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Molecular physiology of Arc/Arg3.1: The oligomeric state hypothesis of synaptic plasticity

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Abstract

The immediate early gene, Arc, is a pivotal regulator of synaptic plasticity, memory, and cognitive flexibility. But what is Arc protein? How does it work? Inside the neuron, Arc is a protein interaction hub and dynamic regulator of intracellular signaling in synaptic plasticity. In remarkable contrast, Arc can also self-assemble into retrovirus-like capsids that are released in extracellular vesicles and capable of intercellular transfer of RNA. Elucidation of the molecular basis of Arc hub and capsid functions, and the relationship between them, is vital for progress. Here, we discuss recent findings on Arc structure–function and regulation of oligomerization that are giving insight into the molecular physiology of Arc. The unique features of mammalian Arc are emphasized, while drawing comparisons with *Drosophila* Arc and retroviral Gag. The Arc N-terminal domain, found only in mammals, is proposed to play a key role in regulating Arc hub signaling, oligomerization, and formation of capsids. Bringing together several lines of evidence, we hypothesize that Arc function in synaptic plasticity—long-term potentiation (LTP) and long-term depression (LTD)—are dictated by different oligomeric forms of Arc. Specifically, monomer/dimer function in LTP, tetramer function in basic LTD, and 32-unit oligomer function in enhanced LTD. The role of mammalian Arc capsids is unclear but likely depends on the cross-section of captured neuronal activity-induced RNAs. As the functional states of Arc are revealed, it may be possible to selectively manipulate specific forms of Arc-dependent plasticity and intercellular communication involved in brain function and dysfunction.

KEYWORDS

activity-regulated cytoskeleton-associated protein (Arc), Gag protein, memory and cognition, oligomerization, protein interaction hub, protein structure–function, retrovirus-like capsid, synaptic plasticity

1 | INTRODUCTION

Understanding brain mechanisms in learning and memory is one of the great challenges of neuroscience. Representation of information in neural networks is widely thought to rely on, in part, synaptic plasticity—the ability of the synapse to change in strength in response to use or disuse.[1–3] The generation of long-term functional changes in synaptic transmission requires neuronal activity-dependent alterations in gene transcription and regulation of protein synthesis. Synaptic plasticity, like cell differentiation and growth, is a complex cellular response orchestrated by numerous and functionally diverse genes.[4–9] As briefly summarized below, the activity-regulated cytoskeleton-associated protein (Arc; aka activity-regulated gene Arg3.1) has emerged as a pivotal regulator of synaptic plasticity with important functions in memory formation and post-natal cortical maturation.

1.1 | Arc gene expression dynamics and function in synaptic plasticity

Arc was discovered due to robust enhancement of its mRNA expression in the rat hippocampus following electrically-induced seizures in vivo.[10,11] In mammals, Arc is predominantly expressed in excitatory glutamatergic neurons of the central nervous system. Arc belongs to a class of immediate early genes in which RNA polymerase II docks near the transcriptional start site, allowing transcription within 2–5 minutes of cellular or behavioral stimulus.[12,13] The Arc pre-mRNA has a single coding-exon and a 3′UTR harboring two introns that are spliced to generate the mature Arc mRNA. No alternative splice variants are known. After the stimulus, Arc mRNA shows a wide somatodendritic distribution, with transport along the dendritic arbour at rates up to 70 μm/min.[14] Arc mRNA accumulates beneath stimulated post-synaptic sites where it undergoes activity-dependent translation.[14–18] Arc mRNA is subject to translation-dependent degradation, due to the presence of the exon junction complex on spliced Arc mRNA which recruits machinery for RNA decay.[19] Stimulus-evoked translation of Arc in neuronal dendrites occurs in short bursts, which aligns well with translation-dependent RNA decay.[20,21] The newly synthesized protein also undergoes rapid ubiquitination and proteasomal degradation with an estimated half-life between 30 and 60 min.[22–25] Together these studies indicate rapid induction and tight spatial and temporal control of Arc expression.

Various forms of long-term plasticity are impaired when the Arc gene is ablated, or the RNA is knocked down. Arc is causally implicated in the neuronal activity-induced enhancement of synaptic transmission in long-term potentiation (LTP), decreased transmission in long-term depression (LTD), as well as homeostatic synaptic scaling.[25–35] Arc is also required for LTP induced by exogenous application of brain-derived neurotrophic factor (BDNF) in the dentate gyrus,[25,36] and LTD induced by application of the group I metabotropic glutamate receptor (mGluR) agonist, DHPG, in the hippocampal CA1 region.[31] All these Arc-dependent forms of plasticity are associated with enhanced Arc expression in the post-synaptic compartment of excitatory glutamatergic neurons.

1.2 | Arc function in memory and post-natal cortical maturation

Numerous studies have demonstrated learning task-specific expression of Arc mRNA in subpopulations of cells engaged in memory encoding.[37–45] Loss of function approaches using constitutive Arc knockout, conditional gene deletion, or knockdown by antisense RNA support a role for Arc in long-term memory formation, whereas learning (acquisition) itself does not require Arc.[26,27,46] Studies employing local, brain region-specific Arc knockdown in the adult brain support a casual role for Arc expression in hippocampal-dependent spatial memory formation,[26,46] while Arc expression in the amygdala is necessary for Pavlovian fear memory.[47] Memory processes triggered by a reminder cue (reconsolidation and extinction) are similarly dependent on brain region-specific Arc expression.[48–50] Arc knockin mice harboring mutations in the two predominant ubiquitination sites have impaired degradation of Arc in the ubiquitin proteasomal system.[51] These mice learn and remember a spatial task normally and have a lower threshold for hippocampal mGluR-dependent LTD, but they are impaired in a reversal-learning task which requires switching to a new learning strategy. This suggests that ~~the~~ Arc protein turnover rate influences plasticity and contributes to cognitive flexibility.[51]

Arc also has important functions in neocortical circuits. During motor learning, Arc expression in neuronal ensembles of the secondary motor cortex is necessary for the consolidation of task-specific motor responses.[42,52–54] During post-natal maturation of the visual cortex in rodents, Arc expression supports ocular dominance column formation and orientation tuning of visual inputs, as well as receptive field plasticity.[33,55–58] Collectively, research indicates a role for Arc in post-natal maturation of the visual cortex as well as consolidation of hippocampal-dependent memory (spatial learning and contextual fear) and hippocampal-independent motor learning. However, causal roles for Arc-dependent forms of plasticity in behavior remain to be established.

1.3 | Arc as a protein interaction hub

At the molecular level, Arc is proposed to act as a protein interaction hub.[59] Arc has more than 30 validated interaction partners, as shown by affinity purification methods or by direct binding assays such as surface plasmon resonance. According to this model, Arc regulates intra-cellular signaling and plasticity by binding to different effector proteins. For example, Arc interaction with the clathrin adaptor protein, AP2, dynamin 2, and endophilin, facilitates clathrin-mediated endocytosis of AMPA-type glutamate receptors and promotes decreases in synaptic strength in LTD and synaptic scaling[28,31,60,61] (Figure 1A). Arc can also be targeted to inactive synapses, by binding to inactive calcium/calmodulin-dependent protein kinase-β (CaMKII-β), which enables a selective weakening of inactive synapses.[35,62] During LTP at the medial perforant path input to the dentate gyrus, stabilization of nascent actin filaments and consolidation of LTP depends on Arc synthesis.[25,63] Newly synthesized Arc binds to the actin cross-linking protein, drebrin A,[64] and regulates the activity of the actin-severing protein, cofilin[25] (Figure 1B). Arc also enters the nucleus and associates with the histone acetylases, TIP60, and CREB binding protein.[65,66] Nuclear Arc was shown to downregulate transcription of AMPA receptor GluA1 subunits to support a dendrite-wide synaptic downscaling,[66] while recent work implicates Arc in the regulation of chromatin state[65,67,68] (Figure 1C). In sum, convergent evidence supports a role for Arc as a neuronal activity-induced hub that regulates both synaptic and nuclear mechanisms in the post-synaptic neuron (Figure 1A–C).

Post-translational modifications of Arc have also been identified, shedding light on regulation of nucleocytoplasmic localization,[69,70] protein–protein interactions,[35,64,71,72] and turnover.[24,73–75] Furthermore, recombinant Arc protein is capable of reversible self-association, raising the possibility that function is determined by its oligomeric state.[76]

1.4 | Arc as a retrovirus-like capsid

In a bioinformatic analysis from 2006, Arc was identified as one of 103 human protein isoforms with conserved homology to retroviral Group-specific antigen (Gag) polyprotein.[77] The work suggested that Arc protein and modern retroviruses originated from ancient Ty3/Gypsy retrotransposon elements. Current evidence indicates that the Arc gene has been repurposed in separate evolutionary events towards mammalian Arc and *Drosophila* , which has two Arc genes (dArc1 and dArc2).[78–81] In an exciting new development, Arc protein from mammals and *Drosophila* were shown to self-assemble into retrovirus-like capsid structures that contain Arc mRNA. Arc capsids are released from neurons in extracellular vesicles (EVs), which transmit the capsid and release the RNA into neighboring cells[80,81] (Figure 1D). In primary mouse hippocampal neuronal cultures, immunogold electron microscopic (immuno-EM) labelling showed native Arc protein inside a subpopulation of EVs isolated from the culture medium.[80] Depolarization of cultures by KCl treatment increased Arc-EV release into the medium. When neuronal cultures from Arc knockout mice were treated with EVs isolated from a wildtype (WT) neuron culture medium, somatodendritic expression of Arc was observed. Treatment with DHPG (a mGluR agonist) further enhanced translation, indicating activity-dependent translation of internalized Arc mRNA. At the *Drosophila* larval neuromuscular junction, dArc1 protein and mRNA are enriched in exosome-like EVs produced in the pre-synaptic bouton.[81] Immuno-EM analysis of lysed EVs showed capsid structures that contained Arc. Using selective pre- and post-synaptic genetic manipulations, Ashley and colleagues provide evidence that dArc1 capsids bearing *dArc1* RNA are transferred to post-synaptic muscle fibers, and that this transfer is required for Arc protein expression in muscle. Functionally, the *Drosophila* study provides evidence that Arc capsids/EVs are required for synapse maturation and activity-dependent synapse formation.[81,82] Thus, Arc capsid transmission in EVs represents a novel form of intercellular communication, with Arc mRNA as one of the cargos (Figure 1D).

1.5 | Hub versus capsid: A tantalizing dichotomy

A tantalizing dichotomy now exists between Arc as an intra-cellular signaling hub on one hand[59] and a capsid vehicle for intercellular RNA transfer on the other[82,83] (Figure 1). Resolving the relationship between these seemingly disparate functional states is vital for progress. Here, we discuss recent discoveries on Arc structure–function and regulation of oligomerization that are yielding exciting new insights into the molecular physiology of the protein and the path towards applications in systems neuroscience. The unique features of mammalian Arc are emphasized while drawing comparisons with dArc and retroviral Gag. Finally, we provide working models of mammalian Arc function in plasticity related to its oligomeric state: monomer/dimer, tetramer, large oligomer, and capsid.

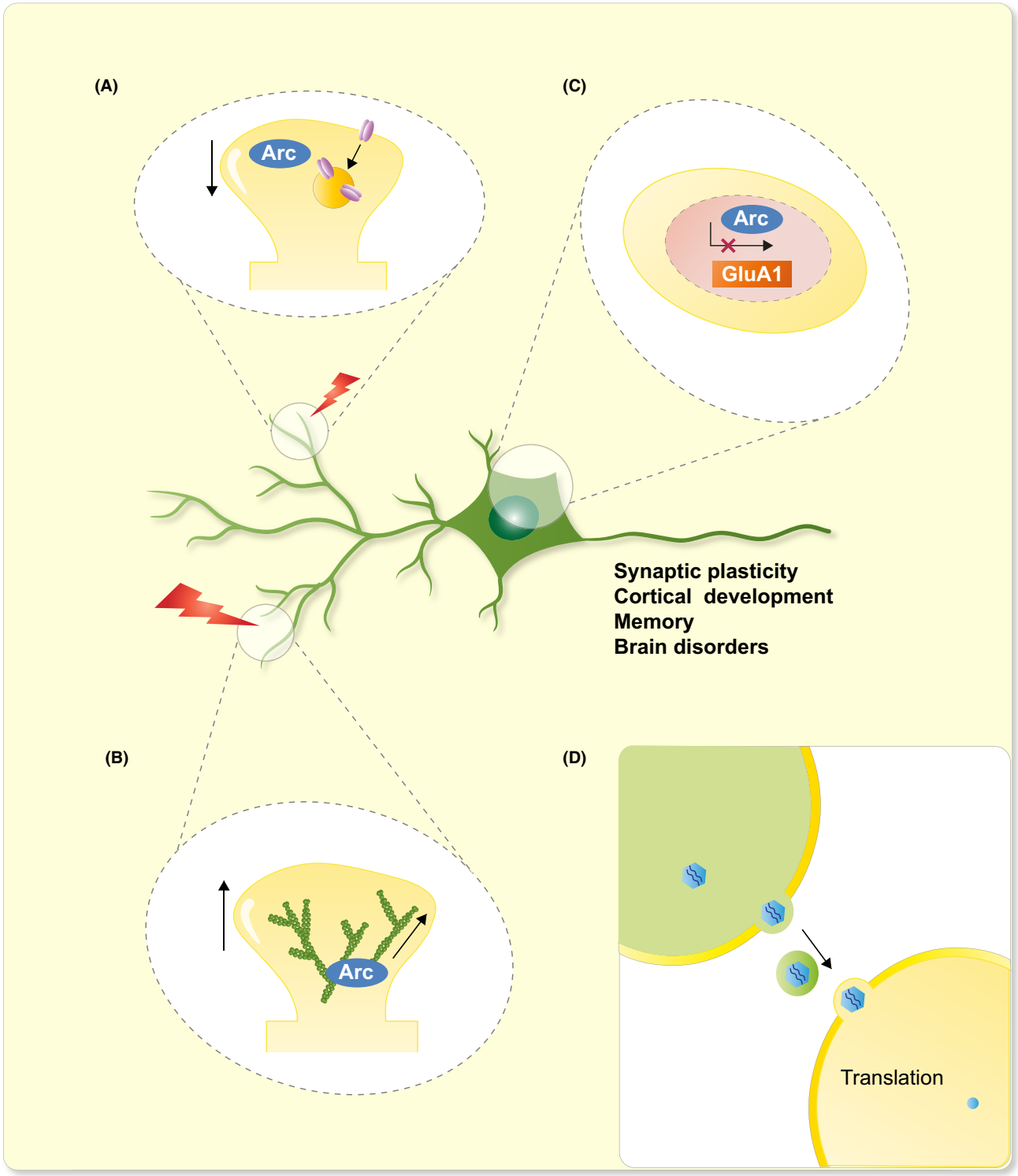


FIGURE 1 The Arc hub protein in neuronal function. Panels A, B, and C illustrate the overarching function of Arc as a hub protein in post-synaptic glutamatergic neurons during long-term synaptic plasticity. (A) Arc interacts with proteins of the endocytic machinery and facilitates clathrin-mediated endocytosis of AMPA-type glutamate receptors, resulting in local decreases in synaptic strength (LTD). (B) Arc interacts with F-Actin-binding proteins, and Arc synthesis following synaptic activation is required for the stabilization of newly polymerized actin ~~Actin~~ filaments in post-synaptic dendritic spines and stable synaptic strengthening in LTP. (C) Arc enters the nucleus of the post-synaptic neuron where it interacts with histone acetylases and inhibits transcription of AMPAR GluA1 subunits. This implicates a function in dendrite-wide homeostatic scaling. (D) Arc forms virus-like capsid structures which encapsulate mRNA, are released in vesicles and taken up by neighboring cells. This suggests a function in intercellular signaling. The relationship between Arc hub and capsid functions is unclear.

2 | MAMMALIAN ARC PROTEIN STRUCTURE

Arc is highly conserved in mammals but has low sequence homology to other proteins. Structural analysis has been hindered by the strong aggregation propensity and insolubility of the protein in standard buffer conditions. In 2015, Myrum et al. provided the first biochemical and biophysical analysis of recombinant human Arc, showing a negatively charged N-terminal (NT) domain and positively charged C-terminal (CT) domain separated by a disordered linker region[76] (Figure 2A). Arc has a loose tertiary structure within and between its domains, suggesting a floppy protein with conformational flexibility.

The predicted evolutionary relationship to Gag was borne out by crystal structure analysis of the rat Arc CT, revealing 3D homology to the Gag capsid (CA) domain of human immunodeficiency virus (HIV) and Rous sarcoma virus.[79] Based on this homology the Arc CT is also known as the CA domain. Gag CA is comprised of two domains, the CA-NTD and CA-CTD, which self-assemble to form a capsid shell.[85] Arc CA also has two domains, the N-lobe and C-lobe, both of which are homologous to Gag CA-CTD.[79] Notably, the Arc N-lobe has evolved a ligand binding pocket that does not exist in retroviral Gag. The crystal

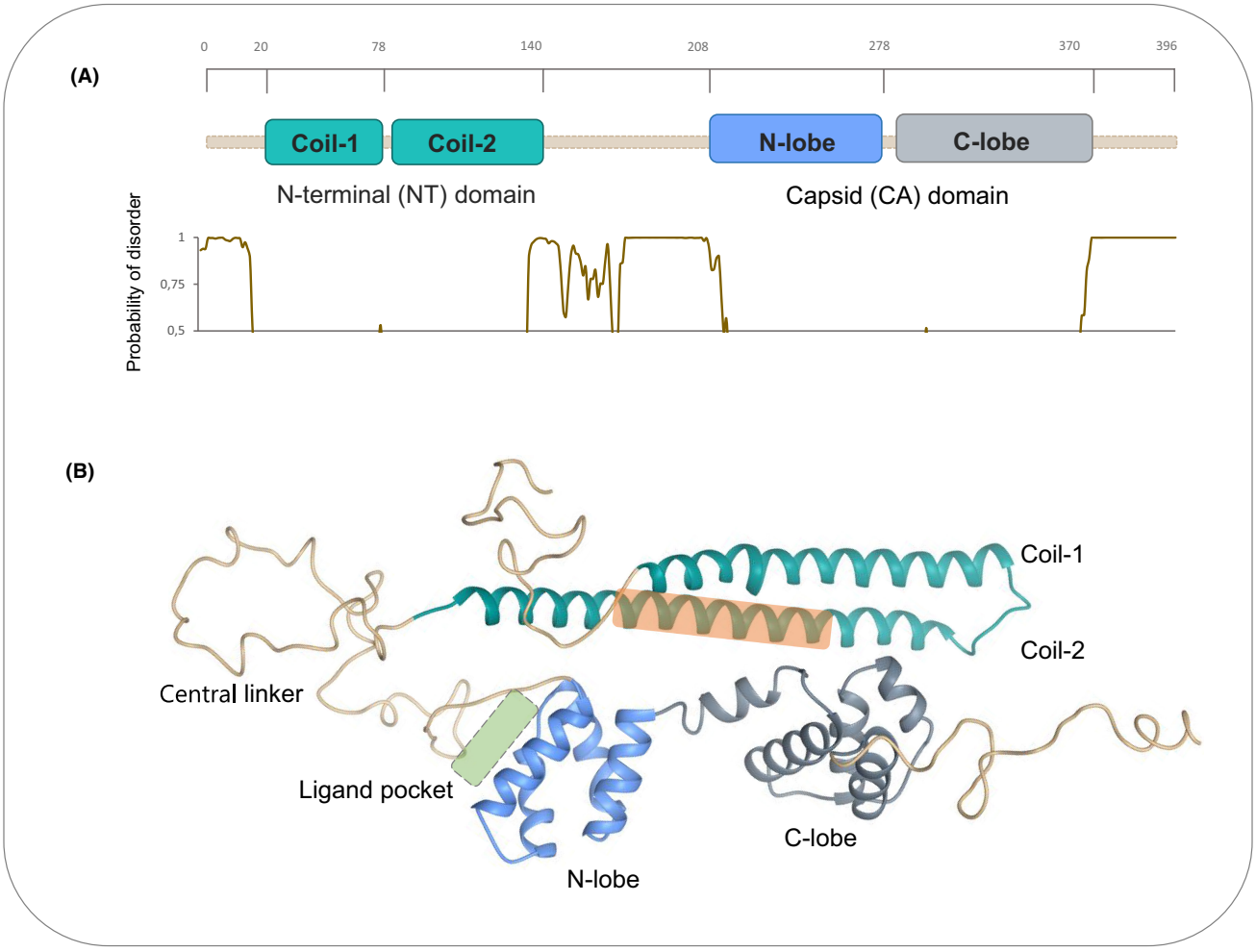


FIGURE 2 Mammalian Arc domain structure and full-length hybrid 3D structure. (A) Simple representation of Arc domains. Boxes represent structured domains and stippled lines indicate unstructured, potentially flexible regions. The bottom panel represents a prediction of disordered regions of human Arc based on its amino acid sequence using ODINPred (https://st-protein.chem.au.dk/odinpred). (B) Hybrid structural 3D model of the Arc protein (Ref. [84]). The structure is based on small angle X-ray scattering (SAXS) analysis of monomeric full-length Arc and subregions, crystal structure of the capsid (CA) domain, and a homology model of the N-terminal (NT) domain. The NT domain is predicted to be an antiparallel, alpha-helical coil. The highlighted orange region of Coil-2 is important for Arc self-association (see Section 3.1). The CA comprises two separate globular domains termed the N-lobe and C-lobe. The ligand binding pocket of the N-lobe is marked in green. The Arc NT and CA domains are connected by an unstructured central linker region and flanked by unstructured N- and C-termini.

structure of the isolated N-lobe was obtained in complex with peptides of TARP γ-2 (a.k.a. stargazin), a transmembrane auxiliary subunit of AMPA-type glutamate receptors, and CaMKII-α. Identification of the N-lobe binding motif led to the validation of additional ligands: the N-methyl-D-aspartate glutamate receptor (NMDAR) subunit 2A (GluN2A), the scaffolding protein guanylate kinase anchoring protein (GKAP), and the actin-binding protein WAVE1.[79,86] As a multiligand binding site, the N-lobe provides a compelling structural basis for hub signaling along with other established interaction sites on Arc. Arc N-lobe binding to stargazin is implicated in AMPAR endocytic trafficking.[79] However, the mechanisms regulated by Arc interaction with stargazin and other N-lobe ligands remain to be defined.

An atomic resolution structure of a full-length Arc has yet to be achieved. However, lower resolution structural information of full-length Arc and subregions was obtained by small angle X-ray scattering (SAXS) analysis alongside synchrotron radiation circular dichroism (SRCD) spectroscopy.[84] The insolubility of Arc is caused by the NT region as all fragments lacking the region are monomeric. For SAXS analysis, the soluble full-length monomer was obtained by short exposure to high pH, which neutralizes the positive charge on the NT. In a hybrid model of Arc monomer (Figure 2B), the oppositely charged domains interact, making a compact "closed" structure that stabilizes the linker region. Based on SRCD analysis and structural predictions, the Arc NT domain was proposed to be an antiparallel alpha-helical coiled coil. The compact state of Arc and relative size of the domains were further validated by intra-molecular fluorescence lifetime FRET imaging in CA1 pyramidal neurons in hippocampal slices.[84] Given the flexibility of the protein,[76] it is possible that Arc also has an "open" conformational state with an extended linker and different orientations of the domains. Such conformational changes might serve to activate or inhibit domain-specific functions of Arc in response to cellular factors and stimuli.

A major question is whether ligand binding to the N-lobe induces structural and functional changes. Using SAXS to assess conformational changes, Hallin and colleagues mixed partner peptides (GluN2A, stargazin, GKAP, and WAVE1) with the isolated CA, the separate N- and C-lobes, or CA flanked by the linker region and C-terminus. The peptides bound selectively to the N-lobe but did not elicit a major conformational change.[84] However, a subsequent molecular dynamic simulation of the N-lobe crystal structure, with and without bound stargazin peptide, showed greater flexibility in the absence of ligand.[86] The change in flexibility occurs in the N-terminal strand of the N-lobe adjoining the linker region. Upon binding of stargazin to the N-lobe pocket, the N-terminal strand folds back on the peptide to form a beta-sheet which stabilizes the structure. This observation from molecular dynamic simulations leaves open the possibility that ligand binding impacts the flexibility of the linker and orientation between domains.

In studies using pure phospholipid liposomes, the highly basic NT region was found to mediate Arc interaction with phospholipid membrane.[84,87] Palmitoylation of cysteine residues (C95, C96, and C98) in the NT domain allowed insertion of Arc into the hydrophobic core of the bilayer, and mutation of residues impaired Arc-dependent LTD in hippocampal neuronal cultures.[87] Because of the insolubility of full-length Arc, no data are available on the effects of ligand binding or membrane binding on protein conformation.

Given the evolutionary link with retroviruses, it is useful to contrast and compare Arc structure–function with that of HIV Gag. The relationships are summarized in Figure 3. Retroviral capsids consist of large lattices of hexamers that assemble into approximately spherical structures of variable size.[88] The capsid shell harbors and protects the viral genome which enters the host cell during infection. In the case of HIV, capsids are assembled by the Gag polyprotein precursor, which contains four major domains: matrix (MA), capsid (CA), nucleocapsid (NC), and p6. Each domain plays a distinct and crucial role in the formation of the immature virion.[89,90]

3 | MAMMALIAN ARC OLIGOMERIZATION AND CAPSID FORMATION

Many proteins form oligomers where two or more monomers of a protein interact to form a larger complex. Smaller oligomers like dimers and tetramers are the most prevalent, and a survey of *Escherichia coli* proteins suggests that only one-fifth of proteins are solely monomeric.[91] Oligomeric forms can provide increased stability of the protein, create new interfaces for binding partners and regulate protein activity. Importantly, many proteins form reversible oligomers, in which the various oligomeric forms exist in equilibrium. The equilibrium may shift according to the intra-cellular environment, protein concentration, partner availability, and post-translational modifications.

Recombinant Arc has been shown to form reversible oligomers,[76,92] consistent with a physiological role. This differs from uncontrolled aggregation, where monomers assemble into irreversibly bound clusters, as seen in β-amyloid deposits.[93] Myrum and colleagues monitored

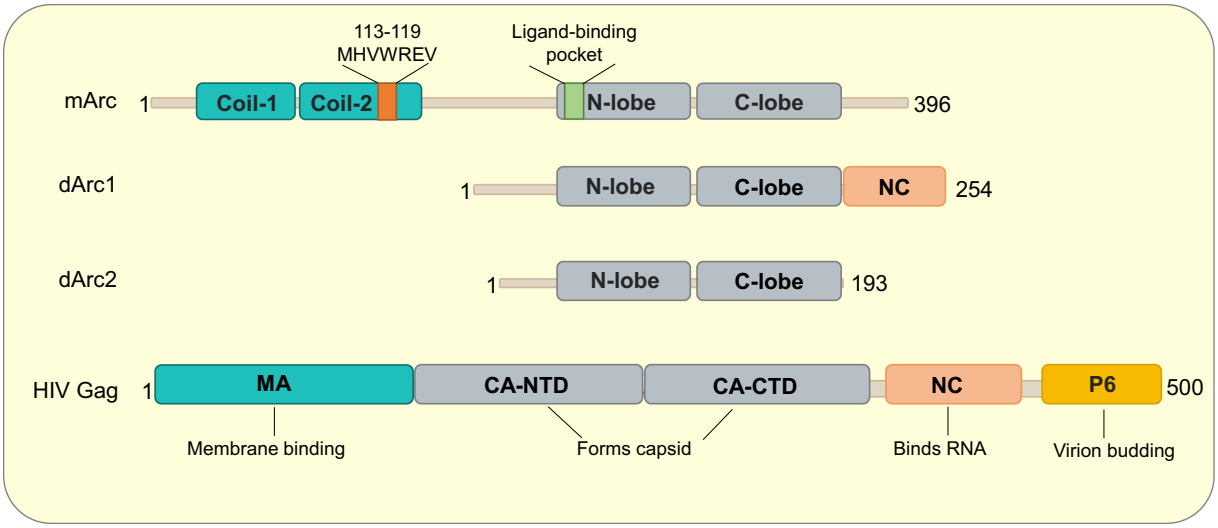


FIGURE 3 Structural comparison of Arc proteins and HIV Gag protein. Color coding is used to depict structural or functional similarity of Arc and HIV Gag domains. The mammalian Arc (mArc) features several notable evolutionary adaptations. (1) The mArc N-lobe has evolved a peptide ligand binding pocket (marked in green) which is not found in *Drosophila* Arcs (dArc1/2) or retrotransposon Ty3. (2) mArc lacks the zinc knuckle domain which is found at the C-terminus of dArc1 and homologous to the Gag nucleocapsid domain (NC) important for binding of viral RNA. (3) mArc has an approx. 206 amino acid long N-terminal region which contains a flexible linker and a structured NT domain. The unique mArc NT self-associates and is important for higher-order oligomerization, formation of retroviral-like capsids, and may be the site of RNA binding. Arc self-association and capsid formation are ~~is~~ disrupted by a 7-amino acid substitution mutation in NT Coil-2 (marked in red). Additionally, the mArc NT-domain associates with cellular membranes as does the MA (matrix) domain of the HIV Gag protein. CA-NTD = capsid N-terminal domain, CA-CTD = capsid C-terminal domain.

the size of human Arc by dynamic light scattering (DLS), showing monomers, dimers, and oligomers ranging up to 40 units, depending on the buffer conditions. In physiological phosphate buffer, a 12-unit oligomer predominated.[76] Negative-stain transmission EM analysis of purified protein in salt buffer shows irregular-shaped particles. However, subsequent work on the purified protein and functional studies demonstrated the existence of Arc capsids.[80,81] These discoveries sparked a further investigation of the mechanism of Arc self-association and the function of oligomers.

3.1 | Regulation of oligomerization by an Arc N-terminal helical coil motif

The isolated Gag CA self-associates through dimerization motifs,[94] which are also predicted in Arc CA.[95] Surprisingly, however, the isolated Arc CA is monomeric,[84,95,96] and the NT-region is needed for oligomerization and capsid formation.[80,84,97] A recent study identified a critical role for Coil-2 in the antiparallel coiled coil.[97] Employing affinity purification of ectopically expressed Arc in HEK293 cells, the isolated Coil-2 region undergoes self-association, whereas Coil-1 and other segments of the Arc protein do not have this property. Coil-2 harbors a 28-amino-acid stretch, the oligomerization region, that is necessary for self-association of full-length Arc (illustrated in Figures 2 and 3). Alanine substitution scanning further identified a 7-amino acid oligomerization motif ($_{113}$MHVWREV$_{119}$), critical for the self-association of full-length Arc. Interestingly, protein cross-linking treatment of HEK293 cells transfected with the alanine substituted oligomerization motif mutant (Arc$^{s113\text{-}119A}$) or Arc WT revealed a low abundant dimer along with monomer for both proteins. Analysis of purified Arc$^{s113\text{-}119A}$ by different methods (DLS, SAXS, size exclusion chromatography, and transmission EM) showed a nearly homogenous dimer population, with no higher-order species. Both transmission and cryoEM analysis showed the presence of capsids of approx. 30 nm in diameter in Arc WT samples, whereas no capsids were found in samples of Arc$^{s113\text{-}119A}$.[97] Seeking to identify the molecular structure of the Arc–Arc interface, a peptide of the oligomerization region was synthesized, and an atomic resolution crystal structure was obtained, showing a dimer arranged into an anti-parallel coiled coil. Point mutations of critical residues in the interaction surface (M113 and W116) inhibited self-association of full-length Arc. The work suggested that Arc self-association above the dimer stage and capsid formation requires NT–NT interactions, mediated by the Coil-2 interface (Figure 4, step 2).

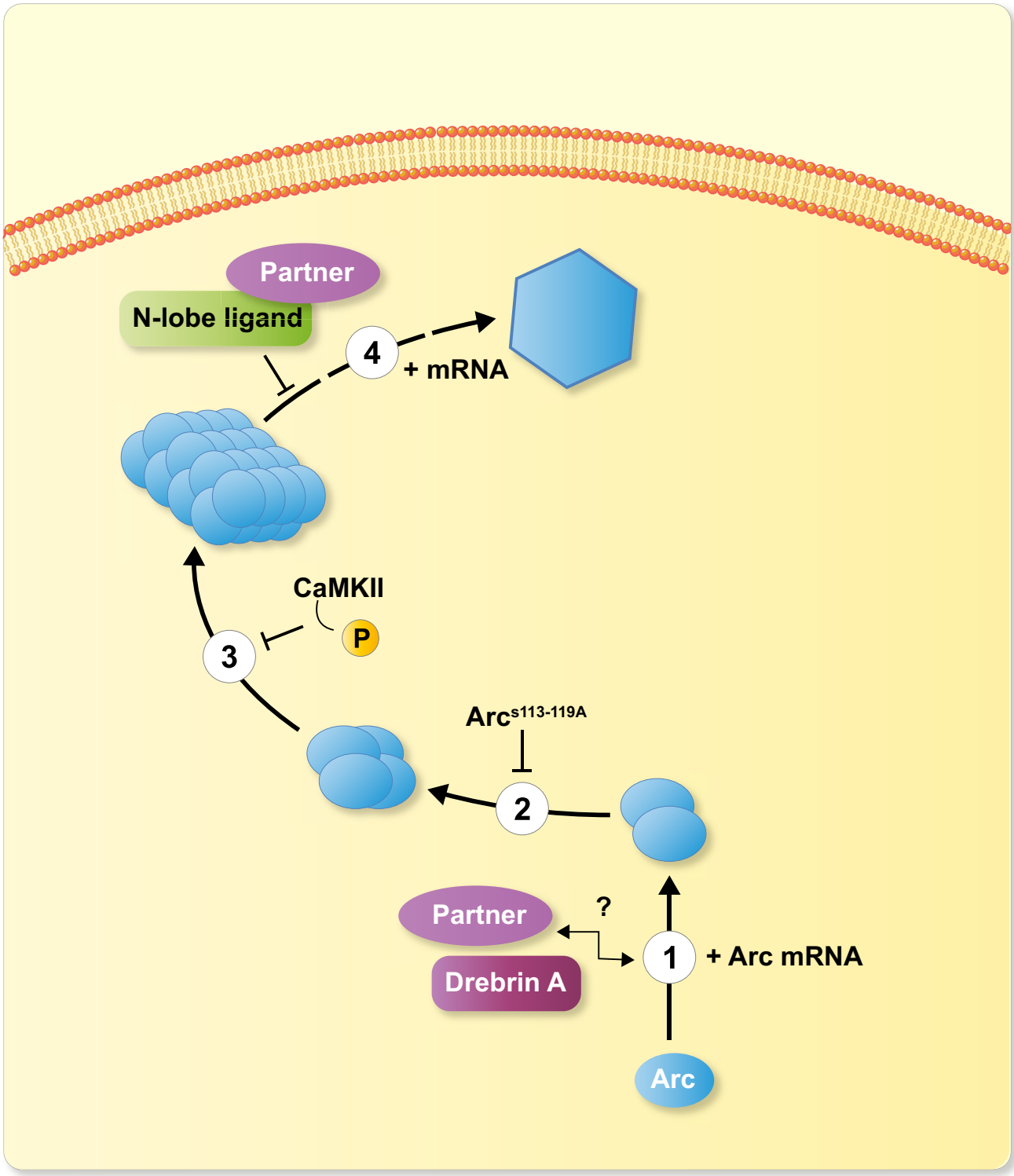


FIGURE 4 Regulation of Arc oligomerization—A working model. The figure illustrates how the formation of different-sized Arc oligomers is regulated by partner proteins, mRNA, and post-translational modifications. We refer to Section 3 for a full description of the different steps. (1) The mechanism of dimer formation is unknown, but is suggested to involve interaction with Arc mRNA. Dimerization may be promoted by interaction with Arc binding partners, and a working model for dimer function in LTP and association with the F-actin-binding protein drebrin A is described in Section 7.1. (2) Higher-order oligomerization is driven by coil-2 dimerization of the NT-domain. Mutating amino acids 113–119 of Coil-2 prevents the formation of higher-order Arc oligomers, but dimers are still preserved. (3) Arc N-lobe phosphorylation by CaMKIIα blocks the generation of Arc oligomers larger than tetramers. Native full-length Arc can form particles of 32 Arc units (octamer of tetramers). (4) In vitro studies show that Arc forms capsid-like structures, and adding mRNA promotes this capsid assembly. We speculate that large Arc oligomers promote LTD by facilitating N-lobe interaction with protein partners and that such interactions prevent capsid assembly (see Section 7.2).

3.2 | Regulation of oligomerization by peptide ligand binding to Arc N-lobe

Structural analysis of Arc CA by nuclear magnetic resonance (NMR) spectroscopy demonstrated a monomeric bilobar structure.[96] The authors proposed that, in full-length Arc, the CA might obtain a different orientation that enables oligomerization, possibly as a function of ligand binding to the N-lobe. When the isolated CA was exposed to high temperatures, structural rearrangement of the N-lobe was observed by CD spectrum analysis and NMR, while DLS showed the irreversible formation of a larger oligomeric population. Strikingly, these changes were abolished in the presence of the GluN2A peptide, suggesting that ligand binding to the N-lobe initiates a conformational change that inhibits temperature-induced oligomerization of Arc CA (Figure 4, step 4). However, whether ligand binding impacts full-length Arc oligomerization in vitro or in vivo is unknown.

3.3 | Regulation of oligomerization by phosphorylation of Arc CA

CaMKII is a major regulator of signal transduction for plasticity in the post-synaptic compartment and a crucial molecule for the structural organization of the post-synaptic density (PSD).[98] The Arc CA is phosphorylated by CaMKIIα at S260 in the N-lobe, close to the border with the C-lobe.[95] Using DLS to monitor oligomerization, purified full-length Arc formed a stable tetramer whereas constructs lacking the N-terminal half of Arc were monomeric. Reversible temperature-dependent oligomerization was shown, in agreement with a previous report.[76] Further experiments were done under conditions that resulted in 1.8 MDa particles, corresponding to approx. 32 Arc units (an octamer of tetramers). The introduction of the phosphomimetic S260D mutation blocked the generation of oligomers larger than tetramers (Figure 4, step 3).[95] Interestingly, similar inhibition of higher-order oligomerization was observed upon mutation of Gag CA-like dimerization motifs in the N-lobe and C-lobe. Coupling these observations, the authors propose that phosphorylation at S260 promotes the formation of an intra-molecular salt bridge that prevents the CA–CA intermolecular interactions needed for oligomerization above the tetramer stage.[95]

CaMKIIα catalyzed phosphorylation at S260 was further implicated in adaptive learning and synaptic plasticity. Arc knockin mice harboring non-phosphorylatable S260A exhibited impaired learning during fear conditioning training.[95] In the analysis of DHPG-induced LTD in the CA1 region of hippocampal slices, LTD magnitude was enhanced in knockin mice relative to WT, whereas LTP induction was not affected. In Purkinje cells in cerebellar slices, ectopic expression of Arc WT protein induced synaptic depression which occluded with LTD induced by treatment with the protein kinase C activator phorbol-12,13-diacetate (PDA), while expression of Arc S260D phosphomimetic failed to induce synaptic depression. Combining biochemistry and electrophysiology, the work suggests that oligomers larger than tetramers are necessary for full expression of LTD, and phosphorylation of S260 inhibits oligomerization and dampens LTD. Eriksen and colleagues[97] reported that disruption of the oligomerization motif in Coil-2 blocks Arc-induced enhancement of transferrin endocytosis in HEK293 cells. Notably, mutation of the adjacent endophilin binding site in Coil-2 disrupts endocytosis without disrupting Arc self-association. Thus, current evidence suggests that higher-order Arc oligomers facilitate endocytosis and LTD.

Biochemical analysis of Arc protein–protein interactions using GST-pulldown showed that mutations that inhibit high-order oligomerization and dampen LTD are not associated with loss of binding to endophilin or stargazin, two binding partners implicated in AMPAR endocytosis and LTD.[28,79] The enhancement of LTD in oligomerization-competent Arc could be due to the recruitment of additional binding partners and/or more efficient endocytosis due to the concentration of binding partners in a multivalent oligomeric scaffold.

A recent study showed that Arc is phosphorylated by the serine/threonine kinase TNIK (Traf2 and NCK-interacting kinase) at Ser67 and T278.[70] Ser67 is located in a previously identified nuclear retention domain in NT Coil-1.[66] Studies with overexpression of Arc-Myc-FLAG in Neuro2a cells indicate a role for S67 phosphorylation in the nuclear export of Arc, allowing accumulation of Arc in an F-actin fraction. T278 is located in the short linear hinge between the N-lobe and C-lobe. Transmission EM analysis of recombinant Arc WT protein showed round capsid-like structures, whereas samples of ArcT278D phosphomimetic showed aggregates but were devoid of capsids.[70] DLS analysis also showed large diameter aggregates and a smaller diameter subpopulation. In the study of Zhang and colleagues,[95] T278 was identified as a protein kinase C (PKC) phosphorylation site in Arc transfected HEK293 cells. While evidence for PKC phosphorylation of Arc in neurons is lacking, DLS analysis showed that recombinant ArcT278D is able to form tetramers but not higher-order oligomers.[95] The discrepancy between studies in the DLS results for ArcT278D may reflect differences in the buffers used,[70] yet the studies converge in indicating a potential role for T278 phosphorylation in inhibiting capsid formation. Finally, it is interesting to note that in Gag CA, the flexibility of the short hinge segment between lobes is

important for capsid assembly.[85] In Arc CA, glycine 277 is predicted to confer flexibility between lobes, and substitution with alanine at this site again results in a tetramer peak by DLS.[95] It has therefore been proposed that phosphorylation of adjacent T278 may impair oligomerization by restricting flexibility between lobes.[95]

3.4 | Regulation of oligomerization by Arc interaction with mRNA

Pastuzyn and colleagues found that bacterially expressed Arc copurifies with RNA.[80] Fewer capsids were obtained after an RNA stripping procedure, while the addition of purified mRNA of enhanced green fluorescent protein (EGFP) increased the yield of regular-shaped capsids. This suggested that the interaction of Arc with RNA can promote capsid assembly. In brain lysates, Arc mRNA coimmunoprecipitated with Arc protein.[80] Hence, Arc interaction with its cognate RNA may be physiologically relevant.

The impact of RNA on the size of Arc oligomers was investigated in vitro using single-molecule total internal reflection fluorescence (smTIRF) microscopy.[97] In this method, a self-labelling SNAP tag on the Arc N-terminus was covalently labeled with a fluorescent dye, while biotinylation of the Arc C-terminus allowed tethering to a glass coverslip. The size of each Arc oligomer was determined by photobleaching, where the number of photobleaching steps corresponds to the number of dye molecules. Under these conditions, Arc WT and Arc$^{s113\text{-}119A}$ were mainly dimeric or trimeric. However, the addition of purified EGFP mRNA to Arc WT induced the formation of oligomers containing 40–70 Arc molecules, while adding the purified Arc mRNA coding region induced larger assembles in the range of 70–100 units, with the largest containing up to 170 molecules. This mRNA-induced facilitation of higher-order oligomerization (Figure 4, step 4) was almost completely abolished in the Arc$^{s113\text{-}119A}$ mutant. While it is still unknown whether these large oligomers measured by photobleaching represent capsids or subcapsid structures, the size range fits with the estimate of 130 units per Arc capsid.[97]

In HIV, recognition of viral genomic RNA is mediated by trans-acting zinc finger motifs in the Gag NC domain.[90,99] Additionally, non-specific binding to host RNA is thought to function as a scaffold for Gag-Gag interactions and oligomerization.[90,100–102] This interaction is mediated by a patch of basic residues at the NC N-terminus.[90,100] Although Arc does not have amino acid sequence similarity to Gag NC and lacks a zinc finger, the NT oligomerization motif is flanked by positively charged patches that might interact with polyanionic RNA. The fact that mRNAs for both EGFP and Arc facilitate oligomerization points to an electrostatic interaction rather than RNA sequence-specific binding to the protein.[97] Interestingly, the replacement of the NC domain with a leucine zipper coiled-coil is able to reinstate nucleic acid-induced Gag multimerization.[103,104] Thus, Arc Coil-2 has a similar function to Gag NC in mediating RNA-induced multimerization.

While the dimer is proposed as the building block for oligomeric assembly, the mechanism of Arc dimer formation is unknown. Dimer formation does not require the Coil-2 oligomerization motif or the predicted CA interaction motifs. However, results from smTIRF suggest that dimerization involves interaction with Arc mRNA[97] because protein samples treated with purified Arc mRNA contain dimers but are devoid of monomers (Figure 4, step 1). As Arc monomers were abundant in samples treated with EGFP mRNA, dimerization may depend on binding to specific sequences in Arc mRNA.

4 | MAMMALIAN ARC CAPSID RELEASE, UPTAKE, AND CARGO

Viral capsids are released from the host cell through the budding of the plasma membrane. The process of virion release depends on the type of virus, but in the case of HIV its release relies on different domains of the Gag protein. The budding process is promoted by the interaction of the basic MA domain with the lipid bilayer, and by the p6 domain which recruits the endosomal sorting complex required for transport. Consequently, the viral capsid is packed within a membrane envelope which can be secreted from the host cell.

Arc capsids are packed into exosome-like EVs of cultured cortical neurons and released.[80] By transferring vesicle-containing medium to neuronal cultures from Arc knockout mice, it was shown that Arc capsids can be taken up by recipient cells by endocytosis, and the internalized Arc RNA is translated, with enhanced translation upon application of DHPG. Interestingly, Arc mRNA was also transmitted into cells after the addition of purified Arc containing non-enveloped capsids to the medium. This is surprising as membrane proteins are normally required for the transfer of mRNA across endosomal membranes.

The packaging and release of Arc capsids may be facilitated by the attachment of the Arc NT to the plasma membrane.[84,87] Recent experiments using giant unilamellar vesicles, a model system for the eukaryotic cell membrane, show that purified Arc promotes the formation of small vesicles that penetrate into the interior.[105] Such a mechanism may facilitate the formation of mRNA-containing EVs from the plasma membrane. This study

also demonstrates the intrinsic ability of Arc to change membrane curvature independently of associated proteins. Perhaps the intrinsic ability of Arc to curve membranes explains the ability of non-enveloped capsids to deliver RNA to the neuronal cytoplasm.[105,106]

In the study of Pastuzyn et al., treatment of purified Arc with RNaseA did not affect the abundance of Arc mRNA or bacterial (Asn) mRNA, indicating that these transcripts are encapsulated and shielded from enzymatic degradation.[80] The abundance of Arc/Asn mRNA inside capsids was proportional to expression in bacterial lysates, consistent with a non-specific mechanism of encapsulation. A full characterization of cargo from endogenous Arc capsids has not been achieved but will be key to unraveling function. The small volume of Arc capsids is estimated to give an RNA carrying capacity of 10 kb, which is enough to accommodate only 2–3 copies of Arc mRNA. Even for microRNAs and other small RNAs, the carrying capacity would be low. However, low carrying capacity is a general feature of EVs. In various physiological and pathological processes involving intercellular RNA transfer by EVs, the transmission of RNA within vesicles is at low copy number.[107,108]

Intercellular transfer of Arc protein and mRNA in the intact mammalian brain has not been shown. However, a recent study on dorsal root ganglion (DRG) responses to inflammation support a possible physiological role for Arc-containing EVs.[109] Arc is translated in axons of DRG neurons in response to inflammatory mediators. In Arc knockout mice, excessive skin vasodilation occurs in response to inflammatory challenge, and this response is inhibited by injection of a mixed EV pool that includes Arc-positive EVs but is not affected by control injection with Arc-negative EVs. It is not yet known whether endogenous Arc capsids are involved, as EVs were obtained by overexpression of Arc in a nociceptor cell line derived from DRG neurons.

5 | DROSOPHILA ARC PROTEINS AND CAPSID STRUCTURES

Drosophila has two Arc proteins, dArc1 and dArc2, encoded by separate genes. The mammalian and dArc proteins independently evolved from the domestication of different Ty3-Gypsy retrotransposons, resulting in proteins with distinct structures.[78] dArcs have a bilobar CA with N-lobe and C-lobe. However, the dArc N-lobe lacks the peptide ligand binding pocket of mammalian Arc. dArc proteins also lack the large NT domain of mammalian Arc (Figure 3). dArc1, but not dArc2, contains a structured zinc-finger pair at its C-terminus homologous to the NC domain of retroviruses and is predicted to be involved in RNA binding. This feature might explain why only dArc1 capsids are enriched in Arc mRNA.[81,110] A high-resolution crystal structure of the dArc1 CA reveals a homodimer and a dimerization interface in the C-lobe homologous to retroviral CA.[78]

In another major advance, single particle cryo-EM was used to resolve the structure of dArc capsids.[110] The dArc proteins both assemble into icosahedral capsids, with the capsid shell formed by 240 copies of the dArc CA. While there is no NT domain, there is a short N-terminal stretch of 41 residues in dArc1 and 28 residues in dArc2. These predicted amphipathic α-helices form flexible spikes that extend outwards and inwards from the capsid shell. The spikes occlude openings in the shell and could be involved in membrane interactions and regulation of access to the capsid interior.[110] The interior of capsids is highly basic which could promote non-specific encapsulation of RNA, while the zinc fingers in dArc1 confer binding to dArc1 mRNA.

In mammals, the N-terminal strand of the N-lobe is exposed for ligand binding, whereas in *Drosophila* it tucks inside the N-lobe hydrophobic core. dArc does not bind stargazin peptide and lacks the binding site motif.[111] Interestingly, the mammalian Arc N-lobe in complex with ligand adopts a β-strand structure similar to dArc N-lobe.[78,111] As discussed in Hallin et al.,[86] this raises the possibility that dArc N-lobe could bind peptide ligands under conditions that liberate the N-terminal strand, albeit with a binding specificity different from that of mammalian Arc N-lobe.[111]

Another recent study performed structural characterization of the dArc lobe domains.[111] While the mammalian Arc lobes are monomeric, the isolated N- and C-lobes of dArc1 and dArc2 are all oligomeric in solution. Using constructs with a truncated N-terminal tail, the dArc2 N-lobe formed a novel domain-swapper dimer in the crystal structure. This contrasts with the penta/hexameric structures in dArc2 capsids, indicating a critical role for the N-terminal strand in capsid assembly. In HIV, the CA protein has multiple functions beyond its structural role in capsids. In the early phases of HIV-1 infection, CA contributes to viral trafficking and uncoating, recognition of host cellular proteins and nuclear import of the viral pre-integration complex.[112] Notably, the domain-swapped dArc2 dimer has structural homology to flavivirus C protein dimer,[111] with postulated roles in host cell transcription, mRNA splicing, and decay,[113] while one chain of the dArc2 dimer resembles histone core protein monomer. It will therefore be interesting to evaluate possible non-capsid roles of dArc2 in nuclear regulation of gene expression, for instance upon disassembly of dArc2 capsids in muscle at the neuromuscular junction.

6 | PIVOTAL ROLE OF ARC NT DOMAIN IN OLIGOMERIZATION AND HUB FUNCTION

In the domestication of mammalian Arc from ancient retrotransposon elements, the NT domain has evolved functional properties of multiple Gag domains. Arc NT has only 18% sequence similarity to retroviral MA, from which it is predicted to have evolved, yet they share physicochemical properties. The MA domain is critical for targeting Gag to the plasma membrane. The targeting involves a patch of basic residues in the MA highly basic region and membrane anchoring by myristoylation.[90,114] Similarly, Arc interaction with the phospholipid membrane is mediated by electrostatic interactions with the N-terminal region[84] and palmitoylation of Coil-2 in the NT domain.[87] As discussed, the Coil-2 oligomerization motif also has functional similarity to Gag CA in mediating self-association, and to Gag NC in mediating RNA-facilitated oligomerization. These unique properties suggest a major, multifaceted role for the NT domain in regulating mammalian Arc function (Figure 5).

The Arc dimer is proposed as the basic assembly unit and the addition of dimer units is supported by the Coil-2 oligomerization interface and facilitated by mRNA. The assembly from tetramers to higher-order oligomers further depends on CA-CA interactions. The most parsimonious model is that NT–NT interactions mediated by Coil-2 induce a conformational change that enables CA-CA interactions in further oligomerization. Functionally, evidence suggests that Arc oligomers larger than tetramers are necessary for the full expression of LTD. Biochemical data shows that the transition from tetramers to 32-unit oligomers is arrested by phosphorylation of CA by CaMKII, TNIK, and PKC, and possibly by ligand binding to the N-lobe (Section 3). In this model, the NT serves to activate the capacity for CA oligomerization while phosphorylation of CA inactivates it.

The long, disordered linker region between the NT and CA also warrants mention as a target for regulation. Extracellular signal-regulated kinase (ERK), downstream of NMDAR and BDNF activation of TrkB receptors, drives Arc transcription and translation.[21,115–118] The Arc linker region has a linear binding motif for ERK and is phosphorylated by ERK in vitro and in vivo on S206, at the border with the N-lobe.[69] In hippocampal neuronal cultures, S206 phosphorylation promotes cytosolic localization relative to nuclear localization of activity-induced Arc. Following LTP induction in the dentate gyrus of live rats, endogenous Arc undergoes enhanced S206 phosphorylation in the cytoskeletal fraction. Interestingly, the distal end of the linker (residues 195–206) binds AP2μ and dynamin 2 critical for clathrin-mediated endocytosis of AMPARs, as well as calnexin, an integral transmembrane protein of the endoplasmic reticulum. Calnexin is a negative regulator of clathrin-mediated endocytosis in

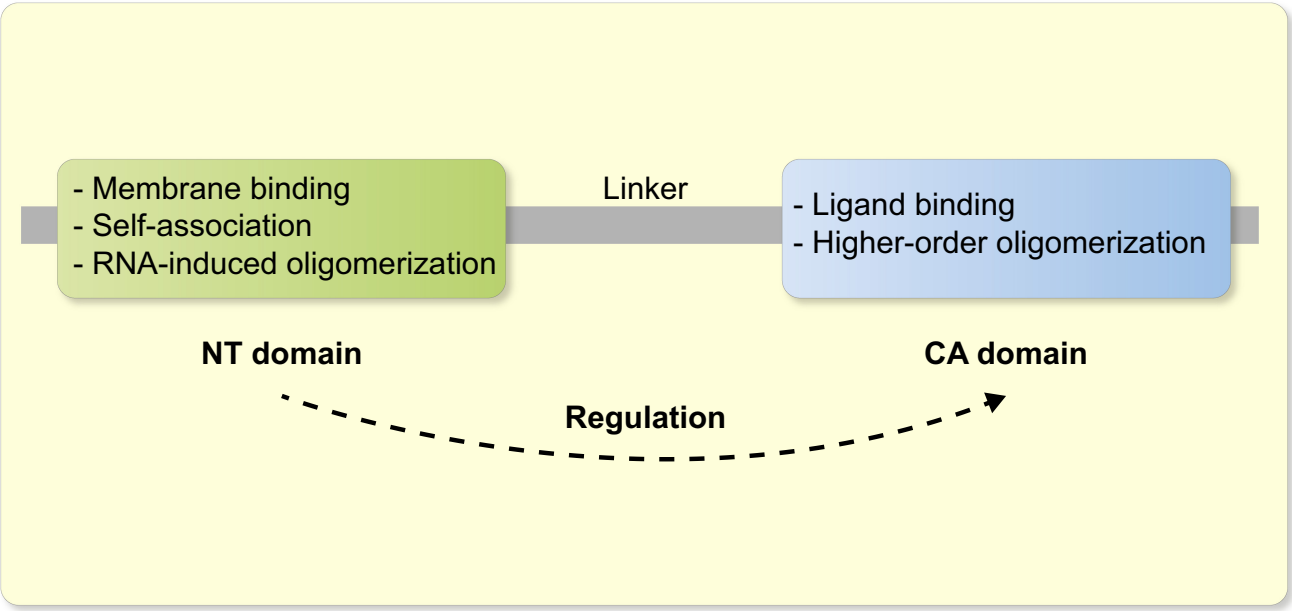


FIGURE 5 Pivotal role of NT domain in mammalian Arc function. Mammalian Arc has evolved functional properties of multiple HIV Gag domains (see Figure 3 for comparison). The large NT domain is unique to mammals and confers properties of membrane binding, self-association, and RNA-induced oligomerization. The NT domain is proposed to regulate the CA domain function of higher-order oligomerization and N-lobe ligand binding. The central disordered linker between mArc domains is also a prospective target of regulation as it binds several Arc protein partners and undergoes phosphorylation during in vivo LTP.

neurons, in addition to its canonical role as a chaperone in protein quality control.[119] Arc binds to the cytosolic tail of calnexin in BDNF-treated neurons in vitro and after LTP induction in vivo.[120] Notably, binding of the HIV-1 protein Nef to the C-tail of calnexin disrupts chaperoning function.[121] While the relationships between the binding partners, phosphorylation, and oligomerization remain to be explored, the linker segment closest to the N-lobe appears to be a nexus for regulation.

7 | MODEL OF ARC OLIGOMER FUNCTION IN MAMMALIAN SYNAPTIC PLASTICITY

Arc is known as a dynamic regulator of intra-cellular mechanisms of synaptic plasticity with assumed cell-autonomous functions, be they synaptic mechanisms or nuclear mechanisms. An outstanding issue that has puzzled researchers for a long time is how stimulus-induced Arc subserves opposite modifications of synaptic efficacy in various forms of LTD, LTP, and homeostatic synaptic scaling.[122]

With recent advances, it seems clear that understanding the relationship between oligomerization and hub protein–protein interactions is key to resolving Arc's molecular functions. We propose that oligomerization occurs in discrete stages, with each stage representing a distinct functional state of the Arc hub. The cell biological function is determined by the specific set of binding partners, the concentration of the binding partners, and subcellular localization (dendrites/spines and soma/nucleus) including targeting to membranes, actin cytoskeleton, and multiprotein complexes such as the PSD.

We propose that Arc has five distinct states in the neuron (1) a nascent pool of Arc monomer and dimer at sites of translation, (2) a dimer that regulates actin cytoskeleton dynamics in LTP, (3) a tetramer that facilitates AMPAR endocytosis and supports basic LTD function of low amplitude, (4) a larger oligomer (32 units) that amplifies LTD, and (5) a capsid (130 units) that functions in intercellular signaling. Regulation of oligomeric state would occur by post-translational modifications, availability of binding partners, and local concentration of Arc. Below we outline a working hypothesis of bidirectional regulation of synaptic plasticity based on the Arc oligomeric state (Figure 6).

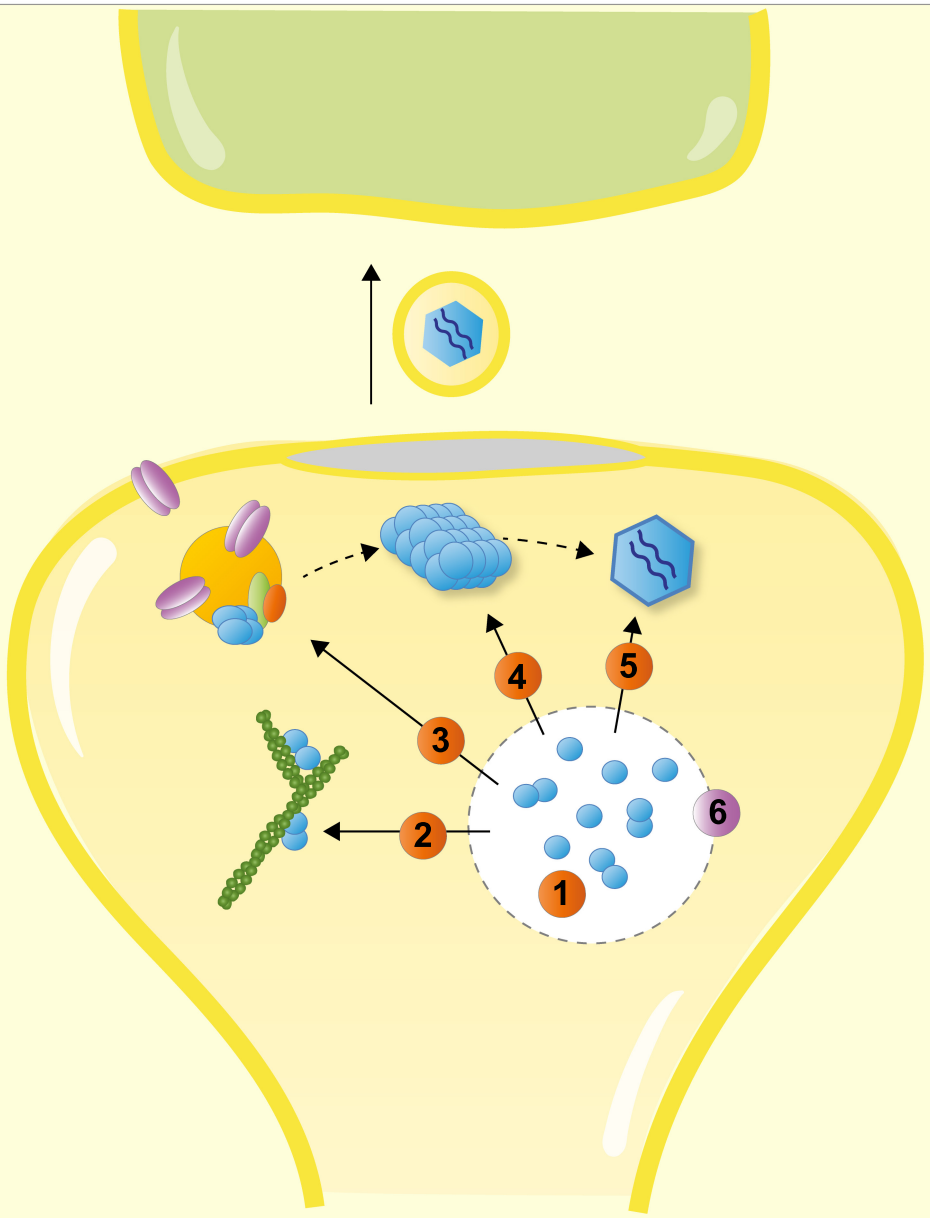
7.1 | Working model of Arc monomer/dimer function in LTP

Several lines of evidence suggest rapid turnover of newly synthesized Arc to support a time-window of LTP consolidation at the perforant input to the dentate gyrus.[122] Local infusion of Arc antisense oligodeoxynucleotides during the LTP maintenance phase results in reversion of LTP to baseline by 30 minutes along with marked inhibition of Arc protein synthesis.[25] Arc synthesis is required for the stabilization of nascent F-actin filaments in the perforant path termination zone on granule cell dendrites,[25] which in turn is required for stable structural enlargement of dendritic spines.[63] The dynamic role of the new Arc in actin filament reorganization fits with the function of a monomer or dimer, rather than a more metabolically stable oligomer (Figure 6, state 2). Although Arc does not bind F-actin directly, it associates with the F-actin-binding protein drebrin A.[64,70] Drebrin A is a major regulator of F-actin stability and spine stability. Arc is SUMOylated during LTP, which favors interaction with drebrin A.[64] Thus, selective targeting of F-actin regulation in LTP could occur through SUMOylation and association with drebrin A. ERK could also target Arc for function in LTP; ERK activity promotes local dendritic accumulation of Arc mRNA at stimulated perforant path synapses,[18,123] controls Arc translation in the synaptic compartment during LTP maintenance,[116] and phosphorylates Arc S206 in the cytoskeletal fraction.[69]

7.2 | Working model of Arc tetramer and large oligomer in LTD

Arc associates with components of the endocytic machinery (AP2, dynamin 2, and endophilin 3) to enhance internalization of AMPARs during LTD.[60] Recombinant Arc has been shown to facilitate the polymerization of dynamin 2 and stimulate its GTPase activity in vitro.[124] We propose the following sequence of events. Arc initially interacts with AP2 in the process of recruiting clathrin to the membrane and selecting cargo. Arc then dissociates from AP2, oligomerizes, and serves as a scaffold for dynamin 2 and endophilin 3, which in turn results in the scission of endocytic vesicles containing receptors for internalization (Figure 6, state 3). The smallest unit of the oligomer supporting endocytosis and LTD could be a tetramer, which based on available evidence is stabilized by CA phosphorylation.[95,97] The full expression of LTD requires larger oligomers, potentially a 32-unit octamer of tetramers[95] (Figure 6, state 4). The enhanced LTD could be due to the concentration of binding partners on a multivalent Arc scaffold or to the recruitment of new partners.

We speculate that new partners are recruited to 32-mer oligomeric Arc by selective exposure of the N-lobe ligand binding site. Arc interacts with the auxiliary subunit of AMPARs, TARP γ-2 (orstargazin). Stargazin and other TARP family members play an important role in



1. Nascent translated Arc monomers
2. Dimers - LTP function
3. Tetramers, phosphorylated, basic LTD function
4. Large oligomers (32 units), PSD regulation, enhanced LTD function
5. Capsids (130 units), intercellular signaling
6. Regulation of oligomerization and switching between states by PTMs, protein-protein interactions, RNA binding, membrane binding and local Arc concentration.

FIGURE 6 Working hypothesis on Arc oligomer function in synaptic plasticity. Arc's ability to self-associate into different oligomeric states is likely to be a key mechanism in terms of its cellular functions, synaptic strengthening, and weakening. We propose that Arc has 5 states in the neuron: (1) Arc monomers and dimers at the site of Arc translation in the spine. (2) Arc dimer role in F-Actin cytoskeletal regulation during LTP. (3) Arc tetramers supporting AMPAR endocytosis and consequent LTD. (4) Arc 32-mers supporting enhanced (full) LTD through recruitment of PSD partners and enhanced lateral mobility of AMPARs (dotted arrows imply a shift between oligomeric states). (5) Large Arc capsids encapsulating mRNA and functioning in intra-cellular signaling. (6) Across all states of Arc, its function and oligomeric state are regulated by Arc protein concentration, availability of protein interaction partners and mRNA, post-translational modifications (PTMs) of the Arc protein, and its interaction/position at the cellular membrane. We refer to Sections 7.1–7.3 for a detailed description.

regulating trafficking and plasma membrane surface mobility of AMPARs by interacting with PSD-95 and related scaffolding proteins.[125,126] The binding of the stargazin cytoplasmic tail to PSD95 serves to tether AMPARs in the post-synaptic membrane, across from pre-synaptic sites of glutamate release. Competitive binding of the Arc N-lobe to the stargazin cytoplasmic tail might disrupt tethering, resulting in increased lateral diffusion of AMPARs away from the PSD and increased endocytosis.[122] The Arc N-lobe is also well-suited for structural remodeling of the PSD through regulation of liquid–liquid phase separation. Interaction of the stargazin cytoplasmic tail with the PSD95 N-terminus regulates phase separation at the synapse.[127] Interaction of GKAP (aka DLGAP1) with the guanylate kinase domain at the PSD95 C-terminus is also crucial for PSD structure.[128,129] GKAP binds PSD95 through its five GKAP repeats, two of which are able to bind the Arc N-lobe.[79,86] Pulldown of Arc complexes from mouse forebrain shows that PSD-95 is the most abundant Arc interactor, with 72 proteins of 107 in the complex containing the Arc N-lobe consensus motif.[130] For full expression of LTD, we propose that higher-order Arc exposes N-lobe binding sites resulting in lateral movement and endocytosis of AMPARs along with structural remodeling of the PSD.

7.3 | On Arc capsid formation and function

The discovery of Arc capsids raises profound questions regarding mechanisms and subcellular sites of assembly, the capture of RNA cargo, and the impact of the released EVs at target sites. The Rous Sarcoma virus seems to start capsid formation by trafficking Gag proteins to the nucleus, where the association with RNA induces dimerization.[131,132] These early particles with RNA are then exported back to the cytosol for assembly to capsid structures. Arc also enters the nucleus where it can be retained or exported back to the cytoplasm through identified nuclear import, retention, and export sequences.[66] There is no data thus far on Arc oligomerization in the neuronal nucleus or evidence for binding to nuclear mRNA. However, fluorescence fluctuation spectroscopy of Arc-EGFP in transfected HeLa cells indicates that Arc is monomeric in the nucleus and monomeric or dimeric in the cytoplasm.[133] If this is the case in neurons, Arc mRNA-induced dimerization would occur in the cytoplasm rather than the nucleus. In HIV Gag, assembly starts in the cytosol before the immature capsid associates with the cellular membrane to complete its formation. Conceivably, pre-existing Arc protein resident in the PSD is utilized in capsids (Figure 6, state 5). However, with an estimated 130 Arc units in a capsid,[97] assembly from newly synthesized protein is more likely.

The formation of Arc capsids in the cytoplasm following transcriptional activation would allow efficient capture of activity-induced RNAs. Current evidence indicates that Arc capsids encapsulate mRNA non-specifically.[80] However, Arc capsids could serve to capture a highly specific plasticity cargo depending on the location and time of capsid formation after the LTP or LTD induction event. LTP in the dentate gyrus is associated with different temporal patterns of expression of mRNA, microRNA, long-non-coding RNA, and differential expression of repeat elements including retrotransposons, simple DNA repeats, and tRNA.[6] The capsid function would therefore depend on the cross-section of RNA, which changes greatly during the first hours after LTP induction. Arc mRNA expression during LTP positively correlates with the expression of specific long-noncoding RNAs and retrotransposons including the endogenous retroviral sequence, MER21B, and the long-interspersed nuclear element–1, L1MD3.[6] It is difficult to speculate on functional outcomes without knowledge of capsid cargo.

In the nervous system, EVs are implicated in several forms of neuron–glia cell interactions, while evidence for inter-neuronal signaling by EVs remains sparse.[83,134–137] Arc capsids may represent a new form of communication specific to activity-dependent modification of neural circuits. The discovery of Arc capsid transfer between cells suggests that this form of Arc works through paracrine signaling, affecting multiple cells. Intercellular transfer of genetic information to neighboring cells may serve as a way of altering cellular state in response to network activity. The functional impact of Arc EVs will be specific to the cargo

profile and the cellular targets (pre-synaptic neuron, adjacent post-synaptic neurons, and glial cell types) in the adult brain. Another interesting area to address is the identity of the target recipient cells for Arc capsids. If the capsid is released from a granule cell dendrite, would it go into another granule cell dendrite, to neighboring glia, or to the perforant path terminal? What effect may this have on plasticity in the hippocampus? At the neuromuscular junction in *Drosophila* larvae, transfer of Arc in capsids from the pre-synaptic boutons to muscle is critical for plasticity, yet the mechanism of Arc function in this context is unknown.

8 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Seminal advances in Arc structure and its biochemical and biophysical characterization provide a basis for understanding how Arc works. Current evidence suggests that the multifunctional nature of Arc relates to hub protein–protein interactions and oligomeric state. Regulation of oligomerization by post-translational modifications and interactions with mRNA have been discovered, with surely much more to be revealed.

The data on oligomerization so far is almost exclusively from in vitro biochemical experiments on purified protein, and knowledge of endogenous Arc oligomerization and oligomeric states in vivo is lacking. Evidence of Arc capsids in mammalian brain tissue is lacking and no information is available on the dynamics of capsid formation and intercellular transfer following synaptic activation, plasticity, or learning. Given the multiple states of the protein and its complex regulation, insights gained from gene knockout and mRNA knockdown approaches are limited.

New tools are needed for imaging Arc activity-state (biosensors) and for probing domain-specific functions and regulatory mechanisms. Tools that would allow labeling or functional manipulation of endogenous Arc would be an advantage. To this end, single-domain antibodies (nanobodies) that bind the Arc CA domain have been developed.[138,139] The recombinant nanobodies bind with high affinity (low nM Kd) and are suitable for expression in mammalian cells as intra-bodies. One of the nanobodies, ArcNb H11, binds selectively to the N-lobe ligand pocket with 35 000 times higher affinity than stargazin, competitively inhibits stargazin binding, and allows affinity purification of intra-cellular Arc. As the molecular function of Arc continues to unfold, strategies for selective manipulation of Arc hub function, oligomerization, and capsid formation can be used to address numerous outstanding issues in systems neuroscience, including the contribution of Arc-dependent synaptic plasticity to neural circuit dynamics and information processing in learning and memory.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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