Review

Arc in synaptic plasticity: from gene to behavior

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The activity-regulated cytoskeletal (*Arc*) gene encodes a protein that is critical for memory consolidation. Arc is one of the most tightly regulated molecules known: neuronal activity controls *Arc* mRNA induction, trafficking and accumulation, and Arc protein production, localization and stability. Arc regulates synaptic strength through multiple mechanisms and is involved in essentially every known form of synaptic plasticity. It also mediates memory formation and is implicated in multiple neurological diseases. In this review, we will discuss how Arc is regulated and used as a tool to study neuronal activity. We will also attempt to clarify how its molecular functions correspond to its requirement in various forms of plasticity, discuss Arc's role in behavior and disease, and highlight critical unresolved questions.

Introduction

The molecular basis of learning and memory involves modifying neuronal synapses in response to electrical activity, a process termed synaptic plasticity. Memory formation has been divided into two temporal phases. Short-term memory formation involves changes to synaptic efficacy by modifying existing proteins. Long-term memory formation requires new gene transcription and protein production to stabilize recent changes. These long-term changes to synaptic strength take several forms. In long-term potentiation (LTP), specific synapses are strengthened. In long-term depression (LTD), specific synapses are weakened. In homeostatic plasticity, neuron-wide shifts in responsiveness maintain the maximal sensitivity of the neuron to future activity-dependent synaptic plasticity.

Arc is specifically required for long-term memory formation and affects all of these forms of synaptic plasticity. *Arc*, also known as *Arg3.1*, is found only in vertebrates but is highly conserved in this group [1,2]. Glutamatergic neurons in the brain express Arc in response to an increase in synaptic activity in a range of behavioral and learning paradigms [3–6]. Localization and stability of the transcript and protein are also highly regulated. Arc protein is not found in presynaptic terminals or axons but is highly expressed in dendrites [7,8], the postsynaptic density [7,9,10] and the nucleus [11,12]. Arc regulates endocytosis of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptors (AMPARs) [13,14], Notch signaling [15], and spine size and type [16].

These distinct actions all modify synaptic strength. Removal of *Arc* in knockout (KO) animals results in an unusual phenotype: short-term learning is normal but lasting memories cannot be formed [17]. Arc, therefore, provides a means to understand the cellular processes of memory consolidation. However, Arc has multiple functions and responds differently to different stimuli and signaling pathways. Although much is known about how Arc is regulated and affects synaptic strength, defining a coherent mechanism for its role in synaptic plasticity and memory consolidation has proven difficult. Recent reviews eloquently described known mechanisms of Arc regulation and function in plasticity [18–24]. Here, we will summarize and update these findings. In addition, we will discuss how Arc's functions might contribute to its effects on synaptic plasticity and its role in disease.

Regulation of Arc transcription

In 1995, two labs independently identified *Arc* as a gene induced by seizures in the hippocampus [1,2]. Arc expression is also induced by the increased neuronal activity that occurs in response to learning [4,25], brain-derived neurotrophic factor (BDNF) [26,27], LTP [7,28], LTD [29,30] and other stimuli. Arc transcripts appear within 5 min of stimulation [4], which makes Arc a 'rapid' immediate early gene. Such genes have transcriptional machinery poised just downstream of the start site, allowing the fastest possible transcriptional activation in response to neuronal activity [31].

The signaling cascades that connect a change in activity to *Arc* transcription are complex and not fully understood. *Arc* is transcribed at a low level under basal conditions and can be further decreased by activating AMPARs [32]. *Arc* transcription is dramatically upregulated by activating the BDNF TrkB receptor [26,27], group 1 metabotropic glutamate receptors (mGluR1s) [29,30,33], muscarinic acetylcholine receptors [34] and NMDA receptors (NMDARs) [35] (Figure 1). The extracellular-signal-regulated kinase (ERK) is a central node of the signaling pathways downstream of these receptors and is required for increases in *Arc* transcription. Once activated, ERK phosphorylates a coactivator of the serum response factor (SRF), such as Elk-1, a ternary complex factor (TCF). This complex binds serum response elements (SREs) in promoter regions to activate transcription [36]. The major SRE-responsive region in *Arc*'s promoter is 6.5 kb upstream of the *Arc* coding sequence [27,37] (Figure 1). Although the sequence

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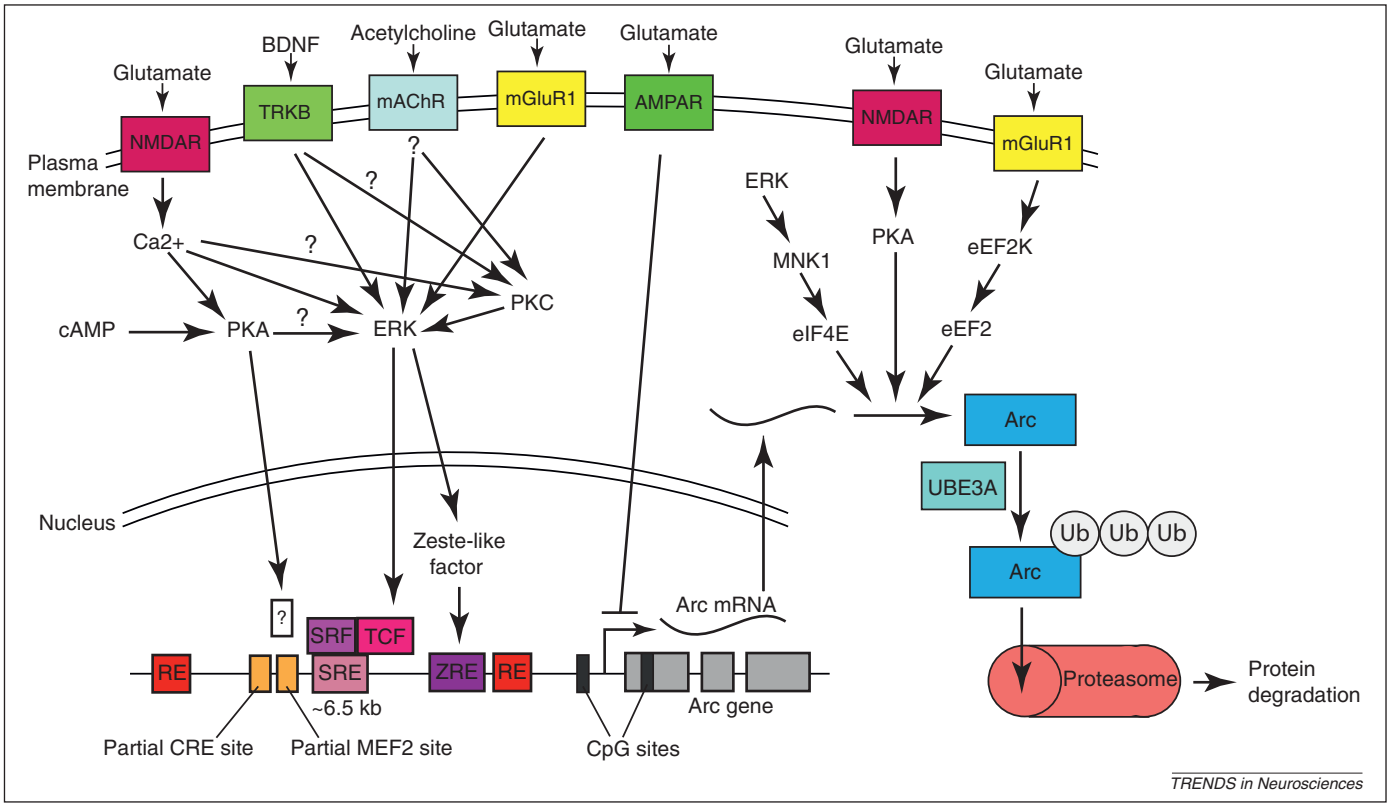

Figure 1. Regulation of Arc expression. Signaling through NMDARs [35], TrkB receptors [26,27] and group 1 metabotropic glutamate receptors (mGluRs) [29,30] promotes *Arc* transcription through one or several downstream signaling kinases, including protein kinase A (PKA) and extracellular-signal-regulated kinase (ERK). ERK acts through a coactivator, such as a ternary complex factor (TCF), to activate serum response factor (SRF), which binds a serum response element (SRE) in the Arc promoter to increase transcription [27,37]. ERK also acts through a Zeste-like factor, which binds a Zeste-like response element (ZRE) [27]. PKA acts near the SRF site through an unidentified enhancer element. The region surrounding the *Arc* gene also contains partial cAMP response element (CRE) and myocyte enhancer factor 2 (MEF2) sites [37] and CpG sites that mediate epigenetic modifications of *Arc* transcription [83]. Response elements (REs) in the promoter of *Arc* [27] and signaling pathways induced after AMPAR activation [32] inhibit transcription. ERK signaling and signaling pathways activated by NMDARs [47] and mGluR1s [30] promote *Arc* translation. Arc protein is ubiquitinated by ubiquitin-protein ligase E3A (UBE3A) [52], which targets it to the proteasome for degradation. Question marks indicate relationships for which the supporting evidence is limited or indirect, or has not been specifically linked to Arc expression. Abbreviations: cAMP, cyclic adenosine monophosphate; mAChR, muscarinic acetylcholine receptor; TrkB, tropomycin-receptor-kinase; mGluR1, metabotropic glutamate receptor; PKA, protein kinase A; PKC, protein kinase C; RE, repressor element; ZRE, Zeste-like response element; SRE, serum response element; MNK1, mitogen-activated protein kinase-interacting kinase 1; eIF4E, eukaryotic initiation factor 4E; eEF2K, eukaryotic elongation factor 2 kinase; eEF2, eukaryotic elongation factor 2; UBE3A, ubiquitin-protein ligase E3A; Ub, ubiquitin.

surrounding this SRE has a TCF binding region [37], this does not exclude activation of the Arc SRE by MAL. MAL is another SRE coactivator regulated by actin, and TCF or MAL binding to a given SRE is difficult to predict based on DNA sequence alone [38].

Additional sites have not been fully characterized but are also important in mediating the response of the *Arc* promoter to neuronal activity. A region 1.4 kb upstream of the start site contains a Zeste-like response element (ZRE). Its cognate transcription factors have not been identified, but it is required for full activity-induced transcription [27]. The region near the SRE response site also contains a site that could bind myocyte-enhancer factor-2, a partial cAMP response element (CRE) site [37], and an unidentified protein kinase A (PKA)-responsive region [27,39]. Finally, other sites repress Arc transcription, including a site 9 kb upstream of the start site and a region near the ZRE [27] (Figure 1). Although we do not fully understand the crosstalk between the many receptors, signaling pathways and promoter regions that contribute to *Arc* transcription, Arc clearly provides a fascinating means of understanding a neuron's response to different forms of synaptic activity.

Regulation of *Arc* mRNA

Once *Arc* mRNA is transcribed, it undergoes further regulation before being translated. At 30 min after stimulation, *Arc* mRNA is exported from the nucleus to the cytoplasm and, by 1 h, can travel to the most distal tips of dendrites [32,40]. The speed of *Arc* mRNA movement (up to 65 μm/min [41]) and its localization to the kinesin motor complex [42] imply active transport. Other components of the *Arc* messenger ribonucleoprotein complex (mRNP) include fragile X mental retardation protein (FMRP) and Pur-alpha, both of which suppress translation [42,43]. An 11-nucleotide A2 response element (A2RE) within the Arc coding region and other dendritically targeted mRNAs, such as Ca^{2+}/Calmodulin-dependent kinase II alpha (CaMKIIα), is a binding site for the A2 mRNP [44]. CArG box binding factor A (CBF-A) also controls localization of A2RE containing transcripts, including Arc and CaMKIIα, through a mechanism regulated by NMDARs and AMPARs [45]. However, because of the unique regulation of Arc mRNA localization, A2RE is likely to be only one of several elements necessary for targeting Arc mRNA.

When Arc was strongly induced (e.g. by a seizure), subsequent laminar stimulation of the perforant pathway

resulted in accumulation of Arc mRNA in the corresponding region of synaptic innervation on dentate granule cells [46]. The mechanism controlling this targeting has not been fully elucidated but appears to require signaling through NMDARs, AMPARs [35] and mitogen-activated protein (MAP) kinase kinase, which phosphorylates ERK [47]. Stabilization of F-actin by Rho kinase is also required indicating that reorganization of the actin cytoskeleton is involved in tagging synapses for *Arc* mRNA localization [47]. However, it is unclear whether *Arc* transcripts are truly targeted to specific synapses or are prevented from continued transport after reaching active synapses, or are simply selectively stabilized at active synapses. Also, it is not clear if this mechanism is relevant only when high-intensity stimuli lead to the production of exceptionally large amounts of *Arc* transcript.

The *Arc* transcript has a half-life of just 47 min [32], presumably owing to its association with the eukaryotic initiation factor eIF4AIII. This core exon junction complex component targets mRNAs for nonsense-mediated decay [48], limiting the amount of protein from one transcript. However, the small amount of transcript available in dendrites under basal conditions is sufficient to mediate at least some forms of plasticity [29]. Although we are clearly far from a complete understanding of the expression pattern of Arc mRNA after different stimuli, the high degree of regulation of the transcription, localization and stability of the mRNA indicates the extreme importance of Arc and calls for further investigation of these mechanisms.

Regulation of Arc protein production and clearance

Similar to *Arc* transcription, Arc translation also seems to be highly controlled by activity levels and specific signaling cascades. Stimulation of NMDARs and Gs-coupled receptors in cultured neurons increases Arc translation in a manner dependent on PKA [49]. LTP-induced *Arc* translation *in vivo* requires ERK signaling through MAP kinase-interacting kinase (MNK), which phosphorylates eIF4E [50]. mGluR-LTD can result in immediate translation of pre-existing mRNA in a manner dependent on eukaryotic elongation factor 2 (eEF2) phosphorylation [30] (Figure 1). Although it is not clear where within a neuron the bulk of Arc translation occurs, at least some is likely to occur in or around spines [51].

Once translated, Arc stability is probably controlled by a PEST sequence that could target it to the proteasome [32] and by a binding region for the ubiquitin-protein ligase E3A (UBE3A) [52]. Six hours after stimulation, UBE3A is synthesized and ubiquitinates Arc, resulting in its degradation (Figure 1). This provides a mechanism by which Arc protein levels can be returned to baseline after prolonged activity.

Regulation of Arc by behavior

Behavioral tasks that induce Arc expression range from a simple sound exposure [53] to complex tasks such as reversal learning in a T-maze [5]. The tight link between synaptic activity and *Arc* transcription led to widespread use of *Arc* RNA as a means of determining what neurons are activated in response to various learning paradigms. *Arc* RNA appears in the nucleus within a few minutes of neuronal activation and is predominately localized to the cytoplasm by 30 min. *In situ* hybridization, therefore, reveals the history of the activity of individual neurons as well as the network of neurons activated during the formation of a new memory. When applied to CA1 neurons, this method is sensitive enough to distinguish between populations of neurons that responded to two different environments and can reveal whether the same neuron was activated twice [40].

The link between Arc expression and neuronal activity levels also allows levels of *Arc* mRNA to be used to gain a better understanding of where and when activity in response to learning is increased. Some evidence points to a second wave of Arc induction hours after the degradation of the first wave of Arc [4,25], which may indicate the reactivation of circuits during consolidation. The levels and persistence of Arc induction appear to differ depending on the brain region and cell type. For example, different regions of the hippocampus (e.g. CA3 and CA1 [54,55]), and even varying cell types within a brain region (e.g. striatopallidal vs. striatonigral neurons within the striatum [5]), showed different Arc induction in response to behavioral tasks. These differences might provide information about the levels of activity in these cells/regions and how and when they function in learning and memory. Furthermore, knockin mice in which green fluorescence protein (GFP) is expressed in place of the Arc protein allow for *in vivo* imaging of neurons activated by a visual stimulus over successive trials [56]. Imaging of Arc expression in behaving animals has also been attempted in transgenic mice expressing luciferase enzyme under the control of the *Arc* promoter [57]. The remarkable correlation between *Arc* induction and the behaviors that induce learning raises the intriguing possibility that *Arc* transcription is, to some extent, tuned to the signals generated by patterns of neuronal activity that produce synaptic plasticity.

Arc function in structural plasticity

Synaptic plasticity takes several forms, including modification of synapse structure and strength. Synaptic structure is modified in neurons in response to changes in neuronal activity, and stabilization of spines is associated with long-term memory formation [58]. The original observations that Arc cofractionates with actin [1] and that changes in actin cytoskeleton are required for changes in spine structure [59] led to an investigation of the role Arc has in structural plasticity. In hippocampal neurons, Arc overexpression increases spine density *in vitro*, and disruption of Arc decreases spine density *in vivo* [16]. Although these effects were modest, Arc had impressive effects on the distribution of spine morphology in this study. Arc specifically increased the proportion of thin spines, so-called learning spines that are more plastic, whereas it decreased the proportion of more stable stubby spines [16]. This shift suggests Arc is required for modifying the cellular response to activity and thereby important for forming new memories or forgetting old ones. Arc also may interact with Wave3 (C. Peebles *et al.*, 2008, Society for Neuroscience abstract, 763.20), a regulator of actin nucleation that could underlie these changes in synaptic structure (Figure 2).

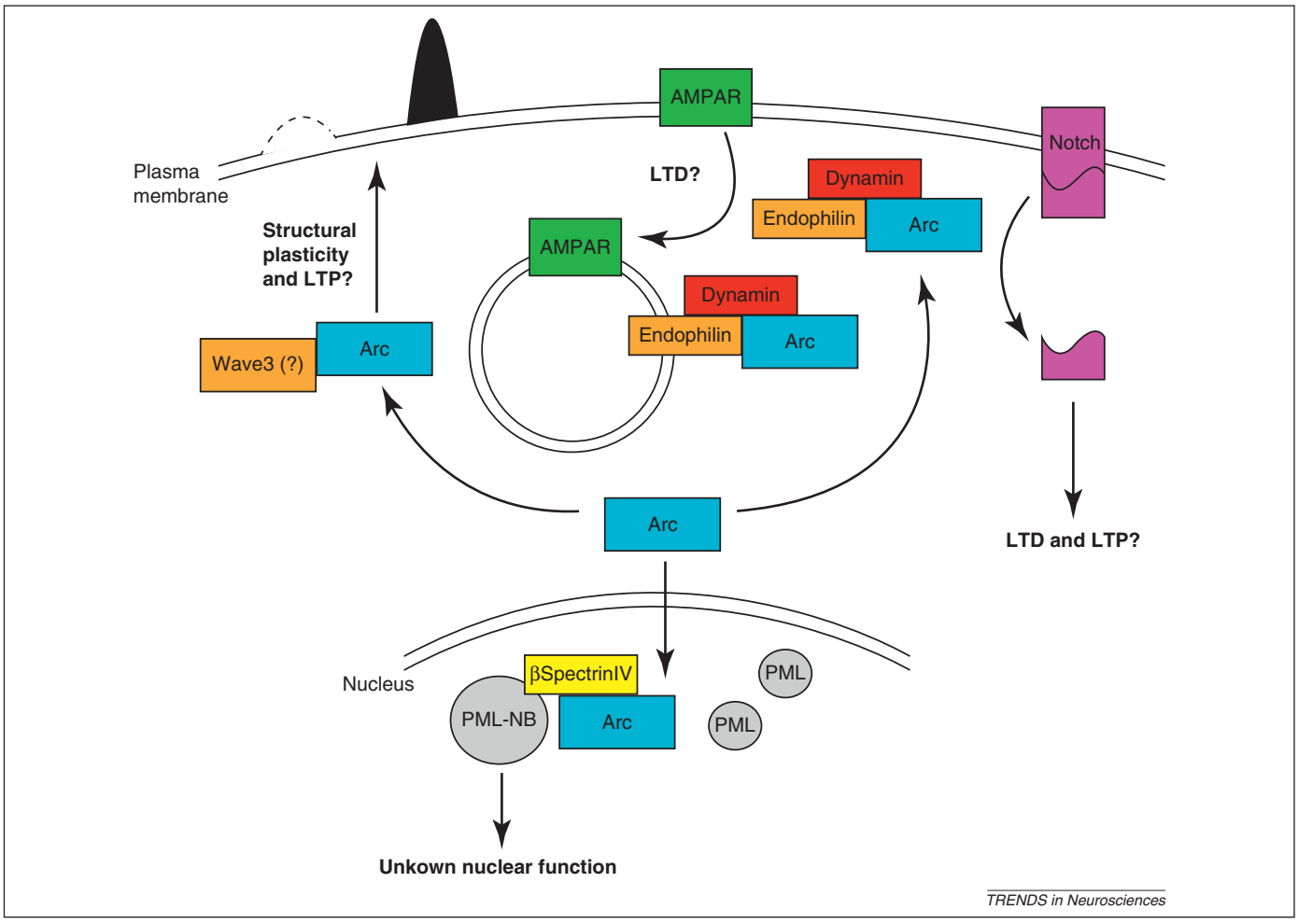

Figure 2. Cellular functions of Arc. Interactions with other proteins might mediate Arc's function in long-term potentiation (LTP), long-term depression (LTD) and structural plasticity. Arc may interact with Wave3 (C. Peebles *et al.*, 2008, Society for Neuroscience abstract, 763.20), which could allow it to regulate spine morphology, increasing the proportion of thin spines and decreasing stubby spines [16]. Arc is known to form a complex with endophilin and dynamin that increases the rate of endocytosis of AMPARs [13,14], possibly mediating Arc's affects on LTD. The Arc and dynamin complex cleaves the Notch receptor to activate Notch signaling pathways [15], which might be involved in synaptic depression and potentiation. Arc protein is also present in the nucleus where it promotes the formation of promyelocytic leukemia nuclear bodies (PML-NBs) through βSpectrinIV [11], which have unknown functions in neurons.

Arc function in Hebbian plasticity

In addition to modifying synaptic structure, Arc also regulates synaptic strength. Knockdown and KO studies show that Arc is required for the late phase of LTP. *Arc* KO mice show a steady decline of potentiation after an initial enhanced response to high frequency stimulation (HFS) [17]. The initial enhancement is not seen with acute manipulations of Arc levels, therefore it is most probably a result of some developmental compensation in response to a lifelong deficit in Arc expression. However, the loss of the late stage of LTP is also seen with short-term knockdown of Arc levels. When Arc antisense oligodeoxynucleotides (ODNs) were infused into the hippocampus of rats 1.5 h before LTP induction, the maintenance phase of LTP, but not early-phase LTP, was lost [60]. Similarly, pretreatment with Arc antisense ODNs caused a permanent loss of late-phase potentiation when infused 2 h after stimulation and completely abolished a form of LTP induced by BDNF [61]. Interestingly, Arc antisense ODNs also caused a transient decrease in early-phase LTP when infused shortly before or after HFS [61]. However, the effects of Arc ODNs in early LTP differ greatly between studies [17,60,61]. These discrepancies may be due to differences in the timing and degree of knockdown because neurons are highly sensitive to levels and timing of Arc expression. Taken together, these studies suggest Arc may play a role in early-phase LTP and strongly support a vital role for Arc in late-phase LTP.

The molecular mechanisms underlying Arc function in LTP could include Arc regulation of cytoskeleton dynamics and spine morphology or Arc function in other signaling cascades. LTP results in actin polymerization and stabilization and increases in spine number [59,62]. Arc localizes to the actin cytoskeleton, and Arc overexpression increases spine number [16]. Interestingly, LTP causes an increase in synapse number on thin spines [63], and Arc specifically promotes thin spine formation [16], as discussed above. In addition, the loss of LTP maintenance in response to *Arc* knockdown can be prevented by adding an actin-stabilizing drug, Jasplakinolide [61]. Yet, Arc has not yet been definitively shown to regulate actin polymerization in response to HFS, and Arc may affect LTP through other signaling cascades. Arc co-immunoprecipitates with, and activates, Notch1 [15], a receptor that is important in regulating

morphology and synaptic plasticity in mature neurons, in addition to its roles in development. Arc appears to promote the cleavage and activation of Notch in response to activity [15] (Figure 2). How this occurs is not yet known, but disruption of Notch1 signaling impairs both LTP and LTD in hippocampal slices [15].

In addition to its crucial function in LTP, Arc is also essential for LTD. In *Arc* KO slices, low frequency stimulation of CA1 pyramidal neurons induced only a short-lasting and smaller LTD than was observed in wild-type controls [17]. In CA1 neurons, Arc is also required for LTD mediated by mGluR1s. Interestingly, this form of LTD does not require new *Arc* transcription but relies on translation of pre-existing dendritic *Arc* mRNA [29]. This translation is regulated by the Ca^{2+}/Calmodulin-dependent eEF2K and FMRP [30]. Arc is also required for LTD in a different system in which LTD is induced in cultured Purkinje neurons with paired glutamate and depolarization [64]. This form of LTD is mediated by transcription that specifically requires the SRE site on the Arc promoter. The differing requirements for transcription of Arc in LTD in these two systems could potentially be the result of the different stimulations used to induce LTD, or the different cell types used, because mechanisms behind Hebbian plasticity can differ between cell types.

Arc might mediate LTD by regulating AMPAR endocytosis. In fact, mGluR-induced LTD requires Arc for endocytosis of AMPARs [29]. Arc expression decreases the amplitude of AMPAR-mediated synaptic currents by acting through endophilin 1 and dynamin 2 to promote AMPAR endocytosis [13,14] (Figure 2). It is unclear if Arc acts on the GluA1 or GluA2 subunits of AMPARs. It is also possible that both subunits are regulated by Arc, and discrepancies between studies could be due to the differing sensitivities of the techniques used [13,14]. Exactly how Arc regulates the endocytic machinery is unknown, and no direct evidence shows that low frequency stimulation causes Arc to localize to endophilin and dynamin. To fully explain how Arc can mediate both LTP and LTD, additional research is needed to better understand the cellular functions of Arc and how they are regulated by different stimuli.

Arc function in homeostatic plasticity

LTP and LTD are self-reinforcing mechanisms and might push synaptic strength to one extreme or another if left unchecked. This can reduce the capacity of the system to respond to additional changes in activity and might also make the network unstable, leading to conditions such as epilepsy. To prevent such extremes, a neuron responds to long-term increases or decreases in activity by scaling its response to activity down or up, respectively, to maintain the same average firing rate. This cell-wide form of plasticity, termed homeostatic scaling, is highly sensitive to levels of Arc. Normal scaling responses to manipulations of neuronal activity *in vitro* are lost in *Arc* KO neurons, as well as in neurons overexpressing Arc [65]. Arc is also necessary for a more synapse-specific form of homeostasis, in which the strength of a synapse is increased in response to a reduction in the firing rate of the presynaptic neuron [66].

It is not yet clear how Arc regulates these cell-wide responses to long-term changes in activity that oppose the changes seen in LTP and LTD. Although increased AMPAR endocytosis might explain a homeostatic response to increased activity, Arc appears to be required for both down- and up-scaling [65]. Even more ambiguous is how Arc mediates the lasting response to short-term stimuli that results in LTP or LTD and, concurrently or sequentially, responds to long-term tonic changes in activity through effects on scaling. Given the difficulty of reconciling the disparate data on Arc's role in plasticity, Arc has been proposed to subserve more than one function, depending on its location in the cell and its interaction partners [18].

One reason to suspect that Arc has additional yet-to-be discovered functions is that, in addition to being localized to dendrites, Arc is also enriched in the nucleus [11,12]. Arc interacts with the nuclear matrix protein βspectrinIVΣ5 to promote the formation of promyelocytic leukemia nuclear bodies (PML-NBs) [11]. PML-NBs are involved in brain development [67] and regulate many nuclear functions, such as transcription and mRNA export [68,69]. Changes in these nuclear functions would have cell-wide effects, making this an attractive mechanism for mediating functions such as homeostasis. However, the function of PML-NBs varies widely between cell types and has not been investigated in neurons, so it is not yet known whether modifying PML-NB formation affects synaptic plasticity. Additionally, the molecular mass of Arc is near the size cut-off for passive diffusion into the nucleus through the nuclear pore complex, so it is not clear if the high level of Arc nuclear localization is due to active transport or simple diffusion and nuclear retention. Clearly, more research is needed to determine exactly how Arc functions in LTP, LTD and scaling and how the protein is localized to different cellular regions to mediate these effects.

Arc function in behavior

Arc's importance in the late phases of LTP and LTD indicates that it is likely to have an important functional role in late phases of learning and memory. Interestingly, *Arc* KO mice learn new behavioral tasks in a similar manner to control mice, but cannot consolidate new memories [17]. For example, at 10 min after exposure to an object, KO and wild type mice have achieved the same preferences for a novel object over the original one, indicating that KO mice can learn and remember within this short time frame. However, at 24 h, wild type mice still preferred a novel object, but KO mice did not. These mice also have disrupted memory consolidation during spatial learning, fear conditioning, and conditioned taste aversion [17] (Table 1). Consolidation can also be disrupted by infusions of *Arc* antisense ODNs given before or after training [60,70–72], which strongly supports the argument that the memory deficits observed in the KO mice are specific to a role for Arc in the mature hippocampus, and are not due to developmental deficits. Even after the period of consolidation, Arc antisense ODNs given during reactivation of a fear memory actually result in loss of the memory [73], demonstrating the extreme sensitivity of memory to Arc expression.

Table 1. Arc functional roles in the brain and in neurological diseases[a]

Function/disease	Model system	Technique/behavioral task	Refs
Memory consolidation	Arc KO mice, knockdown in rats in hippocampus, amygdala or anterior cingulate cortex	Spatial learning, fear conditioning, taste aversion, object recognition, contextual inhibitory avoidance task	[17,60,70,72]
Reconsolidation of memory	Knockdown in lateral nucleus of amygdala	Reactivation of memory in a Pavlovian fear conditioning test	[73]
Response to visual experience or deprivation	Arc KO mice	Ocular dominance plasticity, stimulus-selective potentiation, visual cortex development, orientation specificity, average spike tuning	[56,74,75]
Network excitability	Arc KO mice	EEG recordings and seizure induction	[16]
Alzheimer's disease	hAPP mice, amyloid β treatment *in vitro*	Arc expression in a novel environment, BDNF stimulation	[77,78]
Alcohol-induced plasticity	Arc knockdown in amygdala, alcohol exposure	*Arc* expression after alcohol drinking	[80]
Angelman syndrome	Ube3A KO mice, Arc knockdown *in vitro*	Arc and GluA1 expression	[52]
Fragile X syndrome	Fmr1 KO mice	mGluR LTD induced rapid Arc translation	[30]
Aging	Aged rats	*Arc* gene methylation and expression	[83]

[a]Abbreviations: EEG, electroencephalogram; hAPP, human amyloid precursor protein; Fmr1, fragile X mental retardation protein; KO, knockout.

Arc also has an important function in the response to visual experience (Table 1). After monocular deprivation in wild type mice, the visual cortex shows depression of deprived-eye responses and potentiation of open-eye responses. The visual cortex of *Arc* KO mice did not show these changes and also lacked the normal response to the same stimulus given over several days, including a stimulus-selective response potentiation [74], orientation specificity and average spike tuning [56]. Given Arc's role in mediating homeostatic plasticity, it is also unsurprising that *Arc* KO mice are deficient in a form of homeostatic plasticity seen in the visual cortex in response to changes in light exposure. After 2 days of dark-rearing, wild type mice show an enhanced mini-excitatory post-synaptic potential amplitude that is reversed upon 2 h of light exposure, and both of these adaptations were absent in KO mice [75].

Ultimately, Arc expression may provide a means of inferring how well an animal is learning a task. Animals with high levels of Arc in the striatum or hippocampus showed faster learning in a reversal learning motor-response task [5] or a spatial learning task [76], respectively. However, another study observed that animals with higher levels of Arc in the hippocampus showed slower learning in a lever-pressing task [54]. This apparent discrepancy may be because the latter task does not rely on the hippocampus. In addition, no correlation was found in another hippocampal-independent cued-response task [76]. Yet another study found that animals over-trained in a lever-pressing task had less Arc expression across many brain regions compared with newly trained animals [3]. Thus, although the exact relationship between levels of Arc and the ability to learn is not yet clear, precise control of Arc expression is clearly required for many forms of learning and behavior.

Arc in aging and disease

Although no diseases are known to be caused by mutations directly related to Arc expression, the loss of *Arc* in KO mice leads to hyperexcitability and increased susceptibility to seizures [16]. Interestingly, increased levels of Alzheimer's-related human amyloid precursor protein (hAPP)-derived amyloid β in transgenic mice expressing hAPP [77] and in cultured cortical neurons [78] impaired Arc expression and also led to hyperexcitable networks and seizures [79]. Indeed, the extent to which Arc was depleted in granule cells from hAPP mice assessed histologically *post-hoc* was one of the best predictors of behavioral deficits in individual animals [77]. Moreover, similar to Alzheimer's disease (AD) mouse models, Arc KO mice have reduced calbindin and increased neuropeptide Y (NPY) protein levels in the hippocampus [16], which may indicate adaptive changes that occur to prevent severe epilepsy. Because Arc is required for normal memory formation, this evidence suggests that decreased Arc expression may underlie some of the memory disturbances seen in AD (Table 1). The mechanism by which Arc is reduced in AD is not yet clear. However, adaptive changes in NPY expression in interneurons, which may mitigate hyperexcitability driven by amyloid β, might also interfere with the production of Arc in response to patterns of activity that would normally stimulate plasticity and enduring memories.

Arc might also be involved in neuropsychiatric diseases (Table 1). Decreased Arc induction results in anxiety-like and alcohol-drinking behaviors in rats [80]. Fragile X syndrome, the most common inherited cause of mental retardation and autism, results from a decrease in FMRP, which regulates Arc translation [30]. Angelman syndrome, a neurodevelopmental disorder characterized by motor dysfunction and severe mental retardation, is caused by a mutation in UBE3A, which encodes the ubiquitin ligase that targets Arc for degradation [52]. Mutations and copy number variations in UBE3A are also associated with autism spectrum disorders (ASDs) [81]. As discussed above, Arc is involved in synaptic homeostasis, which may be one of the underlying causes of the neuronal dysfunction seen in ASDs [82]. Given these links, a role for Arc in mental retardation and ASDs warrants further investigation. Even in normal aging, Arc expression is disrupted owing to regional changes in *Arc* methylation [83]. However, it is not always clear whether disruptions in Arc expression are a cause or effect of

neuronal dysfunction. Still, the importance of Arc in learning and memory implies that such disruptions result in major behavioral abnormalities even when Arc is not the primary cause of a disease.

Conclusions and future directions

Arc plays a vital role in the molecular mechanisms underlying memory consolidation. Its expression is tightly linked to neuronal activity, and this finding has led to its use as a tool to study both functional and dysfunctional neurons. Given its enrichment at synapses and in the nucleus, and its pleotropic roles in Hebbian and homeostatic plasticity, Arc probably mediates its effects on behavior through multiple cellular functions, including regulation of AMPAR endocytosis, spine morphology and yet-to-be defined nuclear functions. The extent of its cellular functions remains to be elucidated, and how different stimuli lead to different localization and functions of Arc is also not yet clear. These will be important areas for further research in the pursuit of a better understanding of how neurons encode information and form lasting memories. Arc undeniably still has a lot to teach us about how the brain accomplishes one of its most remarkable feats, the consolidation of memory.

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