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Arc: building a bridge from viruses to memory

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Abstract

Arc (activity-regulated cytoskeleton-associated protein) is a neuron-specific immediate early gene that is required for enduring forms of synaptic plasticity and memory in the mammalian brain. Arc expression is highly dynamic, and tightly regulated by neuronal activity and experience. Local translation of Arc protein at synapses is critical for synaptic plasticity, which is mediated by Arc-dependent trafficking of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid)-type glutamate receptors. To date, few structural or biophysical properties of Arc protein have been investigated. Recent studies, including that of Myrum et al. published in the 468:1 issue of the *Biochemical Journal*, now shed light on some intriguing biophysical properties of Arc. These findings show that Arc contains large N- and C-terminal domains around a flexible linker region and that purified Arc protein is capable of self-oligomerization. Intriguingly, these domains show homology with the viral capsid protein found in the gag polypeptide of most retroviruses. These studies provide insight into how Arc may regulate multiple critical cell biological processes in neurons and reveals unanticipated biology that resembles viral trafficking in cells.

Keywords

amyloid β-peptide; Arc; glutamate receptor; memory; oligomerization; synaptic plasticity

Arc (activity-regulated cytoskeleton-associated protein) is a single-copy gene that is highly conserved in vertebrates and whose expression is induced in divergent behavioural paradigms in many species. Indeed, Arc mRNA and protein induction during behavioural learning is so robust and reproducible that cellular imaging of Arc transcription provides a powerful methodology to detect neural networks that underlie information processing and memory. *Arc* mRNA also accumulates in dendrites and becomes enriched at the site of local synaptic activity where Arc protein is locally translated [1,2]. This exquisite regulation of mRNA and protein localization/expression suggests that Arc plays an important role in brain function. Indeed, Arc-knockout mice demonstrate impaired consolidation of long-term memory, without alteration of short-term memory [3]. These mice also show severe deficits in synaptic plasticity such as LTP (long-term potentiation) and LTD (long-term depression) [4,5]. Arc regulates the trafficking of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-

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propionic acid)-type glutamate receptors (AMPARs), by interacting directly with the endocytic machinery [6]. Synaptic scaling of AMPARs, a form of homoeostatic plasticity, is thought to be important for normal synaptic function as it maintains neuronal output without changing the relative strength of individual synapses [7]. Synaptic scaling of AMPARs *in vitro* is also dependent on Arc [8]. In addition, Arc is critical for experience-dependent plasticity in the visual cortex *in vivo* [9], as Arc-deficient synapses in the visual cortex are rendered insensitive to the effects of both experience and deprivation. This is a striking phenotype, and reminiscent of mice that lack important signalling kinases such as CaMKII (Ca^{2+} /calmodulin-dependent protein kinase II) or receptors such as the NMDA (*N*-methyl-D-aspartate)-type glutamate receptors (NMDARs), which are thought to have pleiotropic roles in synaptic plasticity. This implies that Arc, whose expression is regulated downstream of multiple activity-regulated signalling pathways, is one of the main effector proteins at the synapse required for transducing experience into long-lasting synaptic changes in the brain [10]. Arc protein expression has also been observed in the nucleus of neurons where it may regulate gene expression [11], but its mode of localization and precise function in this compartment requires further characterization. Despite Arc's importance in brain function, little is known about its structural and biophysical properties.

Myrum et al. [12] in this issue of the *Biochemical Journal* provide an initial glimpse into Arc's structural characteristics and outline a potential physical basis for its various functional interactions. Analysis of Arc's secondary structure using CD reveals that the protein is predominantly α-helical, which matches *in silico* predictions. Proteolytic assays of Arc, coupled with peptide identification via MS, show that there are two main domains at the N- and C-termini that are connected by a flexible linker region. Interestingly, the previously mapped binding site for dynamin is localized within this predicted unstructured region [6]. Experiments using different ionic conditions and analysis using CD, DSF (differential scanning fluorimetry) and thermal melts revealed that there are two distinct regions of the protein that are likely to correspond to the major N- and C-terminal domains. These domains were stabilized by the addition of heparin, a polyanion that can be used to mimic the negatively charged plasma membrane or cytoskeletal proteins *in vitro*. The domains were stabilized further by the addition of salt, in this case 150 mM potassium fluoride. Given the basic nature of the amino acids within the N-terminus and its predicted isoelectric point of 9.6, the stabilization via heparin is likely to be due to its association within this domain, whereas the latter effect of increasing ionic strength probably stabilizes the C-terminal domain. This suggests that these two domains have a loose tertiary structure and may partially account for Arc's ability to bind many different proteins. Furthermore, the stabilization of the N-terminus with heparin suggests that Arc may be capable of binding the cell membrane directly, which may have implications for Arc's role in endocytosis. Interestingly, the authors note that the observed thermal properties of the C-terminus are consistent with a protein that may form oligomers. To investigate this latter possibility, the authors employed dynamic light scattering, a technique that is capable of measuring the size and heterogeneity of a protein in solution. At physiological temperatures, the authors observed a nearly homogeneous population that suggests that Arc forms oligomers containing approximately 12 monomer subunits. The presence and size of the oligomers appeared to be dependent on the ionic strength of the solution, as purified Arc in distilled

water shifted back to a monomeric state. These putative Arc oligomers were then directly visualized using electron microscopy (EM) under similar ionic conditions. The EM images show a heterogeneous population of oligomeric Arc assemblies and provide further support for ionic strength as a stabilizing force for oligomerization.

The observation that Arc is capable of forming oligomeric assemblies provides some intriguing possibilities for its function *in vivo*. Although Myrum et al. [12] currently do not have evidence of these assemblies in cells or the brain, they speculate that Arc oligomers may serve as a 'hub' that conveys functionally related proteins within the synapse to specific targets. For example, Arc is known to bind the endocytic proteins dynamin and endophilin [6], which are known to form higher-order assemblies, and the Arc oligomer may catalyse the formation of dynamin–endophilin oligomers. This idea is supported from another recent study that shows Arc can augment dynamin's GTPase activity *in vitro* [13]. Similarly, another Arc-interacting protein, CaMKII [14], has been shown to form homo- and hetero-meric assemblies that are required for its kinase activity [15]. The size of this assembly overlaps well with the predicted size of the observed Arc oligomers, which raises the interesting possibility that CaMKII binds Arc in its assembled state rather than as a monomer. Myrum et al. [12] also show that Arc may undergo structural rearrangement when it binds another binding partner, presenilin 1 (PS1). This interaction has implications for Alzheimer's disease as PS1 forms the catalytic part of the γ-secretase complex, which is required for cleavage of the APP (amyloid precursor protein) into amyloid β-peptide. Arc regulates activity-dependent APP cleavage in dendrites via this interaction [16].

The observation that the C-terminus of Arc has the capacity to oligomerize is intriguing. A recent study describes the crystal structure of two domains within Arc's C-terminus that have a high degree of homology with the HIV-1 capsid protein [17]. Capsid proteins are known to spontaneously self-assemble, and this assembly can be induced *in vitro* by increasing ionic strength. Virus capsids are integral for virus infectivity and intracellular trafficking. The functional relevance of Arc's capsid protein homology needs to be determined. One wild possibility is that Arc is acting as a novel protein storage module in line with the prion-memory hypothesis outlined by Kandel and colleagues [18]. It has been shown that some proteins assemble into amyloid or prion-like oligomers that are necessary for long-term synaptic plasticity [18]. It is possible that Arc oligomers assemble at the synapse in response to neural activity and encode information for long-term storage in neurons.

There are a number of questions raised by these biophysical findings. (i) Does Arc self-assemble into high-molecular-mass complexes in the brain and in neurons *in vivo*? (ii) If so, under what conditions does Arc self-assemble and where in the cell? (iii) What is the functional relevance of Arc oligomers in the context of synaptic plasticity and memory formation? (iv) What is the functional relevance of the Arc–virus connection? Arc is a fascinating protein and addressing these questions will shed light not only on Arc-dependent cell biology, but also on how vertebrate brains evolved their fantastic information storage capabilities.

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Abbreviations:

AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid
AMPAR	AMPA receptor
APP	amyloid precursor protein
Arc	activity-regulated cytoskeleton-associated protein
CaMKII	Ca^{2+} /calmodulin-dependent protein kinase II
NMDA	*N*-methyl-D-aspartate
PS1	presenilin 1

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