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Review

Arc ubiquitination in synaptic plasticity

Angela M. Mabb[a,*], Michael D. Ehlers[b,*]

[a] Neuroscience Institute, Georgia State University, 100 Piedmont Ave., Atlanta, GA 30302, United States
[b] Biogen, Cambridge, MA 02142, United States

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ABSTRACT

The activity-regulated cytoskeleton-associated protein (Arc) is a neuron-expressed activity regulated immediate early gene (IEG) product that is essential for memory consolidation and serves as a direct readout for neural activation during learning. Arc contributes to diverse forms of synaptic plasticity mediated by the trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. Notably, Arc protein expression abruptly increases and then rapidly decreases following augmented network activity. A large body of work has focused on Arc transcription and translation. Far fewer studies have explored the relevance of Arc protein stability and turnover. Here, we review recent findings on the mechanisms controlling Arc degradation and discuss its contributions to AMPA receptor trafficking and synaptic plasticity.

Contents

1. Introduction

In neurons, as in all eukaryotic cells, a major mechanism for protein degradation is via the 26S proteasome [1]. A prerequisite for proteasome-dependent degradation is covalent attachment of the 76-amino acid ubiquitin to a lysine residue of the target protein. In most cases, the attachment of ubiquitin requires an enzymatic cascade consisting of a ubiquitin activating enzyme (E1), ubiquitin conjugating enzyme (E2), and a ubiquitin ligase (E3), where the E3 ligase confers substrate selectivity [1–3] (Fig. 1). Ubiquitination regulates synapse composition [4], and disruption of protein turnover modulates numerous forms of synaptic plasticity, such as long-term depression (LTD) and long-term potentiation (LTP), synaptic changes which are linked to learning-related behaviors [5–7].

It is well established that many forms of learning and memory require the synthesis and breakdown of brain-expressed proteins [8]. Learning-related activation of neuronal ensembles triggers the transcription and translation of immediate early genes (IEGs) [9,10], whose protein products exert biological effects for durations that are poorly understood, and likely dependent on protein half-life. Initial insights into how the breakdown of proteins altered learning-related behaviors were described in *Aplysia*, where a loss of the regulatory subunit (R) of cAMP-dependent protein kinase (PKA) during long term facilitation of the sensory motor circuit was found to be dependent on the breakdown or turnover of proteins [8,11–13]. Subsequent studies in mice and rats showed that inhibition of protein turnover via administration of proteasome

* Corresponding authors.
E-mail addresses: amabb@gsu.edu (A.M. Mabb), michael.ehlers@biogen.com (M.D. Ehlers).

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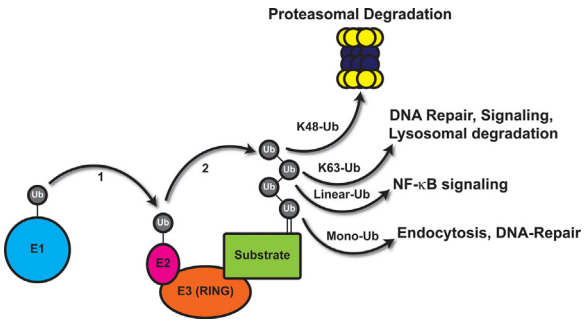

Fig. 1. Protein ubiquitination cascade. An ATP-driven reaction conjugates ubiquitin to a ubiquitin activating enzyme (E1). The E1 then transfers ubiquitin (1) to a ubiquitin conjugating enzyme (E2). The E2 coordinates with a ubiquitin ligase (E3) to covalently attach ubiquitin (2) to a lysine residue to the target substrate. Substrate ubiquitination selectivity and diversity is accomplished with the abundance of E2s (~30–50) and a multitude of E3s (~500–600). Ubiquitination is a reversible process as it can be removed by deubiquitinating enzymes (DUBs) (~100). Shown is an example of ubiquitination of a substrate with a RING-domain containing E3.

inhibitors prevents fear-conditioned memory extinction [14], and disrupts long-term recognition memory consolidation [15], long-term spatial memory consolidation and reconsolidation [16], and formation of context-dependent fear memories in the amygdala [17].

The activity-regulated cytoskeleton-associated protein (Arc) encodes a neuron-specific activity regulated IEG that was originally described as being rapidly induced following growth factor stimulation and electrically-induced seizures [18]. For a more thorough description of Arc induction and function, please refer to other reviews in this journal issue. The induction of Arc mRNA is known to be transient, as it dissipates within hours upon learning and administration of drugs of abuse [18–25]. Although early studies focused predominantly on Arc mRNA expression, the development of viable Arc-specific antibodies allowed researchers to correlate mRNA induction patterns to Arc protein levels [18,26–28]. As expected, the rise and fall of Arc mRNA was strongly correlated with an increase and decrease in Arc protein. Following diverse behaviors, Arc protein is elevated and then rapidly declines [19,26,29–32]. These findings indicated that a coupling of Arc induction and turnover are related to behaviors in a variety of neural circuits, and suggested that disruption of these dynamics likely results in perturbation of normal brain functions, particularly learning.

Although Arc transcription and translation have been well-studied [33], the contribution of Arc degradation to synaptic plasticity and behaviors has received much less attention until recently. Here, we will focus on current findings related to ubiquitin-dependent turnover of Arc and its contributions to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking and synaptic plasticity.

2. Arc-dependent regulation of synaptic plasticity and AMPAR trafficking

Given the observed induction of Arc following a variety of manipulations, Arc is thought to serve as a master regulator of synaptic plasticity [34] (Fig. 2). This is due in part to the ability of Arc to regulate the endocytosis of synaptic AMPA receptors [35–37]. Arc is localized to many subcellular compartments and associates with endosomes [35,38]. Arc interactions with endosome-associated proteins endophilin-2/3, dynamin 2, and recently AP-2 (Fig. 3) have been described to mediate the trafficking of AMPA receptors [35,37,39], a critical driver of multiple forms of synaptic plasticity [40].

Early studies hinted as to how an increase in Arc protein expression might alter plasticity. For instance, activation of type I metabotropic glutamate receptors in CA1 hippocampal neurons with DHPG leads to long term depression (mGluR-LTD) that is coupled to increases in *Arc* translation and a persistent decrease in postsynaptic AMPA receptors [36,41,42]. mGluR-LTD is also controlled by fragile X mental retardation protein (FMRP) [43], an RNA-binding protein that is a negative regulator of dendritic *Arc* translation [44]. *Fmr1* KO mice have enhanced mGluR-LTD that is accompanied with an increase in Arc protein [36,41,45], indicating that abnormal increases in Arc translation might lead to sustained mGluR-LTD. These studies examined Arc overproduction at the level of translation, and whether similar or distinct mechanisms change Arc levels by regulating protein turnover has yet to be established.

Arc translation is also regulated by Src-associated substrate during mitosis of 68 kDa (Sam68), an RNA-binding protein that was found to associate with *Arc* mRNA [46]. Intriguingly, Sam68 KO mice have impaired mGluR-dependent Arc translation and decreases in DHPG-induced mGluR-LTD. Application of the proteasome inhibitor, MG132, to Sam68 KO mice restored DHPG-induced Arc protein levels, mGluR-LTD, and GluA1 endocytosis [47]. Notably, infusion of an *Arc* antisense oligonucleotide in CA1 pyramidal neurons blocked MG132-mediated rescue of DHPG LTD, indicating that Arc is required for DHPG LTD maintenance and altering its basal turnover rates can restore DHPG-induced mGluR-LTD deficits. Under normal conditions, application of DHPG can facilitate short-term synaptic depression, which occurs following subsequent applications of subthreshold levels of DHPG [47]. However, the presence of MG132 following a subthreshold treatment of DHPG-induced synaptic depression turned into LTD following a second subthreshold treatment of DHPG [47]. These results are similar to findings in hippocampal CA1 neurons expressing high levels of Arc following exposure to a novel environment. Arc positive cells were shown to have prolonged and persistent protein synthesis-dependent LTD compared to Arc negative cells [48]. Taken together, these findings indicate that protein-synthesis driven forms of LTD require a coupling of Arc translation and degradation, and that controlling Arc ubiquitination processes may mediate metaplasticity.

Another function of Arc has been described in the regulation of homeostatic synaptic scaling of AMPA receptors, a phenomenon that is critical for re-establishing ocular dominance plasticity after monocular deprivation in the visual cortex [49]. High levels of Arc are correlated with decreases in surface AMPA receptors [50]. Notably, homeostatic upscaling of AMPA receptors induced by the Na^+ channel blocker, tetrodotoxin (TTX) is blunted following overexpression or reduction of an E3 ubiquitin ligase Triad3A/RNF216, possibly due to a dysregulation of Arc [51]. Recently, Arc protein levels were found to decrease into adulthood yet Arc overexpression was able to extend the critical period in the visual cortex, which led to continued expression of juvenile-like LTD [52]. A putative prediction is that disrupting ubiquitin-mediated turnover of Arc might lead to an extended critical period in the visual cortex.

3. Distinct mechanisms mediating arc ubiquitination

3.1. *Mechanisms mediating* Arc *turnover*

Proteasome-dependent turnover of Arc was originally observed in mouse primary cortical neuron cultures, where Arc protein levels were induced with brain-derived neurotrophic factor (BDNF) [53]. Upon BDNF stimulation, co-application of the protein synthesis inhibitor cyclohexamide resulted in a rapid decay of Arc protein. Conversely, co-application of the proteasome inhibitor, MG132, resulted in a sustained and augmented increase in Arc levels compared to BDNF stimulation alone. These findings indicated that, upon BDNF stimulation, Arc is rapidly degraded by the

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