Alzheimer's disease. *Neurobiol Aging* **24(8),** 1023–1027.
5. Davies, C. A., Mann, D. M., Sumpter, P. Q., and Yates, P. O. (1987). A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's Disease. *J Neurol Sci*. **78(2),** 151–164.
6. Minamide, L. S., Striegl, A.M., Boyle, J. A., Meberg, P. J., and Bamburg, J. R. (2000). Neurodegenerative stimuli induce persistent ADF/cofilin-actin rods that disrupt distal neurite function. *Nat Cell Biol.* **2(9),** 628–636.
7. Maloney, M. T., Kinley, A. W., Pak, C. W., and Bamburg, J. R. (2006). ADF/cofilin, actin dynamics and disease. In: dos Remedos C and Chhabra D, eds., *Disorders Caused by Actin and Actin-binding Proteins* New York: Wiley, in press.
8. Vetrivel, K. S. and Thinakaran, G. (2006). Amyloidogenic processing of beta-amyloid precursor protein in intracellular compartments. *Neurology* **66(2 Suppl 1),** S69–S73.
9. Chyung, J. H., Raper, D. M., and Selkoe, D. J. (2005). Gamma-secretase exists on the plasma membrane as an intact complex that accepts substrates and effects intramembrane cleavage. *J Biol Chem.* **280(6),** 4383–4392.
10. Ehehalt, R., Keller, P., Haass, C., Thiele, C., and Simons, K. (2003). Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. *J Cell Biol.* **160(1),** 113–123.
11. Glenner, G. G., and Wong, C. W. (1984). Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochem Biophys Res Commun.* **122(3),** 1131–1135.
12. Mattson, M. P. (2004). Pathways towards and away from Alzheimer's disease. *Nature* **430,** 631–639.
13. Tanzi, R. E. and Bertram, L. (2005). Twenty years of the Alzheimer's Disease hypothesis: A genetic perspective. *Cell* **120,** 545–555.
14. Schmechel, D. E., Saunders, A. M., Strittmatter, W. J., et al. (1993). Increased amyloid beta-

peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* **90,** 9649–9653.
15. Strittmatter, W. J., Saunders, A. M., Schmechel, D., et al. (1993). Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A* **90(5),** 1977-1981.
16. Glenner, G. G. and Wong, C. W. (1984). Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun.* **120(3),** 885–890.
17. Bernstein, B. W. and Bamburg, J. R. (2003). Actin-ATP hydrolysis is a major energy drain for neurons. *J Neurosci.* **23(1),** 1–6.
18. Niwa, R., Nagata-Ohashi, K., Takeichi, M., Mizuno, K., and Uemura, T. (2002). Control of actin reorganization by Slingshot, a family of phosphatases that dephosphorylate ADF/cofilin. *Cell* **108(2),** 233–246.
19. Ghola, A., Birkenfield, J., and Bokoch, G. M. (2005). Chronophin, a novel HAD-type serine protein phosphatase, regulates cofilin-dependent actin dynamics. *Nature Cell Biol.* **7(1),** 21–29.
20. Huang, T. Y., DerMardirossian, C., and Bokoch, G. M. (2006). Cofilin phosphatases and regulation of actin dynamics. *Curr Opin Cell Biol.* **18(1),** 26–31.
21. Hawkins, M., Pope, B., Maciver, S. K., and Weeds, A. G. (1993). The interaction of human actin depolymerizing factor with actin is pH regulated. *Biochemistry* **32(38),** 9985–9993.
22. Hayden, S. M., Miller, P. S., Brauweiler, A., and Bamburg, J. R. (1993). Analysis of the interactions of actin depolymerizing factor with G- and F-actin. *Biochemistry* **32(38),** 9994–10,004.
23. Carlier, M. -F., Laurent. V., Santolini, J., et al. (1997). Actin Depolymerizing Factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility. *J Cell Biol.* **136(6),** 1307-1322.
24. McGough, A., Pope. B., Chiu, W., and Weeds, A. (1997). Cofilin changes the twist of F-actin: implications for actin dynamics and cellular function. *J Cell Biol*. **138(4),** 771–781.
25. Bobokov, A. A., Muhlrad, A., Pavlov, D. A., Kokabi, K., Yilmaz, A., and Reisler, E. (2006). Cooperative effects of cofilin (ADF) on actin structure suggest allosteric mechanism of cofilin function. *J Mol Biol*. **256(2),** 325–334.
26. Edwards, D. C., Sanders, L. C., Bokoch, G. M., and Gill, G. N. (1999). Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signaling to actin cytoskeletal dynamics. *Nature Cell Biol.* **1(5),** 253–259.
27. Dan, C., Kelly, A., Bernard, O., and Minden, A. (2001). Cytoskeletal changes regulated by the PAK4 serine/threonine kinase are mediated by LIM kinase 1 and cofilin. *J Biol Chem*. **276(34),** 32,115–32,121.
28. Arber, S., Barbayannis, F. A., Hanser, H., et al. (1998). Regulation of actin dynamics through phosphorylation of cofilin by LIM-kinase. *Nature* **393(6687),** 805–809.
29. Yang, N., Higuchi, O., Ohashi, K., et al. (1998). Cofilin phosphorylation by LIM-kinase 1 and its role in Rac-mediated actin reorganization. *Nature* **393(6687),** 809–812.
30. Soosairajah, J., Maiti, S., Wiggan, O., et al. (2005). Interplay between components of a novel LIM kinase-slingshot phosphatase complex regulates cofilin. *EMBO J*. **24(3),** 473–486.
31. Meng, Y., Zhang, Y., Tregoubov, V., et al. (2002). Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. *Neuron* **35(1),** 121–133.
32. Bamburg, J. R., and Wiggan, O. P. (2002). ADF/cofilin and actin dynamics in disease. *Trends Cell Biol.* **12(12),** 598–605.
33. Bellugi, U., Lichtenberger, L., Mills. D., Galaburda, A., and Korenberg, J. R. (1999). Bridging cognition, the brain and molecular genetics: evidence from William's syndrome. *Trends Neurosci.* **22,** 197-207.
34. Zhao, L., Ma, Q. L., Calon, F., et al. (2006). Role of p21-activated kinase pathway defects in the cognitive deficits of Alzheimer disease. *Nat Neurosci.* **9(2),** 234–242.
35. Mann, G., (1894). Histochemical changes induced in sympathetic, motor, and sensory nerve cells by functional activity. *J Anat Physiol. London* **19,** 100–108.
36. Fukui, Y., and Katsumaru, H. (1979). Nuclear actin bundles in *Amoeba, Dictyostelium* and human HeLa cells induced by dimethyl sulfoxide. *Exp Cell Res*. **120(2),** 451–455.
37. Iida, K., Iida, H., and Yahara, I. (1986). Heat shock induction of intranuclear actin rods in cultured mammalian cells. *Exp Cell Res.* **165(1),** 207-215.
38. Nishida. E., Iida, K., Yonezawa, N., Koyasu, S., Yahara, I., and Sakai, H. (1987). Cofilin is a component of intranuclear and cytoplasmic actin rods induced in cultured cells. *Proc Natl Acad Sci U S A* **84(15),** 5262–5266.

39. Ohta, Y., Nishida, E., Sakai, H., and Miyamoto, E. (1989). Dephosphorylation of cofilin accompanies heat shock-induced nuclear accumulation of cofilin. *J Biol Chem.* **264(27),** 16,143–16,148.
40. Iida, K., Matsumoto, S., and Yahara, I. (1992). The KKRKK sequence is involved in heat shock-induced nuclear translocation of the 18-kDa actin-binding protein, cofilin. *Cell Struct Funct.* **17(1),** 39–46.
41. Ono, S., Abe, H., Nagaoka, R., and Obinata, T. (1993). Colocalization of ADF and cofilin in intranuclear actin rods of cultured muscle cells. *J Muscle Res Cell Motil.* **14(2),** 195–204.
42. Moriyama, K., Iida, K., and Yahara, I. (1996). Phosphorylation of Ser-3 of cofilin regulates its essential function on actin. *Genes Cells* **1(1),** 73–86.
43. Aizawa, H., Fukui, Y., and Yahara, I. (1997). Live dynamics of *Dictyostelium* cofilin suggests a role in remodeling actin latticework into bundles. *J Cell Sci.* **110 (Pt 19),** 2333–2344.
44. Sameshima, M., Kishi, Y., Osumi, M., Mahadeo, D., and Cotter, D. A. (2000). Novel actin cytoskeleton: actin tubules. *Cell Struct Funct.* **25(5),** 291–295.
45. Maloney, M. T., Minamide, L.S., Kinley, A. W., Boyle, J. A., and Bamburg, J. R. (2005). 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.* **25(49),** 11,313–11,321.
46. Deshpande. A., Mina, E., Glabe, C., and Busciglio, J. (2006). Different conformations of amyloid beta induce neurotoxicity by distinct mechanisms in human cortical neurons. *J. Neurosci.* **26(22),** 6011–6018.
47. Lacor, P. N., Buniel, M. C., Chang, L., et al. (2004). Synaptic targeting by Alzheimer's-related amyloid beta oligomers. *J Neurosci.* **24(45),** 10,191–10,200.
48. Masliah, E. (2000). The role of synaptic proteins in Alzheimer's disease. *Ann N Y Acad Sci.* **924,** 68–75.
49. Mucke, L., Masliah, E., Yu, G. Q., et al. (2000). High-level neuronal expression of abeta 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J Neurosci* **20(11),** 4050–4058.
50. Jang, D. H., Han, J. H., Lee, S. H., et al. (2005). Cofilin expression induces cofilin-actin rod formation and disrupts synaptic structure and function in Aplysia synapses. *Proc Natl Acad Sci U S A.* **102(44),** 16,072–16,077.
51. Yang, A. J., Knauer, M., Burdick, D.A., and Glabe, C. (1995). Intracellular A beta 1-42 aggregates stimulate the accumulation of stable, insoluble amyloidogenic fragments of the amyloid precursor protein in transfected cells. *J Biol Chem.* **270(24),** 14,786–14,792.
52. Hartmann, J., Erb, C., Ebert, U., et al. (2004). Central cholinergic functions in human amyloid precursor protein knock-in/presenilin-1 transgenic mice. *Neuroscience* **125(4),** 1009-1017.
53. Lambert, M. P., Barlow, A. K., Chromy, B. A., et al. (1998). Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proc Natl Acad Sci U S A.* **95(11),** 6448–6453.
54. Walsh, D. M., Klyubin, I., Fadeeva, J. V., et al. (2002). Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* **416(6880),** 535–539.
55. Wang, H. W., Pasternak, J. F., Kuo, H., et al. (2002). Soluble oligomers of beta amyloid (1-42) inhibit long-term potentiation but not long-term depression in rat dentate gyrus. *Brain Res.* **924(2),** 133–140.
56. Lesné, S., Koh, M. T., Kotilinek, L., et al. (2006). A specific amyloid-beta protein assembly in the brain impairs memory. *Nature* **440(7082),** 352–357.
57. Podlisny, M. B., Ostaszewski, B. L., Squazzo, S. L., et al. (1995). Aggregation of secreted amyloid beta-protein into sodium dodecyl sulfate-stable oligomers in cell culture. *J Biol Chem*. **270(16),** 9564–9570.
58. Podlisny, M. B., Walsh, D. M., Amarante, P., et al. (1998). Oligomerization of endogenous and synthetic amyloid beta-protein at nanomolar levels in cell culture and stabilization of monomer by Congo red. *Biochemistry* **37(11),** 3602–3611.
59. Walsh, D. M., Klyubin, I., Shankar, G. M., et al. (2005). The role of cell-derived oligomers of Abeta in Alzheimer's disease and avenues for therapeutic intervention. *Biochem Soc Trans.* **33(Pt 5),** 1087-1090.
60. Cleary, J. P., Walsh, D. M., Hofmeister, J. J., et al. (2005). Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat Neurosci.* **8(1),** 79-84.
61. Klyubin, I., Walsh, D. M., Lemere, C. A., et al. (2005). Amyloid beta protein immunotherapy

neutralizes Abeta oligomers that disrupt synaptic plasticity in vivo. *Nat Med*. **11(5),** 556–561.
62. Busciglio, J., Lorenzo, A., and Yankner, B. A. (1992). Methodological variables in the assessment of beta amyloid neurotoxicity. *Neurobiol Aging* **13(5),** 609-612.
63. Grace, E. A., Rabiner, C. A., and Busciglio, J. (2002). Characterization of neuronal dystrophy induced by fibrillar amyloid beta: implications for Alzheimer's disease. *Neuroscience* **114(1),** 265–273.
64. Grace, E. A. and Busciglio, J. (2003). Aberrant activation of focal adhesion proteins mediates fibrillar amyloid beta-induced neuronal dystrophy. *J Neurosci*. **23(2),** 493–502.
65. Calderwood, D. A., Shattil, S. J., and Ginsberg, M. H. (2000). Integrins and actin filaments: reciprocal regulation of cell adhesion and signaling. *J Biol Chem*. **275(30),** 22,607-22,610.
66. Giancotti, F. G. and Ruoslahti, E. (1999). Integrin signaling. *Science* **285(5430),** 1028–1032.
67. Turner, C. E. (2000). Paxillin and focal adhesion signaling. *Nat Cell Biol,* **2(12),** E231–E236.
68. Chen, G. C., Turano, B., Ruest, P. J., Hagel, M., Settleman, J., and Thomas, S. M. (2005). Regulation of Rho and Rac signaling to the actin cytoskeleton by paxillin during Drosophila development. *Mol Cell Biol*. **25(3),** 979-987.
69. Heredia, L., Helguera, P., de Olmos, S., et al. (2006). Phosphorylation of ADF/cofilin by LIM-kinase mediates amyloid β-induced degeneration: A potential mechanism of neuronal dystrophy in Alzheimer's disease. *J Neurosci*. **26(24),** 6533–6542.
70. Gong, Y., Chang, L., Viola, K. L., et al. (2003). Alzheimer's disease-affected brain: presence of oligomeric A beta ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proc Natl Acad Sci U S A*. **100(18),** 10,417-10,422.
71. Ohno, M., Chang, L., Tseng, W., et al. (2006). Temporal memory deficits in Alzheimer's mouse models: rescue by genetic deletion of BACE1. *Eur J Neurosci* **23(1),** 251–260.
72. Wang, Y., Shibasaki, F., and Mizuno, K. (2005). Calcium signal-induced cofilin dephosphorylation is mediated by Slingshot via calcineurin. *J Biol Chem.* **280(13),** 12,683–12,689.
73. Demuro, A., Mina, E., Kayed, R., Milton, S. C., Parker, I., and Glabe, C. G. (2005). Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. *J Biol Chem*. **280(17),** 17,294–17,300.
74. Roselli, F., Tirard, M., Lu, J., et al. (2005). Soluble beta-amyloid 1-40 induces NMDA-dependent degradation of postsynaptic density-95 at glutamatergic synapses. *J Neurosci*. **25(48),** 11,061–11,070.
75. Xie, C. W. (2004). Calcium-regulated signaling pathways: role in amyloid beta-induced synaptic dysfunction. *Neuromolecular Med*. **6(1),** 53–64.
76. Cook, C. N., Hejna, M. J., Magnuson, D. J., and Lee, J. M. (2005). Expression of calcipressin1, an inhibitor of the phosphatase calcineurin, is altered with aging and Alzheimer's disease. *J Alzheimers Dis*. **8(1),** 63–73.
77. Stokin, G. B., Lillo, C., Falzone, T. L., et al. (2005). Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. *Science* **307(5713),** 1282–1288.
78. Takahashi, R. H., Almeida, C. G., Kearney, P.F., et al. (2004). Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain. *J Neurosci.* **24(14),** 3592–3599.
79. Cataldo, A. M., Petanceska, S., Peterhoff, C. M., et al. (2003). App gene dosage modulates endosomal abnormalities of Alzheimer's disease in a segmental trisomy 16 mouse model of down syndrome. *J Neurosci.* **23(17),** 6788–6792.
80. Cataldo, A. M., Barnett, J. L., Pieroni, C., and Nixon, R. A. (1997). Increased neuronal endocytosis and protease delivery to early endosomes in sporadic Alzheimer's disease: neuropathologic evidence for a mechanism of increased beta-amyloidogenesis. *J Neurosci.* **17(16),** 6142–6151.
81. Cataldo, A. M., Peterhoff, C. M., Troncoso, J. C., Gomez-Isla, T., Hyman, B. T., and Nixon, R. A. (2000). Endocytic pathway abnormalities precede amyloid-β deposition in sporadic Alzheimer's disease and Down syndrome. Differential effects of ApoE genotype and presenilin mutations. *Am J Pathol.* **157(1),** 277-286.
82. Cataldo, A. M., Petanceska, S., Terio, N. B., et al. (2004). Abeta localization in abnormal endosomes: association with earliest Abeta elevations in AD and Down syndrome. *Neurobiol Aging* **25(10),** 1263–1272.
83. Whitehouse P. J., Struble, R. G., Clark, A. W., and Price, D. L. (1982). Alzheimer disease: plaques, tangles, and the basal forebrain. *Ann Neurol*. **12(5)**, 494.

84. Mann, D. M., Yates, P. O., and Marcyniuk, B. (1984). Alzheimer's presenile dementia, senile dementia of Alzheimer type and Down's syndrome in middle age form an age related continuum of pathological changes. *Neuropathol Appl Neurobiol.* **10(3),** 185–207.
85. Casanova, M. F., Walker, L. C., Whitehouse, P. J., and Price, D. L. (1985). Abnormalities of the nucleus basalis in Down's syndrome. *Ann Neurol.* **18(3),** 310–313.
86. Mufson, E. J., Bothwell, M., and Kordower, J. H. (1989). Loss of nerve growth factor receptor-containing neurons in Alzheimer's disease: a quantitative analysis across subregions of the basal forebrain. *Exp Neurol* **105(3),** 221–232.
87. Jhamandas, J. H., Cho, C., Jassar, B., Harris, K., MacTavish, D., and Easaw, J. (2001). Cellular mechanisms for amyloid beta-protein activation of rat cholinergic basal forebrain neurons. *J Neurophysiol.* **86(3),** 1312–1320.
88. Cooper, J. D., Salehi, A., Delcroix, J. D., et al. (2001). Failed retrograde transport of NGF in a mouse model of Down's syndrome: reversal of cholinergic neurodegenerative phenotypes following NGF infusion. *Proc Natl Acad Sci U S A.* **98(18),** 10,439-10,444.
89. Sofroniew, M. V., Howe, C. L., and Mobley, W. C. (2001). Nerve growth factor signaling, neuroprotection, and neural repair. *Annu Rev Neurosci.* **24,** 1217-1281.
90. Hiruma, H., Katakura, T., Takahashi, S., Ichikawa, T., and Kawakami, T. (2003). Glutamate and amyloid beta-protein rapidly inhibit fast axonal transport in cultured rat hippocampal neurons by different mechanisms. *J Neurosci.* **23(26),** 8967-8977.
91. Arias, C., Becerra-Garcia, F., and Tapia, R. (1998). Glutamic acid and Alzheimer's disease. *Neurobiology (Bp).* **6(1),** 33–43.
92. Lancelot, E., and Beal, M. F. (1998). Glutamate toxicity in chronic neurodegenerative disease. *Prog Brain Res.* **116,** 331–347.
93. Yankner, B. A., Duffy, L. K., and Kirschnur, D. A. (1990). Neurotoxic effect of amyloid beta protein: reversal by tachykinin neuropeptides. *Science* **250(4978),** 279-282.
94. Kasa, P., Papp, H., Zombori, J., Mayer, P., and Checler, F. (2003). C-terminal fragments of amyloid-beta peptide cause cholinergic axonal degeneration by a toxic effect rather than by physical injury in the nondemented human brain. *Neurochem Res.* **28(3-4),** 493–498.
95. Salehi, A., Delcroix, J. D., Belichenko, P. V., et al. (2006). Increased APP expression in a mouse model of Down's syndrome disrupts NGF transport and causes cholinergic neuron degeneration. *Neuron* **51,** 29-42.
96. Sun, X., Tong, Y., Qing, H., Chen, C. H., Song, W. (2006). Increased BACE1 maturation contributes to the pathogenesis of Alzheimer's disease in Down syndrome. *FASEB J.* **20(9),** 1361–1368.
97. Sun, X., He, G., and Song, W. (2006). BACE2, as a novel APP theta-secretase, is not responsible for the pathogenesis of Alzheimer's disease in Down syndrome. *FASEB J.* **20(9),** 1369-1376.
98. Arron, J. R., Winslow, M. M., Polleri, A., et al. (2006). NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21. *Nature* **441(7093),** 595–600.
99. Kamal, A., Stokin, G. B., Yang, Z., Xia, C. H., and Goldstein, L. S. (2000). Axonal transport of amyloid precursor protein is mediated by direct binding to the kinesin light chain subunit of kinesin-I. *Neuron* **28(2),** 449-459.
100. Kamal, A., Almenar-Queralt, A., LeBlanc, J. F., Roberts, E. A., and Goldstein, L. S. (2001). Kinesin-mediated axonal transport of a membrane compartment containing beta-secretase and presenilin-1 requires APP. *Nature* **414(6864),** 643–648.
101. Papp, H., Pakaski, M., and Kasa, P. (2002). Presenilin-1 and the amyloid precursor protein are transported bidirectionally in the sciatic nerve of adult rat. *Neurochem Int.* **41(6),** 429-435.
102. Sheng, J. G., Price, D.L., and Koliatsos, V. E. (2003). The beta-amyloid-related proteins presenilin 1 and BACE1 are axonally transported to nerve terminals in the brain. *Exp Neurol.* **184(2),** 1053–1057.
103. Inomata, H., Nakamura, Y., Hayakawa, A., et al. (2003). A scaffold protein JIP-1b enhances amyloid precursor protein phosphorylation by JNK and its association with kinesin light chain 1. *J Biol Chem.* **278(25),** 22,946–22,955.
104. Matsuda, S., Matsuda, Y., and D'Adamio, L. (2003). Amyloid beta protein precursor (AbetaPP), but not AbetaPP-like protein 2, is bridged to the kinesin light chain by the scaffold protein JNK-interacting protein 1. *J Biol Chem.* **278(40),** 38,601–38,606.
105. Kawarabayashi, T., Shoji, M., Yamaguchi, H., et al. (1993). Amyloid beta protein precursor

accumulates in swollen neurites throughout rat brain with aging. *Neurosci Lett.* **153(1),** 73–76.
106. Roberts, G. W., Gentleman, S. M., Lynch, A., Murray, L., Landon, M., and Graham, D. I. (1994). Beta amyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. *J Neurol Neurosurg Psychiatry* **57(4),** 419-425.
107. Smith, D.H., Chen, X. H., Iwata, A., and Graham, D. I. (2003). Amyloid beta accumulation in axons after traumatic brain injury in humans. *J Neurosurg.* **98(5),** 1072–1077.
108. Chen, X. H., Siman, R., Iwata, A., Meaney, D. F., Trojanowski, J. Q., and Smith, D. H. (2004). Long-term accumulation of amyloid-beta, beta-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma. *Am J Pathol.* **165(2),** 357-371.
109. Stokin, G. B. and Goldstein, L. S. (2006). Linking molecular motors to Alzheimer's disease. *J Physiol Paris* **99(2-3),** 193–200.
110. Gouras, G. K., Almeida, C.G., and Takahashi, R. H. (2005). Intraneuronal Abeta accumulation and origin of plaques in Alzheimer's disease. *Neurobiol Aging.* **26(9),** 1235–1244.
111. Borchelt, D. R., Ratovitski, T., van Lare, J., et al. (1997). Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron* **19(4),** 939-945.
113. Delcroix, J. D., Valletta, J., Wu, C., Hunt, S. J., Kowal, A. S., and Mobley, W. C. (2003). NGF signaling in sensory neurons: evidence that early endosomes cary NGF retrograde signals. *Neuron* **39,** 69-84.
114. Koo, E. H. and Squazzo, S. L. (1994). Evidence that production and release of amyloid beta-protein involves the endocytic pathway. *J Biol Chem.* **269(26),** 17,386–17,389.
115. Vetrivel, K. S., Cheng, H., Lin, W., et al. (2004). Association of gamma-secretase with lipid rafts in post-Golgi and endosome membranes. *J Biol Chem.* **279(43),** 44,945–44,954.
116. Prasher, V. P., Farrer, M. J. Kessling, A. M., et al. (1998). Molecular mapping of Alzheimer-type dementia in Down's syndrome. *Ann Neurol.* **43(3),** 380–383.
117. Rosso, S., Bollati, F., Bisbal, M., et al. (2004) LIMK1 regulates Golgi dynamics, traffic of Golgi-derived vesicles, and process extension in primary cultured neurons. *Mol Biol Cell*. **15(7),** 3433–3449.
118. Knebl, J., DeFazio, P., Clearfield, M. B., et al. (1994). Plasma lipids and cholesterol esterification in Alzheimer's disease. *Mech Ageing Dev.* **73(1),** 69-77.
119. Frears, E. R., Stephens, D. J., Walters, C. E., Davies, H., and Austen, B. M. (1999). The role of cholesterol in the biosynthesis of beta-amyloid. *Neuroreport* **10(8),** 1699-1705.
120. Sawamura, N., Morishima-Kawashima, M., Waki, H., et al. (2000). Mutant presenilin 2 transgenic mice. A large increase in the levels of Abeta 42 is presumably associated with the low density membrane domain that contains decreased levels of glycerophospholipids and sphingomyelin. *J Biol Chem.* **275(36),** 27,901–27,908.
121. Runz, H., Rietdorf, J., Tomic, I., et al. (2002). Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. *J Neurosci.* **22(5),** 1679-1689.
122. Burns, M., Gaynor, K., Olm, V., et al. (2003). Presenilin redistribution associated with aberrant cholesterol transport enhances beta-amyloid production *in vivo*. *J Neurosci.* **23(13),** 5645–5649.
123. Jin, L. W., Shie, F. S., Maezawa, I., Vincent, I., and Bird, T. (2004). Intracellular accumulation of amyloidogenic fragments of amyloid-beta precursor protein in neurons with Niemann-Pick type C defects is associated with endosomal abnormalities. *Am J Pathol.* **164(3),** 975–985. Erratum in: *Am J Pathol.* **165(4),** 1447.
124. Cole, S. L., Grudzien, A., Manhart, I. O., Kelly, B. L., Oakley, H., and Vassar, R. (2005). Statins cause intracellular accumulation of amyloid precursor protein, beta-secretase-cleaved fragments, and amyloid beta-peptide via an isoprenoid-dependent mechanism. *J Biol Chem.* **280(19),** 18,755–18,770.
125. Cole, S. L. and Vassar, R. (2006). Isoprenoids and Alzheimer's disease: A complex relationship. *Neurobiol Dis*. **22(2),** 209-222.
126. Simons, M., Keller, P., De Strooper, B., Beyreuther, K., Dotti, C. G., and Simons, K. (1998). Cholesterol depletion inhibits the generation of beta-amyloid in hippocampal neurons. *Proc Natl Acad Sci U S A.* **95(11),** 6460–6464.
127. Buxbaum, J. D., Geoghagen, N. S., and Friedhoff, L. T. (2001). Cholesterol depletion with physiological concentrations of a statin decreases the formation of the Alzheimer

amyloid Abeta peptide. *J Alzheimers Dis.* **3(2),** 221–229.
128. Fassbender, K., Simons, M., Bergmann, C., et al. (2001). Simvastatin strongly reduces levels of Alzheimer's disease beta -amyloid peptides Abeta 42 and Abeta 40 *in vitro* and *in vivo*. *Proc Natl Acad Sci U S A.* **98(10),** 5856–5861.
139. Kojro, E., Gimpl, G., Lammich, S., Marz, W., and Fahrenholz, F. (2001). Low cholesterol stimulates the nonamyloidogenic pathway by its effect on the alpha -secretase ADAM 10. *Proc Natl Acad Sci U S A.* **98(10),** 5815–5820.
130. Abad-Rodriguez, J., Ledesma, M. D., Craessaerts, K., et al. (2004). Neuronal membrane cholesterol loss enhances amyloid peptide generation. *J Cell Biol.* **167(5),** 953–960.
131. Bodovitz, S. and Klein, W. L. (1996). Cholesterol modulates alpha-secretase cleavage of amyloid precursor protein. *J Biol Chem.* **271(8),** 4436–4440.
132. Racchi, M., Baetta, R., Salvietti, N., et al. (1997). Secretory processing of amyloid precursor protein is inhibited by increase in cellular cholesterol content. *Biochem J.* **322 (Pt 3),** 893–898.
133. Galbete, J. L., Martin, T. R., Peressini, E., Modena, P., Bianchi, R., and Forloni, G. (2000). Cholesterol decreases secretion of the secreted form of amyloid precursor protein by interfering with glycosylation in the protein secretory pathway. *Biochem J.* **348(Pt 2),** 307-313.
134. Riddell, D. R., Christie, G., Hussain, I., and Dingwall, C. (2001). Compartmentalization of beta-secretase (Asp2) into low-buoyant density, noncaveolar lipid rafts. *Curr Biol.* **11(16),** 1288–1293.
135. Cordy, J. M., Hussain, I., Dingwall, C., Hooper, N. M., and Turner, A. J. (2003). Exclusively targeting beta-secretase to lipid rafts by GPI-anchor addition up-regulates beta-site processing of the amyloid precursor protein. *Proc Natl Acad Sci U S A.* **100(20),** 11,735–11,740.
136. Pedrini, S., Carter, T. L., Prendergast, G., Petanceska, S., Ehrlich, M. E., and Gandy, S. (2005). Modulation of statin-activated shedding of Alzheimer APP ectodomain by ROCK. *PLoS Med.* **2(1),** e18., 0069–0078.
137. Bi, X., Baudry, M., Liu, J., et al. (2004). Inhibition of geranylgeranylation mediates the effects of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors on microglia. *J Biol Chem.* **279(46),** 48,238–48,245.
138. Kato, T., Hashikabe, H., Iwata, C., Akimoto, K., and Hattori, Y. (2004). Statin blocks Rho/Rho-kinase signalling and disrupts the actin cytoskeleton: relationship to enhancement of LPS-mediated nitric oxide synthesis in vascular smooth muscle cells. *Biochim Biophys Acta.* **1689(3),** 267-272.
139. Ryder, J., Su, Y., Liu, F., Li, B., Zhou, Y., and Ni, B. (2003). Divergent roles of GSK3 and CDK5 in APP processing. *Biochem Biophys Res Commun.* **312(4),** 922–929.
140. Vicent, D., Maratos-Flier, E., and Kahn, C. R. (2000). The branch point enzyme of the mevalonate pathway for protein prenylation is overexpressed in the ob/ob mouse and induced by adipogenesis. *Mol Cell Biol.* **20(6),** 2158–2166.
141. Ridley, A. J. (2001). Rho proteins: linking signaling with membrane trafficking. *Traffic* **2(5),** 303–310.
142. Beal, M. F. (2005). Mitochondria take center stage in aging and neurodegeneration. *Ann Neurol*. **58(4),** 495–505.
143. Espisoto, L., Raber, J., Kekonius, L., et al. (2006). Reduction in mitochondrial superoxide dismutase modulates Alzheimer's disease-like pathology and accelerates the onset of behavioral changes in human amyloid precursor protein transgenic mice. *J. Neurosci.* **26(19),** 5167-5179.
144. Bernstein, B. W., Chen, H., Boyle, J. A., and Bamburg, J. R. (2006). Formation of Actin-ADF/Cofilin rods transiently retards decline of mitochondrial potential and ATP in stressed neurons. *Am. J. Physiol. Cell Physiol.* **291,** C828–C839 .
145. Bowen, D. M., White, P., Spillane, J. A., et al. (1979). Accelerated ageing or selective neuronal loss as an important cause of dementia? *Lancet* **1(8106),** 11–14.
146. Hirai, K., Aliev, G., Nunomura, A., et al. (2001). Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci.* **21(9),** 3017-3023.
147. Swerdlow, R. H. and Kish, S. J. (2002). Mitochondria in Alzheimer's disease. *Int Rev Neurobiol.* **53,** 341–385.
148. Lovell, M. A., Xiong, S., Markesbery, W. R., and Lynn, B. C. (2005). Quantitative proteomic analysis of mitochondria from primary neuron cultures treated with amyloid beta peptide. *Neurochem Res.* **30(1),** 113–122.
149. Kondo, T., Shirasawa, T., Itoyama, Y., and Mori, H. (1996). Embryonic genes expressed in Alzheimer's disease brains. *Neurosci Lett.* **209(3),** 157-160.
150. Banerjee, J. and Ghosh, S. (2006). Phosphorylation of rat brain mitochondrial voltage-

dependent anion as a potential tool to control leakage of cytochrome c. *J Neurochem*., Jun 19; **98,** 670–676.
151. Chua, B. T., Volbracht, C., Tan, K. O., Li, R., Yu, V. C., and Li, P. (2003). Mitochondrial translocation of cofilin is an early step in apoptosis induction. *Nat Cell Biol.* **5(12),** 1083–1089.
152. Yang, E., Kim, H., Lee, J., et al. (2004). Overexpression of LIM kinase 1 renders resistance to apoptosis in PC12 cells by inhibition of caspase activation. *Cell Mol Neurobiol.* **24(2),** 181–192.
153. Gourlay C. W. and Ayscough, K. R., (2005). The actin cytoskeleton in ageing and apoptosis. *FEMS Yeast Res* **5(12),** 1193–1198.
154. Gourlay, C. W., Carpp, L. N., Timpson, P., Winder, S. J., and Ayschough, K. R. (2004). A role for the actin cytoskeleton in cell death and aging in yeast. *J Cell Biol.* **164(6),** 803–809.
155. Gourlay, C. W. and Ayscough, K. R. (2005). A role for actin in aging and apoptosis. *Biochem Soc Trans.* **33(6),** 1260–1264.
156. Gourlay, C. W. and Ayschogh, K. R. (2005). Identification of an upstream regulatory pathway controlling actin-mediated apoptosis in yeast. *J Cell Sci.* **118(10),** 2119-2132.
157. Tsujimoto, Y. and Shimizu, S. (2002). The voltage-dependent anion channel: an essential player in apoptosis. *Biochimie* **84,** 187-193.
158. Zalk, R., Israelson, A., Garty, E. S., Azoulay-Zohar, H., and Shoshan-Barmatz, V. (2005). Oligomeric states of the voltage-dependent anion channel and cytochrome c release from mitochondria. *Biochem J*. **386(Pt 1),** 73–83.
159. Koya, R. C., Fujita, H., Shimizu, S., et al. (2000). Gelsolin inhibits apoptosis by blocking mitochondrial membrane potential loss and cytochrome c release. *J Biol Chem.* **275(20),** 15,343–15,349.
160. Kusano, H., Shimizu, S., Koya, R. C., et al. (2000). Human gelsolin prevents apoptosis by inhibiting apoptotic mitochondrial changes via closing VDAC. *Oncogene* **19(42),** 4807-4814