The roles of the actin cytoskeleton in fear memory formation

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The formation and storage of fear memory is needed to adapt behavior and avoid danger during subsequent fearful events. However, fear memory may also play a significant role in stress and anxiety disorders. When fear becomes disproportionate to that necessary to cope with a given stimulus, or begins to occur in inappropriate situations, a fear or anxiety disorder exists. Thus, the study of cellular and molecular mechanisms underpinning fear memory may shed light on the formation of memory and on anxiety and stress related disorders. Evidence indicates that fear learning leads to changes in neuronal synaptic transmission and morphology in brain areas underlying fear memory formation including the amygdala and hippocampus. The actin cytoskeleton has been shown to participate in these key neuronal processes. Recent findings show that the actin cytoskeleton is needed for fear memory formation and extinction. Moreover, the actin cytoskeleton is involved in synaptic plasticity and in neuronal morphogenesis in brain areas that mediate fear memory. The actin cytoskeleton may therefore mediate between synaptic transmission during fear learning and long-term cellular alterations mandatory for fear memory formation.

Keywords: actin cytoskeleton, fear memory, synaptic plasticity, amygdala, hippocampus

Long-term memory (LTM) formation is believed to involve alterations of synaptic efficacy produced by modifications in neural transmission caused by physiochemical and/or structural modifications of synaptic communication within neuronal networks (Konorski, 1948; Hebb, 1949; Dudai, 1989; Bliss and Collingridge, 1993; Martin et al., 2000; Tsien, 2000; Kandel, 2001; Lamprecht and LeDoux, 2004). A prime challenge is to identify molecules involved in sustaining synaptic alterations and memory formation. Actin is a most attractive candidate to play a key role in memory formation as it is responsive to synaptic signaling, such as triggered during learning, and consequently may mediate cellular events that underlie changes in synaptic efficacy, such as synaptic transmission and morphology.

Actin cytoskeleton is involved in many key cellular processes including cellular morphogenesis, motility, division, and intracellular transport. Actin exists in two states in cells, either as a globular monomer (G-actin) or following head-to-tail interaction as a polymer to form filamentous F-actin. Actin remodeling and the structure of F-actin network are tightly regulated by actin-binding proteins (Luo, 2000; Dillon and Goda, 2005). These actin cytoskeleton-regulatory proteins mediate between intrinsic and extrinsic cellular signals and actin-dependent cellular functions. Thus, by forming such intricate network of filaments responsive to regulatory signals, actin mediates a large variety of cellular functions from supporting cellular morphology to providing contractile forces needed for cellular activities including cell division and transport of vesicles. Actin monomers and filaments are abundant in presynapses and postsynapses and act to regulate key neuronal processes such as alterations in synaptic transmission and morphology (Luo, 2002; Dillon and Goda, 2005; Cingolani and Goda,

2008). Changes in synaptic transmission and neuronal morphology are involved in the process of memory formation (Lamprecht and LeDoux, 2004).

This review is focused on the roles of the actin cytoskeleton in fear memory formation, in particular in the lateral amygdala (LA) and hippocampus brain regions shown to be involved in fear conditioning. Fear conditioning is a useful behavioral paradigm used to study brain mechanisms underlying fear memory formation. In fear conditioning an association is formed between a neutral conditioned stimulus (CS), such as a tone, and an aversive unconditioned stimulus (US), typically a mild footshock (LeDoux, 2000; Davis and Whalen, 2001; Schafe et al., 2001; Sah et al., 2003; Rodrigues et al., 2004; Maren, 2005). Fear conditioning leads to LTM of the CS that acquires affective properties and will subsequently elicit responses that typically occur in the presence of danger. The lateral nucleus of the amygdala receives information about the CS and US from thalamus and cortex and cells in LA are responsive to CS or US and some LA cells respond to both stimuli (e.g., LeDoux et al., 1984; LeDoux et al., 1990a; Turner and Herkenham, 1991; Mascagni et al., 1993; Romanski and LeDoux, 1993; Romanski et al., 1993; Shi and Cassell, 1997; McDonald, 1998; Shi and Davis, 1998; Doron and LeDoux, 2000; LeDoux, 2000; Linke et al., 2000). Damage or functional inactivation of the LA during acquisition prevents the learning from taking place (e.g., LeDoux et al., 1990b; Helmstetter and Bellgowan, 1994; Muller et al., 1997; Fanselow and LeDoux, 1999; Wilensky et al., 1999; Nader et al., 2001), and neural activity changes in LA by learning (e.g., Quirk et al., 1995; Quirk et al., 1997; Collins and Pare, 2000; Repa et al., 2001). LA is connected directly or indirectly to other amygdala nuclei including the central nucleus of the amygdala (CE) shown to participate in fear

memory formation and also to serve as output nucleus to brain areas involved in fear responses (e.g., LeDoux, 2000; Wilensky et al., 2006; Ciocchi et al., 2010; Haubensak et al., 2010; Duvarci et al., 2011). The hippocampus is involved in contextual fear conditioning where the environmental contex is associated with an aversive event (e.g., Kim and Fanselow, 1992; Phillips and LeDoux, 1992).

As noted above, actin is involved in neuronal transmission and morphogenesis and in synaptic plasticity (Luo, 2002; Dillon and Goda, 2005; Cingolani and Goda, 2008) neuronal processes that have been shown to be implicated in fear memory formation in LA and hippocampus (see below). These findings beg the questions: is the actin cytoskeleton an essential component of the molecular events needed for long-term fear memory formation in these brain regions? If so, which cellular mechanisms are modulated by actin cytoskeleton and how they mediate fear memory formation?
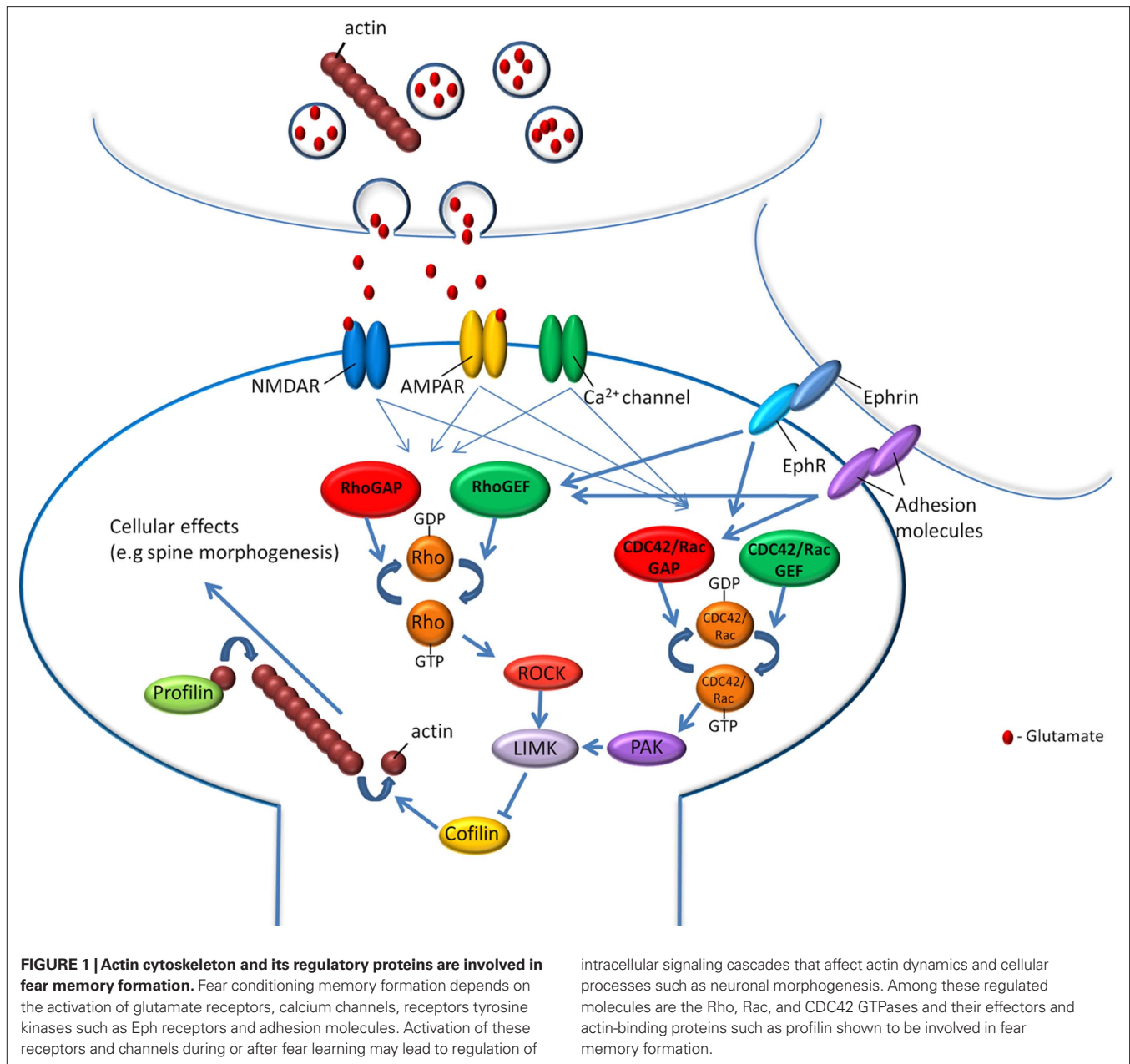
ACTIN AND FEAR MEMORY FORMATION
Several studies have shown that the actin cytoskeleton is needed for both cued fear conditioning (tone–footshock pairing) and contextual fear conditioning memory formation in amygdala and hippocampus. It was shown that intra-hippocampal infusion of actin cytoskeleton assembly inhibitors (latrunculin A or cytochalasin D) impaired the consolidation of contextual fear memory (Fischer et al., 2004). Moreover, microinjection of these compounds into the hippocampus impaired the extinction of contextual fear memory, a form of learning whereby the animal re-learns that the context is not fearful (Fischer et al., 2004). Microinfusion of cytochalasin D, an actin polymerization inhibitor, into rat LA immediately before fear conditioning training interfered with the formation of long-term fear memory (LTM) but not short-term fear memory (STM; Mantzur et al., 2009). Furthermore, microinfusion of cytochalasin D into rat LA immediately after fear conditioning dampened LTM. Cytochalasin D had no effect on fear conditioning memory retrieval when injected immediately before LTM test. Rehberg et al. (2010) showed that auditory cued but not contextual fear memory is disrupted, when the actin depolymerization inhibitor phalloidin was injected into basolateral complex of the amygdala (BLA) 6 h after conditioning. Re-consolidation of memory is also dependent on regulation of actin polymerization (Rehberg et al., 2010). Microinjection of cytochalasin D into the BLA or CA1 was shown to impair the return of fear after reconditioning at the last extinction session indicating that actin polymerization is also needed for reconditioning (Motanis and Maroun, 2011). Actin cytoskeleton was shown to be involved in other types of memory formation (e.g., conditioned taste aversion: Bi et al., 2010; aversive memories of drug withdrawal: Hou et al., 2009). In summary, convincing evidence is available indicating that actin cytoskeleton is involved in fear memory formation.

THE ROLES OF ACTIN REGULATORY PROTEINS IN FEAR MEMORY
How does neuronal activation in amygdala or hippocampus during fear conditioning lead to changes in actin cytoskeleton needed for fear memory formation? Actin cytoskeleton polymerization and depolymerization are tightly controlled by regulatory proteins (Luo, 2000). Other actin-mediated function such as intracellular transport and contractility are also mediated by actin-binding

proteins (Kamm and Stull, 2001; Somlyo and Somlyo, 2003). These regulatory proteins (**Figure 1**) could mediate actin involvement in fear memory formation as they are functionally linked with synaptic receptors that participate in fear conditioning such as the glutamate receptors, Eph receptors, and adhesion molecules such as cadherin (Gerlai et al., 1999; Rodrigues et al., 2004; Schrick et al., 2007; Maguschak and Ressler, 2008; Savelieva et al., 2008). For example, actin dynamics in spines are inhibited by activation of either α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) or N-methyl-D-aspartate (NMDA) glutamate receptors (Fischer et al., 2000). Moreover, activation of either receptor inhibited actin-based protrusive activity from dendritic spine head. In addition, several actin regulatory proteins, such as members of the Rho GTPase family, are activated by glutamate receptor to regulate neuronal morphogenesis. Studies are available suggesting that RhoA mediates the promotion of normal dendritic arbor development by NMDA receptor activation (Li et al., 2000), and recruitment and activation of RhoA underlies spines morphology in a glutamate receptor-dependent manner (Schubert et al., 2006). Two-photon glutamate uncaging leads to long-term volume increase of single spine and to rapid activation of RhoA and Cdc42 in stimulated spine (Murakoshi et al., 2011). Moreover, NMDA or its downstream signaling pathways stimulation may lead to regulation of Rho or Rac GTPases activity (e.g., Tejada-Simon et al., 2006; Nakazawa et al., 2008; Saneyoshi et al., 2008). Eph receptors are also regulators of the Rho/Rac/CDC42 GTPases proteins and affect actin dynamics and neuronal morphology (Shamah et al., 2001; Irie and Yamaguchi, 2002; Penzes et al., 2003; Klein, 2009). Adhesion molecules may regulate Rho/Rac/CDC42 GTPases proteins to affect actin cytoskeleton (e.g., Brusés, 2006).

Indeed, several actin regulatory proteins have been shown to be involved in fear memory formation. Following fear conditioning, the tyrosine phosphorylated p190 RhoGAP becomes associated with a molecular complex in LA (Lamprecht et al., 2002). Importantly, evidence is available that p190 RhoGAP is involved in mediating actin reorganization. Specifically, in p190 RhoGAP mutant mice, polymerized actin accumulates extensively in cells of the neural tube floor, suggesting that p190 RhoGAP plays a role in regulating actin assembly (Brouns et al., 2000). P190 RhoGAP regulates Rho GTPase protein, a molecular switch that controls many key cellular processes including actin dynamics. Inhibition of the Rho GTPase effector, the Rho-associated kinase (ROCK), a kinase that affects actin cytoskeleton (Amano et al., 2010), in LA impaired the formation of long- but not short-term fear memory formation (Lamprecht et al., 2002). Interestingly, the activation of Rho and Rac GTPases led to rearrangement of cerebral actin cytoskeleton, enhanced neurotransmission and synaptic plasticity, and facilitation of fear conditioning (Diana et al., 2007). In addition, RhoB, a member of the Rho GTPase family, is involved in short-term plasticity in hippocampus, in the regulation of cofilin and dendritic and spine morphology (McNair et al., 2010). Intracerebroventricular injection of ROCK inhibitor leads to increase in anxiety-related behaviors (Saitoh et al., 2006). ROCK regulates actin cytoskeleton via other signaling molecules such as the LIM kinase (LIMK) that regulates actin dynamics. LIMK exerts its effect on actin polymerization by phosphorylating and thus inactivating the actin depolymerization factor (ADF)/cofilin

FIGURE 1 | Actin cytoskeleton and its regulatory proteins are involved in fear memory formation. Fear conditioning memory formation depends on the activation of glutamate receptors, calcium channels, receptors tyrosine kinases such as Eph receptors and adhesion molecules. Activation of these receptors and channels during or after fear learning may lead to regulation of intracellular signaling cascades that affect actin dynamics and cellular processes such as neuronal morphogenesis. Among these regulated molecules are the Rho, Rac, and CDC42 GTPases and their effectors and actin-binding proteins such as profilin shown to be involved in fear memory formation.

(Arber et al., 1998; Yang et al., 1998; Sumi et al., 1999). Indeed, in LIMK-1 knockout mice, spine-dendrite F-actin levels were reduced compared to wild type mice (Meng et al., 2002). Furthermore, the knockout mice exhibited significant abnormalities in spine and axonal morphology. In addition, hippocampal long-term potentiation (LTP) is enhanced indicating that synaptic function was altered. The LIMK-1 knockout mice also showed enhanced cued fear conditioning LTM. These results indicate that the regulation of actin polymerization by the LIMK pathway is essential for normal fear memory formation. The LIMK effector cofilin is also involved in fear conditioning. Mice in which n-cofilin was removed from principal neurons of the postnatal forebrain are impaired in long- and short-term fear memory (Rust et al., 2010).

Profilin is another actin cytoskeleton-regulatory protein that regulates actin polymerization by funneling ATP-actin to the growing actin filaments (Witke, 2004). Profilin was shown to be translocated into dendritic spines in cultured hippocampal neurons after neuronal stimulation, LTP and long-term depression (LTD; Ackermann and Matus, 2003; Neuhoff et al., 2005). The translocation of profilin is associated with the suppression of actin dynamics in the spine head and the stabilization of spine morphology. Fear conditioning in rats leads to the movement of profilin into dendritic spines in the LA (Lamprecht et al., 2006a). Profilin-containing spines were shown to be larger compared to spines devoid of profilin. A greater proportion of profilin-containing spines with enlarged PSDs could contribute to the enhancement

of associatively induced synaptic responses in LA following fear learning. Mice with knockdown of one of the profilin isoforms, profilin2, are hyperactive and show increased novelty-seeking behavior (Pilo Boyl et al., 2007). Freezing after fear conditioning is similar in control and knockout mice when number of freezings, but not time of freezing, is measured during LTM test (Pilo Boyl et al., 2007).

Myosin light chain kinase (MLCK) is a calcium/calmodulin-dependent protein kinase that phosphorylates the myosin regulatory light chain (RLC), leading to contraction of the actomyosin filaments (Kamm and Stull, 2001; Somlyo and Somlyo, 2003). MLCK is involved in regulating cellular events related to synaptic transmission, such as neurotransmitter release (Mochida et al., 1994; Ryan, 1999; Polo-Parada et al., 2001), N-methyl-D-aspartate receptor activity (Lei et al., 2001) and potassium channel function (Akasu et al., 1993). In addition, MLCK participates in neural morphogenesis, including the regulation of growth cone motility (Gallo et al., 2002; Zhou et al., 2002) and dendritic branching (Ramakers et al., 2001). MLCK is present in cells throughout the LA and is localized to dendritic shafts and spines that are postsynaptic to the projections from the auditory thalamus to lateral nucleus of the amygdala, a pathway specifically implicated in fear learning (Lamprecht et al., 2006b). Inhibition of MLCK in LA leads to the enhancement of fear memory formation but has no effect on retrieval of fear memory (Lamprecht et al., 2006b). In addition, inhibition of myosin light chain kinase enhances LTP in the auditory thalamic pathway to the LA (Lamprecht et al., 2006b). MLCK inhibition immediately after fear conditioning training has no effect on fear memory formation. The short time window of involvement of MLCK in fear conditioning is consistent with its ability to rapidly regulate synaptic transmission (Ryan, 1999; Lei et al., 2001). In addition, anatomical findings showing that MLCK is located in LA presynaptic terminals and in postsynaptic densities suggest that MLCK might be involved in regulating events in these sites such as vesicle release (Ryan, 1999) or receptor activity (Lei et al., 2001). Moreover, the observation that MLCK inhibition does not affect fear memory retrieval implies that MLCK does not regulate transmission during memory activation, but only during acquisition. Consistent with this view is the observation that application of ML-7 (an MLCK inhibitor) to amygdala slices has no effect on basal transmission but rather specifically on the induction of associative LTP. These findings showing that the inhibition of MLCK enhances conditioning and the synaptic plasticity underlying conditioning indicate that MLCK normally inhibits fear learning.

Other proteins that are involved in actin polymerization and some in spine morphology have been implicated in fear memory formation such as beta-adducin shown to be essential for contextual and cued fear conditioning (Rabenstein et al., 2005), drebrin A needed for context-dependent freezing after fear conditioning (Kojima et al., 2010), Ndr which expression is increased in amygdala 6 h after Pavlovian fear conditioning training (Stork et al., 2004), neurabin needed for contextual fear memory and hippocampal LTP but not auditory fear memory and LTD (Wu et al., 2008) and p21-activated kinase which is not needed for normal short-term contextual fear conditioning but is needed for normal consolidation/retention of fear memory (Hayashi et al., 2004).

Cumulatively, the aforementioned studies show that actin regulatory proteins are involved in fear memory formation. Modulation of the actin cytoskeleton by these proteins may serve as a signaling connection between synaptic activation induced by learning and cellular changes underlying fear memory formation.

To further elucidate possible roles of actin cytoskeleton in fear memory formation its roles in synaptic morphology, transmission and plasticity in amygdala and hippocampus are discussed.

ACTIN CYTOSKELETON IN SYNAPTIC TRANSMISSION

Alteration of synaptic efficacy either by affecting synaptic release of neurotransmitters and/or the level of synaptic receptors for neurotransmitters is associated with memory formation and synaptic plasticity. Changes in synaptic efficacy are induced by fear learning. For example, it was shown that fear-conditioned animals exhibit a presynaptic facilitation of AMPA receptor-mediated transmission in LA neurons (McKernan and Shinnick-Gallagher, 1997) and conditioned fear is accompanied by the enhancement in transmitter release at cortico-amygdala synapses (Tsvetkov et al., 2002). At the postsynapse fear conditioning drives AMPA receptors into the synapses of neurons in the LA, incorporation process that is needed for fear conditioning memory formation (Rumpel et al., 2005; Yeh et al., 2006; Nedelescu et al., 2010).

Actin cytoskeleton is found in pre- and post-synapse and is involved in the regulation of synaptic transmission in these sites and may mediate changes in synaptic efficacy following fear conditioning. In the presynapse actin cytoskeleton contacts synaptic vesicle through short strands of synapsin, a phosphoprotein associated with synaptic vesicle membrane (e.g., Landis et al., 1988; Hirokawa et al., 1989; Doussau and Augustine, 2000). It is possible that actin regulates the availability of the vesicle in the reserve pool (RP) by forming a barrier (e.g., Wang et al., 1996) or may serve as a scaffold protein to retain synapsin in presynapse, thereby indirectly influencing neurotransmission (Sankaranarayanan et al., 2003). Neuronal stimulation may redistribute synapsin enabling access to the RP of vesicles (Greengard et al., 1994; Chi et al., 2001, 2003). Actin may also promote vesicle delivery to the readily releasable pool (RRP) by providing cytoskeletal routes of vesicle to the RRP (Prekeris and Terrian, 1997; Evans et al., 1998; Watanabe et al., 2005). In addition, actin may be involved in the endocytosis of vesicle at the presynapse, possibly by forming a link with dynamin or by promoting the transport of endocytosed vesicles to the internal RP cluster (Shupliakov et al., 2002; Bloom et al., 2003; Engqvist-Goldstein and Drubin, 2003). Synaptic vesicles endocytosed at one bouton can be recruited into the functional pool of nearby boutons where they undergo exocytosis (Darcy et al., 2006). Such distribution of vesicles between nearby boutons requires actin turnover (Darcy et al., 2006).

The postsynaptic actin cytoskeleton may also contribute to synaptic transmission as it is involved in the regulation of glutamate and GABA receptors clustering and trafficking and thereby in the postsynaptic response to neurotransmitters. F-actin depolymerization reduces the number of AMPA and NMDA receptors clusters at excitatory synapses (Allison et al., 1998). Actin also mediates glutamate receptor trafficking via myosins, the main actin-dependent motor proteins. Myosin Va mediates translocation of GluR1-containing AMPA receptor (AMPAR) from the dendritic

shaft into spines and is required for LTP (Correia et al., 2008). Myosin Vb is also involved in AMPAR trafficking (Lisé et al., 2006). Actin regulatory and associated proteins also mediate receptor trafficking. For example, ADF/cofilin-mediated actin dynamics regulates AMPAR receptor trafficking during synaptic potentiation (Gu et al., 2010). The reversion induced LIM protein (RIL) is involved in actin-dependent trafficking of GluR1 (Schulz et al., 2004) and the actin adaptor protein 4.1N stabilizes the surface expression of GluR1 (Shen et al., 2000). Actin also mediates AMPAR internalization. AMPAR internalization can be induced by the actin assembly inhibitor latrunculin A, and this process is blocked by jasplakinolide, a drug which stabilizes actin filaments (Zhou et al., 2001) and myosin VI plays a role in the clathrin-mediated endocytosis of AMPARs (Osterweil et al., 2005). Actin cytoskeleton can also affect inhibitory transmission by mediating GABA receptor trafficking to the synapse (e.g., Graziane et al., 2009).

Taken together, the aforementioned studies show that actin cytoskeleton is involved in regulating synaptic transmission by affecting pre- and post-synapse molecular and cellular events that are also involved in synaptic plasticity and fear memory formation. Additional research is warranted to elucidate whether actin cytoskeleton is needed for presynaptic or postsynaptic changes during and following fear conditioning training.

ACTIN CYTOSKELETON IN SYNAPTIC MORPHOGENESIS

It has been shown that alteration in neuronal morphology is associated with memory formation (Bailey and Kandel, 1993; Lamprecht and LeDoux, 2004) and may serve to modulate neuronal connectivity needed to form or alter memory. Most excitatory synapses in the brain terminate on dendritic spines, which have been the focus of recent work in the mammalian brain. Dendritic spines receive the majority of excitatory synaptic inputs in the brain, compartmentalize local synaptic signaling pathways, and restrict the diffusion of postsynaptic molecules (Nimchinsky et al., 2002; Lamprecht and LeDoux, 2004; Newpher and Ehlers, 2009). Modulation of the number of dendritic spines and/or their morphology has been proposed to contribute to alterations in excitatory synaptic transmission during learning (Lamprecht and LeDoux, 2004). Changes in number and shape of dendritic spines where observed following fear conditioning. For example, contextual fear conditioning leads to a time-dependent increase in dendritic spine density in the CA1 hippocampal region and the anterior cingulate cortex (Restivo et al., 2009; Vetere et al., 2011) and auditory fear conditioning leads to an increase in spinophilin-immunoreactive dendritic spines in the LA (Radley et al., 2006). Postsynaptic density (PSD) area on a smooth endoplasmic reticulum (sER)-free spines increases with fear conditioning while the spines head volume of these spines decreases (Ostroff et al., 2010).

Actin cytoskeleton is involved in neuronal morphogenesis in postsynaptic dendritic spines. The base, neck, and head of mature spine consist of a mixture of branched and linear actin filaments. The neck contains both linear and branched filaments, whereas branched actin filament network is a dominant feature of spine head (Korobova and Svitkina, 2010). The actin cytoskeleton is intimately involved in the formation and elimination, stability, motility, and morphology of dendritic spines (e.g., Halpain et al., 1998; Matus, 2000; Korkotian and Segal, 2001; Luo, 2002; Ethell

and Pasquale, 2005; Tada and Sheng, 2006; Schubert and Dotti, 2007; Honkura et al., 2008; Hotulainen and Hoogenraad, 2010). In addition, actin plays a role in stabilizing postsynaptic proteins (Allison et al., 1998; Kuriu et al., 2006; Renner et al., 2009) and in modulating spine head structure in response to synaptic signaling (Fischer et al., 2000; Star et al., 2002; Okamoto et al., 2004).

Alteration in axonal morphology is also implicated in memory formation and synaptic plasticity (Bailey and Kandel, 1993; Lamprecht and LeDoux, 2004). Actin polymerization mediates morphological changes involved in axonal growth, guidance, shape, collateral branching, branch retraction, and regeneration (Luo, 2002; Letourneau, 2009).

Additional research is warranted to elucidate whether actin is involved in neuronal morphogenesis seen in amygdala and hippocampus following fear memory formation and whether such changes are essential for memory formation. Some supporting evidence comes from studies showing that interference with actin regulatory proteins activity impairs fear memory formation and spine and axonal morphology (e.g., LIMK-1, Meng et al., 2002).

THE ROLES OF ACTIN CYTOSKELETON IN SYNAPTIC PLASTICITY

As mentioned above actin cytoskeleton plays key roles in modulating synaptic transmission and neuronal morphogenesis, cellular processes believed to underlie synaptic plasticity (e.g., Bailey and Kandel, 1993; Lamprecht and LeDoux, 2004). The role of actin cytoskeleton in synaptic plasticity was studied mainly by elucidating its involvement in LTP or LTD, physiological models of memory (e.g., Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Martin et al., 2000). Findings suggest that LTP occur in the LA and hippocampus during fear conditioning. LTP induction at thalamic auditory inputs to the LA enhances auditory-induced responses in the LA in a manner similar to the increase of CS-evoked responses observed during auditory fear conditioning (Rogan and LeDoux, 1995). Fear conditioning-altered auditory CS-evoked responses in LA changes in conjunction with conditioned fear responses (Rogan et al., 1997). Thalamic inputs or cortical inputs to the LA were enhanced in slices from trained animals compared to naive or unpaired animal groups (McKernan and Shinnick-Gallagher, 1997). Moreover, fear conditioning inhibits the induction of LTP at cortical inputs suggesting that LA synapses that have already undergone LTP by training are less capable of showing additional LTP (Tsvetkov et al., 2002; Schroeder and Shinnick-Gallagher, 2004; and Schroeder and Shinnick-Gallagher, 2005). It was shown that contextual fear conditioning increased synaptic response in hippocampal CA1 (e.g., Sacchetti et al., 2001) and that contextual fear conditioning modified the ability to induce LTP in hippocampus (Sacchetti et al., 2002).

To study the roles of actin in LTP Okamoto et al. (2004) used the fluorescence resonance energy transfer (FRET) technique to show that in rat hippocampal dendritic spines LTP induction led to persistent shift of F-actin/G-actin equilibrium toward F-actin within seconds of a tetanic stimulus. In the dentate gyrus, LTP increased F-actin content in dendritic spines lasting up to 5 weeks (Fukazawa et al., 2003). The increase in F-actin correlates with a stable increase in the size of the spine head and inhibition of actin polymerization impaired LTP-induced spine head enlargement (Matsuzaki et al.,

2004; Okamoto et al., 2004; Fortin et al., 2010). LTP also induces changes in axonal morphology and actin cytoskeleton leading to formation of new axonal varicosities and new axonal actin puncta (Colicos et al., 2001; De Paola et al., 2003). The new presynaptic actin puncta become associated with recycling synaptic vesicle pool (Colicos et al., 2001). Long-term facilitation induced the growth of new synapses and presynaptic actin remodeling in *Aplysia* mechanosensory neurons (Hatada et al., 2000). In addition, cytochalasin D, an actin polymerization inhibitor, selectively blocks long-term but not short-term facilitation (Udo et al., 2005).

Actin cytoskeleton is needed for synaptic plasticity in brain areas mediating fear memory formation such as amygdala and hippocampus (LeDoux, 2000; Davis and Whalen, 2001; Schafe et al., 2001; Sah et al., 2003; Rodrigues et al., 2004; Maren, 2005). In LA, 5-HT-induced L-LTP is blocked by the actin inhibitor cytochalasin D (Huang and Kandel, 2007). Furthermore, LTP in interneurons in LA is maintained by trafficking of GluR2-lacking AMPA receptors that require an interaction with SAP97 and the actin cytoskeleton (Polepalli et al., 2010). Inhibition of actin polymerization in hippocampus or disruption of F-actin lead to impairment of LTP formation and facilitation (e.g., Kim and Lisman, 1999; Krucker et al., 2000; Fukazawa et al., 2003; Kramár et al., 2009). In addition, inhibition of actin polymerization affects protein synthesis-independent early LTP, prevents late-LTP, and interferes with synaptic tagging in apical dendrites of hippocampal CA1 (Ramachandran and Frey, 2009). Furthermore, chemical forms of LTP in dissociated hippocampal cultures forms GluR1 and synaptophysin puncta and these cellular and molecular events require actin polymerization (Antonova et al., 2001).

Actin cytoskeleton is also involved in LTD which in many instances induces opposite synaptic, morphological, and molecular events compared to LTP (e.g., Zhou et al., 2004). LTD induces shifts the F-actin/G-actin equilibrium toward G-actin and decreases spine head volume with the disappearance of some spines (Okamoto et al., 2004). Furthermore, LTD-inducing paradigm has stabilizing effects on actin (Star et al., 2002).

Cumulatively, the aforementioned studies show that actin cytoskeleton serves as regulator of synaptic plasticity possibly by affecting synaptic morphology and transmission and thereby tuning synaptic strength. Furthermore, actin cytoskeleton is intimately involved in synaptic plasticity in amygdala and hippocampus areas that mediate fear memory formation. Further studies are needed to elucidate whether actin cytoskeleton is needed for LTP of synapses in the amygdala following fear conditioning and how it can affect plasticity.

FUTURE RESEARCH

Much evidence indicates that the actin cytoskeleton and its regulatory proteins are involved in fear memory formation. However, key questions remain unresolved. For example, are the morphological changes shown to be mediated by actin cytoskeleton needed for fear memory formation? Such changes may include alteration of spines and axonal morphology. Does actin cytoskeleton regulate changes in synaptic transmission needed for fear conditioning memory formation? If so, are they related to presynaptic (changes in vesicle release) or postsynaptic (changes in receptor trafficking) alterations or to both? Studies aimed to elucidate such questions will undoubtedly provide key insights into the roles of actin cytoskeleton in fear memory and also on the cellular processes essential for fear memory formation and greatly contribute to a better understanding of the intricate molecular and cellular processes governing fear memory formation.

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