The arcane relationship between Arc's virus-like properties and its role in AMPA receptor endocytosis.

By Marijke Stokkel

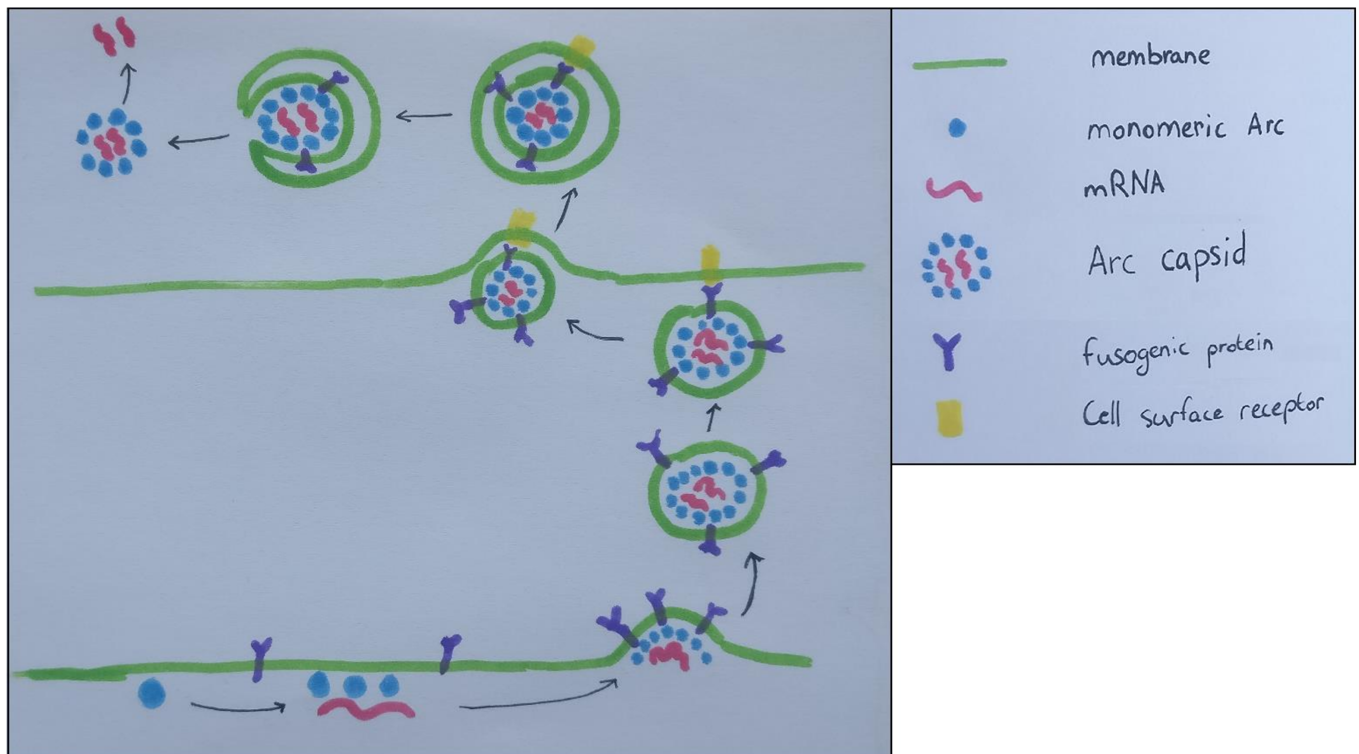

Figure 1. Arc assembles into virus-like particles and is transferred to neighboring cells where it releases its packaged mRNA.

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Supervisor: Dr. Harold MacGillavry

Second examiner: Dr Frederik Verweij

Abstract

Arc is a critical player in synaptic plasticity and is essential for long term memory consolidation. The most well-established method by which Arc influences synaptic plasticity is by enabling the endocytosis of AMPA receptors. Recently it was shown that Arc originates from the Ty3/Gypsy retrotransposon family. Similar to many endogenous retroviruses (ERVs) Arc was shown to bind and package its own mRNA into virus like particles (VLPs). These particles were shown to release from cells and 'Infect' neighboring neurons, where they were observed to undergo activity-dependent translation. Indeed the virus-like properties of Arc is an emerging topic of interest. However, the relationship between the protein's ability to bind and sequester AMPA receptors and to oligomerize and form capsids remains unclear. Here we review factors influencing the oligomerization of Arc and propose that capsid formation and receptor endocytosis are two competing processes which may be balanced by post translational modifications. We compare Arc to other endogenous retroviral proteins and propose a model for the virus-like life cycle of Arc. This is important for understanding the mechanism behind this type of intercellular communication and how it could affect synaptic plasticity.

Leken samenvatting

Arc is een eiwit dat belangrijk is voor de signaaloverdracht in de synaps en het leren. Signalen worden doorgegeven tussen axonen en dendrieten van verschillende neuronen door middel van neurotransmitters. Als een receptor op het dendriet een neurotransmitter bindt gaat het ionenkanaal van de receptor open en depolariseert het membraan waardoor er een signaal door het neuron gaat. Arc kan specifieke neurotransmitter receptoren binden, namelijk AMPA receptoren, waarna de receptoren door endocytose de cel in wordt gebracht. Door het aantal AMPA receptoren aan het membraanoppervlak te reguleren kan Arc de kracht van de signaaloverdracht van de synaps bepalen. Dit is essentieel voor synaptische plasticiteit en maakt leren mogelijk.

Arc is afkomstig van een endogeen retrovirus dat zijn DNA heeft geïntegreerd in het genoom van de gastheer. De meeste endogene retrovirussen zijn inactief en kunnen geen virusdeeltjes meer vormen of zich vermenigvuldigen. Arc daarentegen kan zijn eigen mRNA verpakken in een virus capside en zo een virus-achtig deeltje vormen. Deze virus-achtige deeltjes kunnen het neuron verlaten in een blaasje en naburige neuronen binnendringen. Wanneer deze Arc blaasjes zijn opgenomen komt het mRNA vrij voor translatie. Voor dit literatuur onderzoek is gekeken naar hoe Arc zijn mRNA verpakt in virus-achtige deeltjes en hoe deze worden overgebracht naar andere cellen. Hiervoor vergeleken we Arc met andere eiwitten afkomstig van endogene retrovirussen en andere vergelijkbare retrovirussen zoals HIV-1. Daarnaast vergeleken we Arc's rol in intercellulaire mRNA overdracht met zijn rol in AMPA receptor endocytose.

Interactie met het eigen mRNA blijkt de zelf-associatie en capside vorming van Arc te bevorderen. Net als HIV-1 kan Arc ook ander mRNA binden en verpakken, al gaat dit minder efficiënt. Waarschijnlijk heeft Arc mRNA een speciaal verpakkingssignaal dat andere mRNA moleculen niet hebben waardoor het beter bindt aan het Arc eiwit. Wanneer het mRNA verpakt is in de eiwit capside wordt deze losgelaten in een blaasje en opgenomen door andere cellen via endocytose. De Arc virus-achtige deeltjes gebruiken mogelijk een fusie-eiwit afkomstig van een ander endogeen retrovirus om te binden aan andere cellen en te ontsnappen uit het endosoom.

Modificaties als fosforylatie kunnen de werking van Arc beïnvloeden door bijvoorbeeld de lokalisatie binnen de cel te veranderen of de zelf-associatie die nodig is voor capside vorming te inhiberen. Fosforylatie op specifieke aminozuren kan de zelf-associatie van Arc stoppen waardoor ook de AMPA receptor endocytose wordt geremd. Dit suggereert dat zelf-associatie van Arc belangrijk is voor endocytose. Echter, dit is nog onzeker want er kunnen ook nog andere redenen zijn waarom deze modificatie receptor endocytose remt. Daarnaast blijkt de binding van Arc met eiwitten betrokken bij receptor endocytose de Arc zelf-associatie te remmen. Dit suggereert dat receptor endocytose en capside vorming twee competerende processen zijn. Het is belangrijk om te begrijpen hoe deze twee mechanismen elkaar beïnvloeden omdat dit ons meer inzicht kan geven in hoe Arc betrokken is bij synaptische plasticiteit en leren.

Introduction

Activity-regulated cytoskeleton associated protein, otherwise known as Arc or ARG3.1, is essential for synaptic plasticity[1,2]. Arc is capable of binding to a subtype of neurotransmitter receptors called AMPA receptors. These receptors propagate signals through the synapse in response to neurotransmitter binding. Binding of AMPA receptors by Arc plays a key role in synaptic plasticity, as it leads to receptor internalization resulting in a decrease in synaptic strength. Not only does Arc-mediated AMPA receptor endocytosis affect synaptic strength, the process itself is regulated by synaptic activity, as activation of metabotropic glutamate receptors induces Arc[1,2]. Arc expression is highly localized to active synapses and is induced in distinct phases, namely a rapid and delayed response which have been proposed to enable memory formation and consolidation, respectively[2–4].

The Arc-mediated internalization of AMPA receptors is facilitated by binding of receptor subunit GluA1 and recruitment of clathrin mediated endocytosis machinery[5–7]. Endocytosis is enabled by Arc binding to AP-2, dynamin and endophilin[5–7]. Interaction of Arc with AP-2 is critical during the early stages of receptor internalization, as it allows the formation of the clathrin coated pit[5,8]. In contrast, interaction with dynamin and endophilin occurs at later stages and is responsible for membrane constriction and fission and uncoating of vesicles[9,10]. Once internalized, the AMPA receptors localize to recycling endosomes[7]. This way GluA1 cell surface expression, which is normally increased by chronic neuronal inactivity, is reduced by high Arc expression[7]. By reducing GluA1 surface expression Arc is capable of lowering the amplitude of AMPA receptor-mediated mEPSCs[5]. In contrast, reduced Arc expression leads to increased AMPA receptor signaling and abrogates homeostatic scaling of AMPA Receptors[7].

Arc's function in synaptic activity is intriguing, especially since phylogenetic analyses show that mammalian Arc originates from the Ty3/Gypsy family of retrotransposons[11]. A retrotransposon is a transposable genetic element and differs from a retrovirus in that it lacks an envelope protein and replicates within the same cell[12]. In contrast, a retrovirus does contain a functional envelope protein and can form virus particles that infect neighboring cells[12]. A retrotransposon can become a retrovirus by acquiring an envelope protein and similarly a retrovirus can become a retrotransposon by losing the envelope protein. Retrotransposons and retroviruses can become endogenous through mutations in the inserted DNA that reduce the activity and infectivity of these transposable genetic elements[13]. Endogenous retrotransposons and retroviruses (ERVs) are inherited vertically like other genes in the genome and can adapt to perform cellular functions[12]. This is likely what has occurred for Arc given its high phylogenetic similarity to Ty3/Gypsy. However, despite being adapted to function in neuronal signaling, Arc has retained some of its retrotransposon like functions. Arc subdomains, for instance, are structurally similar to viral Gag proteins and similar to viral Gag and Ty3 the Arc protein is able to oligomerize[14,15,16,17].

Retroviral Gag polyproteins usually contain a matrix (MA), capsid (CA) and nucleocapsid (NC) protein, as well as a p6 domain which require proteolysis to form a mature capsid[18]. In contrast, mammalian Arc only contains a capsid and matrix domain (Figure 3A). Drosophila Arc, which originates from a separate branch of the Ty3/Gypsy family and has been duplicated, contains a NC domain and/or CA domain (Figure 3A)[17,19]. Structural determinations of Arc are limited to subdomains, or require other methods like circular dichroism (CD) and electron microscopy (EM), as the full length protein does not crystallize[16,17]. Nevertheless, a wealth of structural information is already available. It is known that mammalian Arc folds into two distinct regions termed the N-terminal domain (NTD) and C-terminal domain (CTD) which are connected via a linker[16]. These domains are oppositely charged and

in the monomeric protein lie juxtaposed with an unextended linker forming a compact structure[20]. Despite difficulty crystallizing the full length protein, the rat Arc CTD can be co-crystallized with peptides from binding partners like AMPA receptor auxiliary subunit γ2 (TARPγ2) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII)[17]. These structures are superimposable with the HIV Gag protein structure, revealing that similar to HIV Gag the CTD of rat Arc contains two subdomains forming a bi-lobar structure. This structure consists of the N-lobe and C-lobe, of which the N-lobe contains a hydrophobic pocket enabling interaction with TARPγ2 and CaMKII. Whereas the CTD contains peptide binding sites, the NTD consists of long α-helices which are predicted to form anti-parallel coiled-coils capable of interacting with lipid membranes[20].

Not only has Arc retained its retroviral fold throughout evolution, it has also retained some of its retroviral function. In a groundbreaking study by Pastuzyn *et al* it was shown that mammalian Arc protein can bind and encapsidate mRNA in virus-like capsid structures[11]. These capsids can transfer between cells via extracellular vesicles and the transported mRNA can undergo activity dependent translation in recipient cells. Similarly, this Arc-mediated type of intercellular communication was observed in Drosophila Melanogaster. Here, transfer of Drosophila Arc (dArc) capsids was shown to occur from motorneurons to muscles[21]. More importantly, blocking this transfer was shown to have adverse effects, as it abrogated synaptic plasticity[21].

These discoveries have led to an increased interest in Arc, especially concerning oligomerization and capsid formation. Here we review the mechanisms behind Arc's capsid formation and transfer to recipient cells as well as the relationship between Arc's virus-like behavior and its role in AMPA receptor endocytosis. We examine literature regarding Arc or related proteins such as Ty3 Gag, HIV-1 Gag, dArc and PEG10. Based on this we propose a model for the virus-like life cycle of Arc. Additionally we propose that Arc's virus-like properties compete with its ability to endocytose AMPA receptors, which may have functional consequences regarding Arc's role in synaptic plasticity.

1. Capsid structure

1.1 Ty3 retrotransposon capsid structure

How Arc oligomerizes into a capsid may be gleaned from the maturation process and structure of similar capsids such as the Ty3 retrotransposon. Structural analyses of the Ty3 retrotransposon capsid by Dodonova *et al*[22] showed that similar to HIV-1 the Ty3 Gag protein requires proteolysis to form mature capsids. The first step in the formation of the immature HIV-1 capsid is the association of the Gag protein to the plasma membrane forming a curved lattice[23]. Only after the immature HIV-1 particle is released from the cell does proteolysis take place resulting in structural rearrangement of the capsid which is essential for infectivity[23]. Addition of purified mammalian Arc to the outside of giant unilamellar vesicles (GUVs) has been shown to lead to vesiculation into the GUV interior[24]. This indicates that Arc in absence of RNA can interact with membranes and alter curvature in a way that supports exocytosis. It is possible that, similar to HIV-1, Arc distorts the membrane by the formation of a curved lattice. In contrast to HIV-1, the Ty3 capsid did not change size or shape upon maturation after proteolysis[22], indicating that the maturation process may be more subtle. Immature Ty3 particles consisted of a thick electron dense ring of which the interior appeared empty, while mature particles had an electron dense interior with a thin outer ring[22]. This could correspond to the RNA releasing from the Gag domain and condensing in the capsid interior, explaining why proteolysis is required for capsid formation[25].

Detailed structural analysis of the Ty3 capsid revealed interactions and interfaces between capsid proteins and subdomains important for the assembly[22]. As mature Ty3 capsids could not be isolated

the structural analysis by Cryo-ET was performed on a proteolysis resistant Ty3 capsid. These capsids structures were shown to consist of three types of capsomers: pentamers and two types of hexamers. A homology model of the human Arc protein was fitted into the capsid structure to get a more detailed understanding of the structure. This revealed that the different capsomers were not the result of different domain folds, but rather differences in the relative orientation of the CTD and NTD. These differences in relative orientations between domains may be determined by the flexible linker[20]. Regardless of the type of capsomer, the CA-NTDs were shown to be protruding outwards relative to the CA-CTDs which formed the inner layer of the capsomers. This reflects the ability of the NTD to bind lipid membranes[20], which could be essential during capsid assembly. Together this demonstrates how structural information on monomeric proteins can explain how capsids are formed and how capsids structures may reveal mechanism of higher-order assembly.

The pentamers and hexamers of which the Ty3 capsid is build are formed by the asymmetric unit which consists of 9 individual CA domains. The CA-NTDs in this asymmetric unit were shown to exist in two types of conformations in which the 36 N-terminal amino acids mediated intermolecular interactions. In the homology model of monomeric Ty3 Gag these 36 amino acids do not display a defined structure, whereas in the capsid these amino acids form an electron density. This indicates that this amino acid stretch is flexible in the monomeric protein and becomes stabilized in the capsid. In one conformation this amino acid stretch was shown to interact with helix 3 of one NTD and helix 1 of the neighboring NTD. While in the other conformation half of the electron density was not visible indicating that this part may be disordered. By mediating interactions between NTDs in the asymmetric unit these 36 N-terminal amino acids likely play an important role in in the formation of the capsid structure.

1.2 Drosophila Arc capsid structure

Drosophila Arc was shown to cluster separately from the tetrapod branch indicating that it was domesticated from a separate branch of the Ty3/gypsy family[11]. Additionally, Arc was duplicated in Drosophila resulting in two isoforms (dArc1 and dArc2). Despite this the capsids formed by both dArc isoforms were shown to be alike and to share commonalities with the Ty3 capsids. Similar to the Ty3, the NTDs of the dArc capsomers were oriented outwards, with the CTD being oriented more towards the interior of the capsid[26]. Uniquely the dArc capsids displayed 5-8 nm spikes protruding from the capsomers, corresponding to an amino acid stretch upstream of the NTD. Based on the protein sequences these N-termini were predicted to form aliphatic α-helices. It was proposed that these aliphatic α-helices could potentially mediate oligomerization and/or interaction with proteins or membranes. Ty3 capsids were not shown to form these N-terminal spikes and this is likely because it lacks the amino acid sequence corresponding to this region. The rat Arc sequence does contain the charged residues at similar sites to dArc, but whether mammalian Arc forms these spikes in the capsids structure and what its functions may be remains unknown.

1.3 Proposed mammalian Arc capsid structure

Structural determinations have shown that the Ty3 and dArc capsids have a different size and symmetry. The Ty3 capsid with its T=9 symmetry is formed by 540 individual proteins (Figure 2A) and has a radius of 24 nm[22]. Compared to this the dArc capsids are smaller, consisting of only 240 proteins with a radius of 18.5 nm and a T=4 symmetry (Figure 2B)[26]. The difference in capsid size means that the interior of the dArc capsid is smaller than that of Ty3 and therefore less genetic material can be packaged. Erlendsson *et al* proposed that the Drosophila capsids may have shrunk as a result of needing to package less mRNA[26]. This is because the dArc capsids only need to package dArc mRNA and not an entire Gag-Pol polyprotein, as is the case for a Ty3 retrovirus. It seems likely then that the mammalian Arc capsids are also smaller than Ty3 and may be more similar in size to

dArc. Indeed Pastuzyn *et al* found that rat Arc capsids had a radius of 16 nm[11]. Interestingly, the mammalian Arc has a longer protein sequence compared to dArc[19], which means that a longer mRNA sequence needs to be packaged in a smaller capsid interior. Therefore the packing density of mammalian Arc mRNA may be higher than for dArc mRNA.

Despite different CA domain lengths of the dArc isoforms, 110 and 165 residues for dArc1 and dArc2 respectively, both isoforms assemble into T=4 capsids with a 18.5 nm radius. This can be explained by the similar capsomer structures observed between dArc1 and dArc2. rArc on the other hand, despite having a similar length (163 residues), has a different monomeric fold and forms a capsid with a smaller radius (16 nm). While parts of the NTD and CTD of rArc and dArc may overlap the different monomeric fold of rArc is likely to result in different interfaces in the capsomers. Different intermolecular interactions of rArc CA domains may explain why rArc capsids have a smaller radius than dArc. Alternatively, mammalian Arc capsids may have a lower triangulation number requiring fewer proteins to form a capsid, resulting in a smaller capsid.

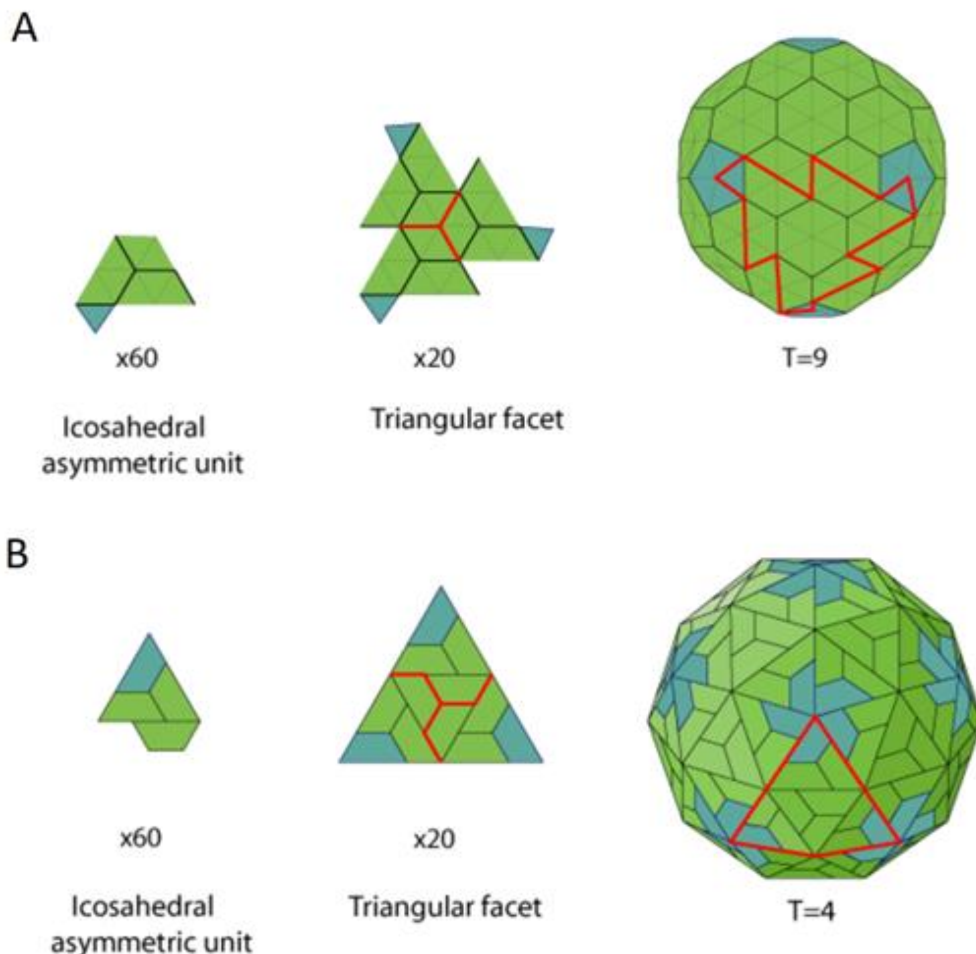

Figure 2. Icosahedral capsid structure of Ty3 (A) and dArc (B) capsids[27,28]. An icosahedron consists of 20 triangular facets which consist of 60 asymmetric units[29]. The asymmetric unit of the Ty3 capsid contains 9 Gag molecules resulting in a capsid of 9*60=540 Gag molecules. The asymmetric unit of the dArc capsid contains 4 Gag molecules resulting in a capsid of 4*60=240 Gag molecules.

2. Factors influencing oligomerization of mammalian Arc

2.1 Post-translational modifications can promote or inhibit Arc oligomerization depending on the modification and site.

In order to mediate capsid release Arc must be able to associate with lipid membranes. While isolated Arc protein has been shown to directly alter membrane curvature in vitro[24], certain post-translational modifications may further promote membrane interactions. In neurons for example, murine Arc has been shown to be palmitoylated in the N-terminal half of the protein enabling the insertion into the lipid bilayer[30]. Palmitoylation is a dynamic process regulated by transferases and thioesterases and plays a critical role in synaptic plasticity[31]. Proteins anchored into membranes by palmitoylation have been shown to diffuse into lipid rafts, which may promote protein-protein interactions by increased localization[32]. This way, palmitoylation-mediated membrane association of Arc may enhance oligomerization or release of Arc capsids in extracellular vesicles.

Not only has Arc been shown to be palmitoylated, it can also be phosphorylated[33,34]. While phosphorylation of Arc may not necessarily affect oligomerization, it may still be able to influence capsid formation by altering subcellular localization. Phosphorylation at S206 for instance, was shown to be increased by synaptic activity in neuroblastoma cells and associated with an increased cytosolic localization in hippocampal neurons[35]. Similarly, phosphorylation of S67 by TNIK appears required for cytosolic localization, as the S67A mutant was shown to be retained in the nucleus[33]. A phosphomimetic S67D mutant of Arc however, did not affect capsid formation or morphology[33]. This indicates that phosphorylation of Arc or lack thereof can alter subcellular localization, which in turn may impede capsid formation at the plasma membrane.

In some instances however, phosphorylation of Arc has been shown to affect oligomerization, namely at S260 and T278[33,34]. Moreover, phosphorylation of S260 by CaMKII was shown to be inducible by excitatory activity in cultured cortical neurons[34]. Phosphorylation of Arc at S260 and T278 was not proposed to interfere with protein ligand interaction as both phosphorylation sites were shown to be distant from the hydrophobic pocket responsible for peptide ligand binding (Figure 3B)[34]. However, phosphorylation of S260 was predicted to inhibit oligomerization as this site was shown to be close to the dimer interface (Figure 3B)[34]. Additionally, this PTM was proposed to form an additional salt bridge competing with intermolecular interactions required for oligomerization[34]. Indeed, phosphomimetic Arc S260D mutants formed tetramers but did not oligomerize[34]. In contrast, S260A mutants displayed temperature-dependent oligomerization similar to WT Arc[34]. In another study by Walczyk-Mooradally *et al,* phosphorylation of T278 was also shown to negatively affect oligomerization, with the T278D mutant forming irregularly shaped aggregates[33]. This demonstrates that dynamic PTMs like phosphorylation, which may be regulated by synaptic activity, can inhibit Arc oligomerization and by extension capsid formation.

Aside from oligomerization the phosphorylation at S260 may also inhibit AMPA receptor endocytosis. The S260D mutant, unlike WT Arc, was shown unable to reduce the amplitude of mEPSCs in Purkinje neurons[34]. Similarly, a R335E mutant that fails to oligomerize also did not reduce mEPSCs[34]. Since Arc reduces mEPSCs through endocytosis of AMPA receptors[5] it may be that Arc requires oligomerization, or at least functional interaction surfaces that mediate oligomerization, for AMPA receptor endocytosis. Alternatively, mutations that inhibit oligomerization may simultaneously abrogate protein interactions required for binding of clathrin mediated endocytosis machinery. While mutations that disrupt oligomerization were not shown to disrupt binding to TARPγ2 or alter subcellular localization[34], the binding of S260D Arc to AP-2, endophilin and dynamin was not verified. The inability of S260 phosphorylated Arc to reduce mEPSC amplitude potentially links Arc oligomerization to AMPA receptor endocytosis, but the underlying cause remains unknown.

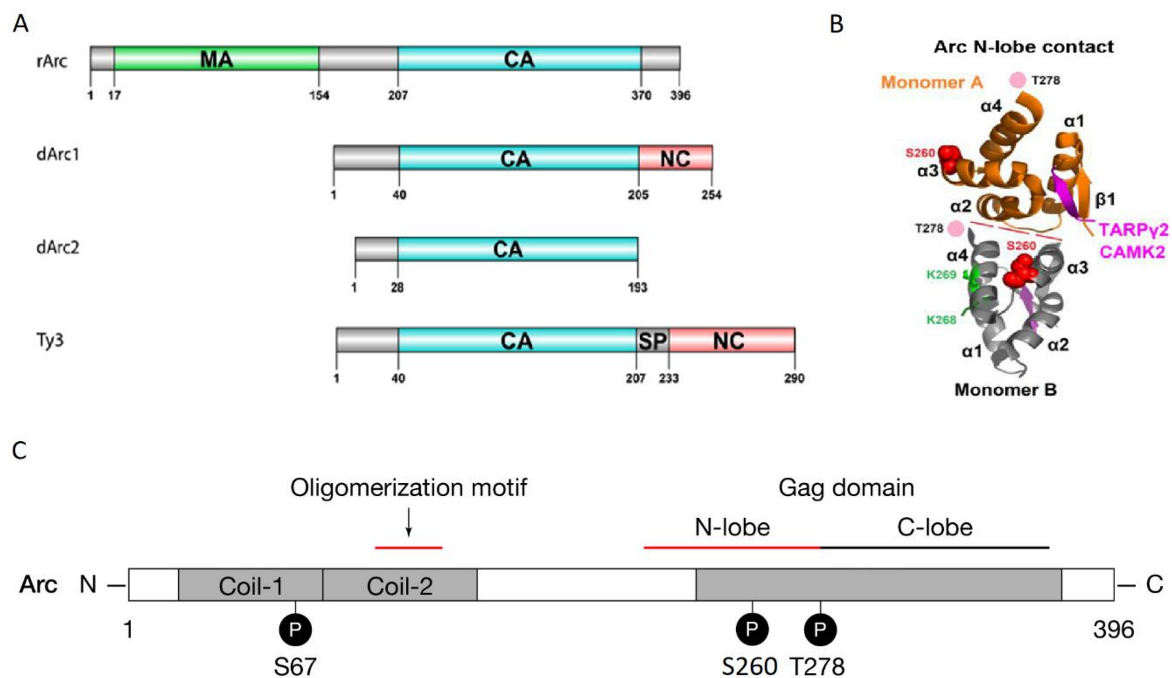

Figure 3. PTMs of Arc can have various effects depending on the domain. (A) Domain alignment of rArc, dArc1, dArc2, Ty3. (B) Structure of the N-lobe contact in dimerized Arc. Phosphorylation site S260 (red) lies at the interface between the monomers, but distant from the peptide binding site (Magenta). (C) Different phosphorylation sites are highlighted on the various mammalian Arc domains. Adapted from Erlendsson *et al* (Nat Neurosci., 2020), Zhang *et al* (Mol cell., 2019) and Walczyk-Mooradally *et al* (JNC 2021)[26,33,34].

2.2 Protein ligand binding by Arc can inhibit oligomerization.

Arc has been described as an interaction hub due to the numerous protein-protein interactions it can establish[36]. Most of these protein ligands include neurotransmitter receptor subunits or associated proteins, such as TARPγ2, CaMKII, WAVE1, GKAP, IQSEC2, GluN2A and GluN2B. These have been shown to bind to the N-lobe[17,20,37]. More specifically, TARPγ2, CaMKII, GluN2A and GluN2B are known to bind to the same hydrophobic pocket in the N-lobe[17,37]. Aside from its well established protein ligands, Arc must also be able to bind other Arc molecules in order to self-oligomerize. This raises the question whether ligand binding competes with Arc oligomerization. One possible way in which binding of protein ligands could compete with Arc oligomerization is by inducing structural changes that prevent oligomerization. This may be domain specific, as binding of peptide ligands to the N-lobe was shown to only induce structural changes within the N-lobe and not in the C-lobe[37]. Moreover, the structural changes observed were the same for different peptides, as binding of GluN2A, TARPγ2, WAVE1 and GKAP all led to the formation of a β-strand structure within the N-lobe (Figure 3B)[37,20]. Interestingly, the N-lobe of isolated Arc was shown to be able to adopt a high energy conformation distinct from the ligand bound state[37]. This could potentially be the conformation Arc adopts during oligomerization and if so this conformation would compete with ligand binding. Indeed addition of GluN2A peptides to purified Arc was shown to suppress oligomerization leading to only monomeric Arc being observed[37].

2.3 Binding of mRNA by Arc induces oligomerization.

In the groundbreaking paper first describing capsid formation by mammalian Arc, stripping the protein of bound mRNA was shown to reduce the number of fully formed capsids[11]. The presence of mRNA was proposed to enhance capsid formation regardless of coding sequence, as addition of

either Arc or GFP mRNA to Arc protein induced capsid formation[11]. Moreover, mRNA packaging by Arc appeared stoichiometry dependent with both highly abundant Arc and AsnA mRNA being found in Arc capsids[11]. However, the extent to which Arc oligomerization is induced by mRNA does appear to differ between mRNA species. Compared to EGFP mRNA, the addition of Arc mRNA to purified Arc protein led to a larger increase in higher-order oligomeric species[38]. It was proposed by Eriksen *et al* that this difference may have arisen from a difference in length between Arc and EGFP mRNA[38]. However, other factors such as Gag binding sites or fold were not taken into account. Aside from a higher ability to induce capsid formation, Arc mRNA also appears to have a higher affinity towards the protein as Arc mRNA and protein were shown to co-immunoprecipitate in lysate[11]. These initial results highlight that mRNA promotes Arc capsid formation, but also that the mechanisms underlying mRNA packaging during capsid formation are still unknown.

Answers may be gleaned from HIV-1, as similar to Arc the HIV Gag protein packages cellular RNA in the absence of viral RNA[39,40]. Moreover, packaging of cellular RNA also appears mostly concentration dependent[39]. However, it was shown that mRNA with a longer 3'UTR has a higher average packaging efficiency than mRNA with shorter 3'UTRs[40]. One possible explanation for this could be that RNAs with longer 3'UTRs have a larger number of available Gag binding sites when bound to ribosomes[40]. Other studies have focused more on the 5'UTR and have found that HIV-1 has multiple RNA binding sites in the 5'UTR in which mutations result in decreased packing[41]. Moreover, combining mutations in different binding sites resulted in synergistic defects in RNA packaging efficiency[41] indicating that the ability of RNA to bind multiple Gag molecules may be required for HIV-1 capsid assembly. Not only do the number of functional Gag binding sites dictate RNA packaging, the 3D structure appears to do so as well. HIV-1 was shown to preferentially package RNA which folds into specific structures that promote RNA dimerization and Gag binding[42].

There may be multiple reasons behind why Arc mRNA has a stronger effect on Arc oligomerization than EGFP mRNA. Arc mRNA may have specific binding sites encoded in the 3'UTR and/or 5'UTR which in EGFP may be more aspecific. Additionally, Arc mRNA could potentially have more binding sites allowing more Arc proteins to be brought into close contact thereby seeding capsid formation. The Arc protein may also have a preference for specific mRNA folds during packaging. However, all of this is yet to be confirmed.

So far little research has been conducted into the interactions between Arc protein and mRNA. Recently another mammalian endogenous retrovirus/retrotransposon capsid protein, namely PEG10, has been identified and shown to bind and package mRNA into capsid structures[43]. In contrast to Arc, mRNA binding sites for the PEG10 protein were investigated and showed that specific regions of the 3'UTR promoted transfection with PEG10 virus like particles (VLPs). The 500bp proximal of the 3'UTR in combination with the intact 5'UTR was sufficient for RNA packaging and could be used to target an mRNA of interest for secretion in PEG10 VLPs[43].

2.4 The N-terminal domain contains a hydrophobic motif essential for oligomerization.

The aggregation propensity and the insolubility of Arc have been shown to be dependent on the NTD[20], indicating that this domain could possibly mediate oligomerization and capsid formation. The NTD contains α-helices predicted to form an antiparallel coiled-coil (Figure 3C)[20]. Co-purification assays showed that coil-2 of the NTD harbors high affinity for WT Arc and the Arc-NTD, but not for the Arc linker region or Arc-CTD[44]. Mutational screening indicated that residues 99-126 may be required for oligomerization and contain an oligomerization motif constituting residues 113-119 in which mutations completely abolish binding to Arc[44]. Crystal structures confirmed the antiparallel coiled-coil structure of this region, which is formed by hydrophobic interactions, with the oligomerization motif lying in the center of the coiled-coil. The hydrophobicity of the coiled-coil

structure appears essential for inter-molecular interactions, as mutations decreasing the hydrophobicity of this region (M113D and W116D) were shown to inhibit association with WT Arc. The identified oligomerization motif was shown to be critical for higher-order oligomerization, with S113-119A Arc only assembling into dimers. Mutations in this motif not only affect Arc oligomerization in isolation, but also in the presence of mRNA, as the S113-119A mutant did not display mRNA enhanced oligomerization.

2.5 Model for capsid formation

As reviewed, numerous factors influence Arc oligomerization and capsid formation. Taking all these factors into account it may be possible to deduce the relationship between Arc capsid formation and Arc mediated receptor endocytosis. We propose that these are two competing processes. The most convincing evidence being that ligand binding in the hydrophobic pocket of the N-lobe inhibits Arc oligomerization[37]. While phosphomimetic mutants (S260D) incapable of oligomerizing do not reduce AMPA receptor-mediated mEPSCs, the underlying cause of this is unexplored and may be separate from the ability to oligomerize. It seems implausible that Arc could bind other Arc molecules and simultaneously be able to recruit endocytosis machinery. This is because the NTD important for oligomerization also contains the binding sites for endophilin-3 and dynamin-2[44,45]. While the effect of peptide binding in the N-lobe on oligomerization has been tested, the effect of peptide binding at other sites is still unknown and may further support our hypothesis. If capsid formation and AMPA receptor endocytosis are indeed two competing processes then it would be expected that binding of mRNA by Arc, which promotes oligomerization, would inhibit AMPA receptor endocytosis. This would raise additional questions as to how the balance of these processes is regulated as Arc mRNA is highly localized in active synapses[46].

Post translational modifications regulate protein function and localization and may likely be involved in balancing Arc's opposing functions. For instance, PTMs may allow or inhibit protein-protein interactions that promote one process over the other. Additionally, PTMs could potentially alter Arc conformation. For instance, PTMs in the linker between the NTD and CTD may reduce the flexibility which may inhibit oligomerization. Lastly, PTMs may target Arc to specific organelles where Arc performs different functions. More research into how post-translational modifications affect Arc function may contribute to our understanding of how AMPA receptor endocytosis and capsid formation by Arc are related.

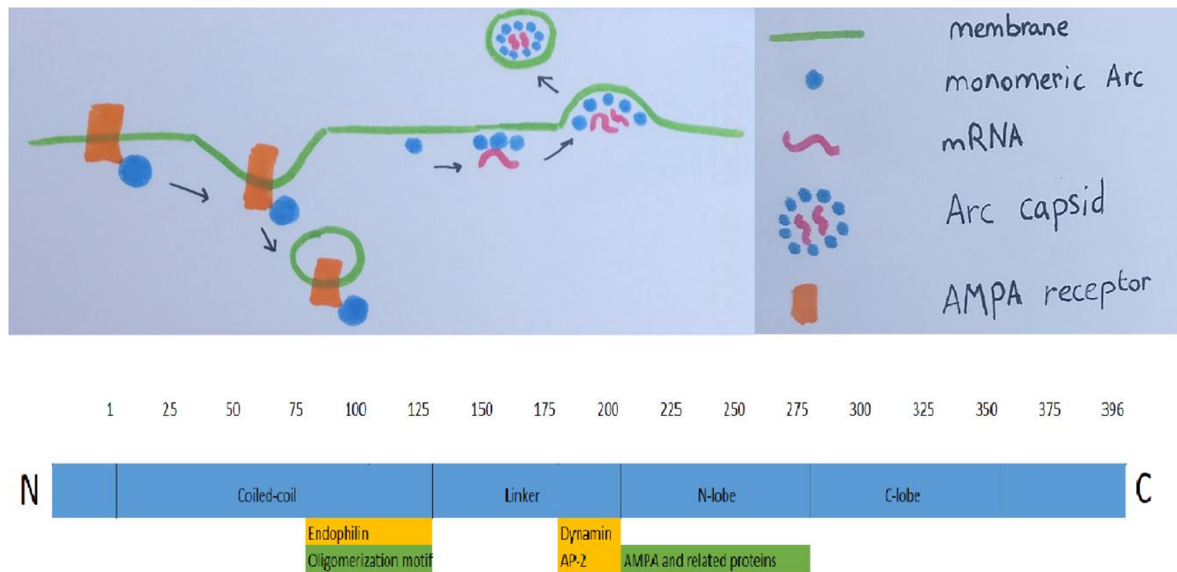

Figure 4. Receptor endocytosis and capsid formation by Arc are competing processes. (Top left) Arc binds an AMPA receptor subunit with its N-lobe in the CTD and recruits endocytosis machinery with its NTD. (Top right) Arc requires the oligomerization motif in the NTD for oligomerization which is promoted by binding of mRNA. Binding of protein ligands to the N-lobe inhibits Arc oligomerization. (Bottom) Arc domains and binding sites. The endophilin binding site required for endocytosis of AMPA receptors overlaps with the oligomerization site required for oligomerization and capsid assembly.

3. Capsid release from cells

In the paper describing dArc1 mRNA transfer across the neuromuscular junction it was proposed that dArc1 capsids are released in exosomes originating from multi vesicular bodies (MVB)[47]. MVBs are formed when vesicles bud into an endosome resulting in intralumenal vesicles (ILVs)[48]. Once the MVB has matured it fuses with the plasma membrane releasing the ILVs as exosomes (Figure 5.1)[48]. While this could be possible, it does require the Arc capsids to be assembled at and invaginated into the endosome. It seems more likely that, as proposed by Pastuzyn *et al*[11], capsids assemble at the plasma membrane where they pinch off from the cell as this requires fewer steps (Figure 5.2). Both processes could take place, as there is currently no literature supporting one mechanism over the other. Research into the localization of Arc translation may reveal more insights into which types of membranes are bound by Arc and where oligomerization and capsid release may take place.

Regardless of the origin of the exosome, the membrane association of Arc is likely to play a key role in driving membrane deformation during oligomerization. Furthermore, it may be possible that Arc oligomerization at the membrane is sufficient for enabling membrane fission and release of exosomes. Isolated Arc has already been shown to be able to support exocytosis[24]. Exocytosis may occur when crowded membrane bound proteins which experience steric pressure induce membrane curvature leading to membrane fission[49]. Arc may experience high steric pressure when brought into close proximity by binding mRNA. However, it would be expected that as the protein oligomerizes into its capsid structure this steric pressure decreases as it adopts favorable protein-protein interactions. In that case membrane interactions with amphipathic helices in the NTD may potentially be enough to drive fission[38]. This is because amphipathic helices that interact with polar phospholipid head groups can expand the bilayer surface relative to the bilayer mid-plane[50]. Thus Arc oligomerization at the membrane may be sufficient in facilitating exocytosis.

Alternatively, Arc may, in a manner similar to HIV-1, utilize endosomal sorting complex required for transport (ESCRT) machinery to facilitate egress. Despite HIV-1 being able to alter membrane

curvature during self-assembly, it still requires ESCRT machinery for membrane fission[51]. Moreover, HIV-1 contains multiple binding motifs for various ESCRT complexes[51]. While Arc is not known to contain these binding motifs, Arc may require ESCRT as well as Arc VLPs were shown to contain ALIX[11]. ALIX is one of many early acting ESCRT factors. These factors are responsible for binding adaptor proteins, concentrating cargo and initiating membrane bending[52]. ALIX recruits CHMP4 which participates in ESCRT dependent membrane fission[52]. The presence of ALIX in Arc vesicles suggests that Arc uses ESCRT to release from cells. However, other interactions between ESCRT proteins and Arc or Arc capsid have yet to be determined.

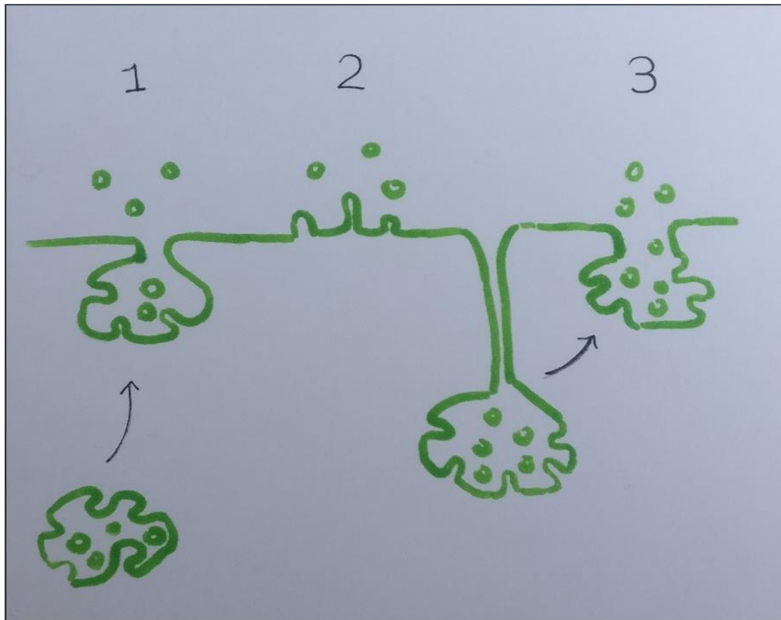

Figure 5. Modes of exosome formation. (1) The endosomal membrane invaginates resulting in the formation of intralumenal vesicles. Once the MVB has matured it fuses with the plasma membrane releasing the contents as exosomes. (2) Vesicle budding from the plasma membrane. (3) Membrane invaginations at intracellular plasma membrane-connected compartments (IPMCs), widening of the IPMC neck releases the vesicles.

4. Capsid attachment to and entry into recipient cells

Pastuzyn *et al* showed that Arc capsids are released from donor cells via extracellular vesicles and taken up in recipient cells by endocytosis. Host attachment and release of genetic material from the endosome are key steps in the life cycle of any virus. How Arc capsids manage to undergo these steps may be even more compelling. Especially as, opposed to regular virus particles, these Arc VLPs are not known to contain proteins dedicated to cell attachment or membrane fusion. Pastuzyn *et al* posited that, similar to non-enveloped viruses, the capsid protein itself, namely Arc, may be able to directly transfer RNA across the endosomal membrane. Several mechanisms were proposed to potentially mediate exit from the endosomes, namely receptor-capsid interactions, or Arc-membrane interactions. The latter was proposed to potentially be mediated by a pH-dependent conformational change in Arc. However, since Arc capsids are released in extracellular vesicles they do not resemble non-enveloped viruses. Moreover, endocytosis of these vesicles results in the capsid being surrounded not by one but by two membranes: the vesicle membrane and the endosomal membrane. Therefore, Arc cannot directly interact with endosomal receptors or the endosomal membrane as this is impeded by the vesicle membrane. Additionally, changes in pH would likely be largest in the space between the vesicle and endosomal membrane, not inside the vesicle surrounding the capsid. If pH-dependent conformational change of the Arc capsid were to occur and

to affect membrane integrity it would only affect the vesicle membrane. In short, Arc capsids do not behave like non-enveloped viruses, as they are released in vesicles.

More likely, the VLPs formed by Arc behave more similar to enveloped retroviruses. Enveloped retroviruses require a fusogenic protein to attach to cell surface receptors and release from the endosome (Figure 6A)[53]. Inside the endosome fusogenic proteins undergo a conformational change which allows the insertion of the protein into the endosomal membrane mediating the fusion between the endosomal membrane and viral envelope (Figure 6B)[53]. Similar to Gag-like proteins, humans are proposed to contain various fusogenic proteins of retrotransposon/retroviral origin[53–55]. Arc VLPs may utilize endogenously expressed fusogenic proteins to attach to and enter cells, as was shown for PEG10[43]. PEG10 is a Gag-like protein of which the CTD has a similar fold as HIV-1 and Ty3 and has been shown to form VLPs containing its own mRNA[43,56]. These VLPs are capable of transfecting cells, but only when co-expressed with a fusogenic protein like the vesicular stomatitis virus envelope glycoprotein (VSVg) or murine Syncytin-A (SYNA)[43]. SYNA, similar to the human homologue Syncytin-1, is an endogenous retroviral envelope protein that mediates trophoblast fusion essential for placental development[54,57,58]. Syncytin is unlikely to mediate attachment and release for Arc VLPs, as it is mostly expressed in placental cells[54,57]. It is however, possible that Arc capsids utilize a different fusogenic protein, as endogenous retroviruses (ERVs) constitute 8% of the human genome[55]. In fact several human ERV envelope proteins have been found to be expressed in human brains under various conditions[59–61].

It may be possible that Arc requires a human ERV envelope protein for entry and release of Arc capsids in recipient cells. It should be noted that many of the HERVs expressed in brain are associated with neurodevelopmental and neurodegenerative disease[62,63]. In bipolar disorder and schizophrenia for instance, the HERV-W envelope protein (Env) levels are elevated compared to healthy controls[64]. The HERV-W Env was also observed in MS lesions[65]. Similarly, the HERV-K Env has been shown to be expressed in cortical and spinal neurons in a subtype of ALS patients, whereas it is not expressed in healthy controls[66]. Expression of this gene in mice led to neuronal degradation and progressive motor dysfunction[66]. Indeed HERVs are becoming increasingly associated with neurodegenerative disease. If Arc were to use a HERV envelope protein for intercellular transport which Env would it be? More importantly, how then does Arc transfer between cells without causing disease? So far disease associations of Arc have been linked to its function in AMPA receptor endocytosis[67]. It seems reasonable then, given HERVs disease associations, that there are possibly neurodegenerative diseases related to Arc virus-like transfer that have previously been overlooked.

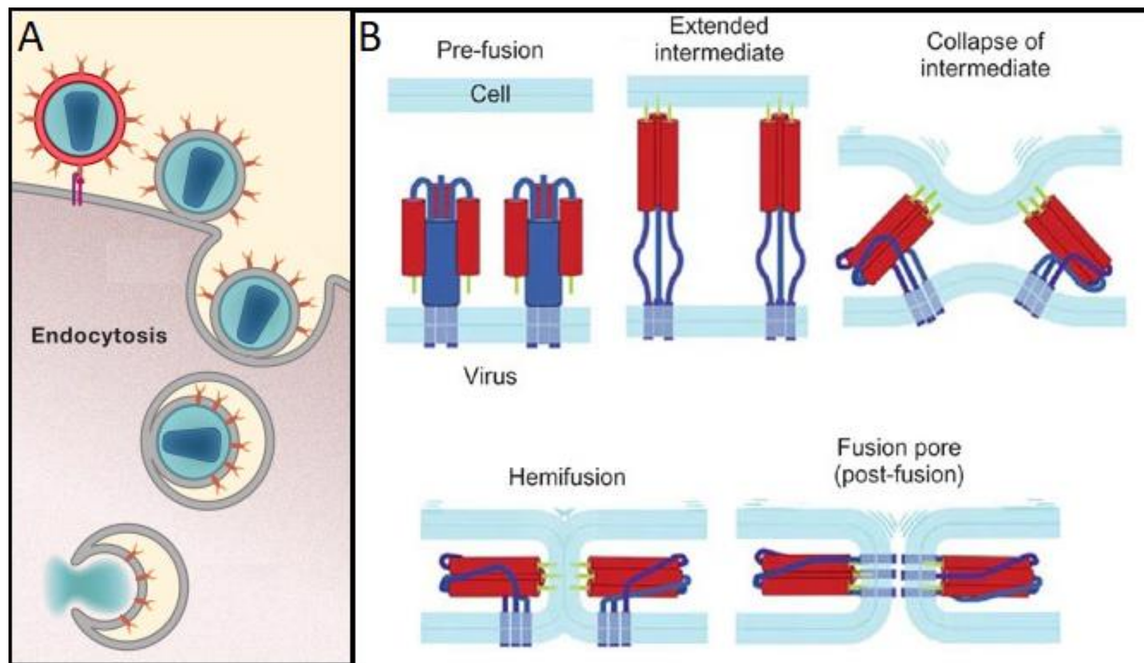

Figure 6. Receptor mediated endocytosis and endosome exit mediated by viral fusion protein. (A) An enveloped virus particle attaches to a cell using a fusion protein. Attachment leads to endocytosis of the virus particle. (B) The fusion protein undergoes a conformational change leading to the insertion into the endosomal membrane. An additional conformational change brings the endosomal membrane and viral envelope in close proximity leading to membrane fusion. Adapted from Uchil and Mothes (Cell 2009) and Harrison (Nat Struct Mol Biol 2008)[68,69].

5. Outlook

Many questions still remain with regard to Arc capsid formation. First, a high resolution structure, such as determined by Cryo-EM, of the mammalian Arc capsid has not yet been solved. This is important as it reveals how Arc molecules interact within the capsid and thus how the protein oligomerizes. Additionally, the capsid structure may demonstrate how certain PTMs or protein-ligand interactions inhibit oligomerization. Second, more research needs to be done regarding mRNA binding by Arc as this promotes oligomerization and capsid formation. Where mRNA is bound to the protein and why Arc mRNA is preferentially bound is still unknown. Answers may be gleaned from oligomerization assays with Arc protein and mRNA deletion mutants. Third, more research is required into how PTMs affect the balance between Arc's opposing functions. If PTMs can inhibit oligomerization, then are there any PTMs that specifically promote oligomerization and capsid formation? Lastly, we proposed that Arc may use cellular proteins during release and a fusogenic envelope protein of endogenous retroviral origin as a means to attach to and enter cells. If this is the case then these protein will be associated with or inside of Arc VLPs and can be identified with LC-MS/MS[70].

Arc and Arc-mediated receptor endocytosis is associated with various neurological diseases including schizophrenia, autism and Angelman Syndrome[67,71,72]. In Angelman Syndrome, mutations in Ube3A were shown to increase Arc expression which was shown to result in increased AMPA receptor endocytosis[67]. This highlights that altered receptor internalization can have negative impacts on synaptic plasticity and can play a key role in neurological disorders. Changes is Arc expression, localization and binding partners is widely accepted to affect receptor internalization. The ability of Arc to form capsids and transfer mRNA between cells may also play a role in this. Given that capsid formation and receptor endocytosis are two competing processes, a disturbance in this balance may potentially result in disease. Although, little is known about the frequency of Arc capsid formation and transport this could potentially open up a new line of research into Arc associated disorders.

References

1. Park, S. *et al.* Elongation factor 2 and fragile X mental retardation protein control the dynamic translation of Arc/Arg3.1 essential for mGluR-LTD. *Neuron* **59**, 70–83 (2008).

2. Ramírez-Amaya, V. *et al.* Spatial exploration-induced Arc mRNA and protein expression: evidence for selective, network-specific reactivation. *J. Neurosci.* **25**, 1761–1768 (2005).

3. Na, Y. *et al.* Real-Time Imaging Reveals Properties of Glutamate-Induced Arc/Arg 3.1 Translation in Neuronal Dendrites. *Neuron* **91**, 561–573 (2016).

4. Nakayama, D. *et al.* Long-delayed expression of the immediate early gene Arc/Arg3.1 refines neuronal circuits to perpetuate fear memory. *J. Neurosci.* **35**, 819–830 (2015).

5. DaSilva, L. L. P. *et al.* Activity-Regulated Cytoskeleton-Associated Protein Controls AMPAR Endocytosis through a Direct Interaction with Clathrin-Adaptor Protein 2. *eNeuro* **3**, 125–140 (2016).

6. Byers, C. E. *et al.* Enhancement of dynamin polymerization and GTPase activity by Arc/Arg3.1. *Biochim. Biophys. Acta - Gen. Subj.* **1850**, 1310–1318 (2015).

7. Chowdhury, S. *et al.* Arc/Arg3.1 Interacts with the Endocytic Machinery to Regulate AMPA Receptor Trafficking. *Neuron* **52**, 445–459 (2006).

8. Kirchhausen, T., Owen, D. & Harrison, S. C. Molecular Structure, Function, and Dynamics of Clathrin-Mediated Membrane Traffic. *Cold Spring Harb. Perspect. Biol.* **6**, (2014).

9. Ferguson, S. M. & De Camilli, P. Dynamin, a membrane-remodelling GTPase. *Nat. Rev. Mol. Cell Biol.* **13**, 75–88 (2012).

10. Milosevic, I. *et al.* Recruitment of endophilin to clathrin-coated pit necks is required for efficient vesicle uncoating after fission. *Neuron* **72**, 587–601 (2011).

11. Pastuzyn, E. D. *et al.* The Neuronal Gene Arc Encodes a Repurposed Retrotransposon Gag Protein that Mediates Intercellular RNA Transfer. *Cell* **172**, 275 (2018).

12. Cullen, H. & Schorn, A. J. Endogenous Retroviruses Walk a Fine Line between Priming and Silencing. *Viruses* **12**, (2020).

13. Hurst, T. P. & Magiorkinis, G. Epigenetic Control of Human Endogenous Retrovirus Expression: Focus on Regulation of Long-Terminal Repeats (LTRs). *Viruses* **9**, (2017).

14. Campillos, M., Doerks, T., Shah, P. K. & Bork, P. Computational characterization of multiple Gag-like human proteins. *Trends Genet.* **22**, 585–589 (2006).

15. Hansen, L. J., Chalker, D. L., Orlinsky, K. J. & Sandmeyer, S. B. Ty3 GAG3 and POL3 genes encode the components of intracellular particles. *J. Virol.* **66**, 1414–1424 (1992).

16. Myrum, C. *et al.* Arc is a flexible modular protein capable of reversible self-oligomerization. *Biochem. J.* **468**, 145–158 (2015).

17. Zhang, W. *et al.* Structural Basis of Arc Binding to Synaptic Proteins: Implications for Cognitive Disease. *Neuron* **86**, 490 (2015).

18. Tedbury, P. R. & Freed, E. O. HIV-1 Gag: An Emerging Target for Antiretroviral Therapy. *Curr. Top. Microbiol. Immunol.* **389**, 171 (2015).

19. Erlendsson, S. *et al.* Structures of virus-like capsids formed by the Drosophila neuronal Arc proteins. *Nat. Neurosci.* **23**, 172–175 (2020).

20. Hallin, E. I. *et al.* Structure of monomeric full-length ARC sheds light on molecular flexibility, protein interactions, and functional modalities. *J. Neurochem.* **147**, 323–343 (2018).

21. Ashley, J. *et al.* Retrovirus-like Gag protein Arc1 binds RNA and traffics across synaptic boutons. *Cell* **172**, 262 (2018).

22. Dodonova, S. O., Prinz, S., Bilanchone, V., Sandmeyer, S. & Briggs, J. A. G. Structure of the Ty3/Gypsy retrotransposon capsid and the evolution of retroviruses. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 10048–10057 (2019).

23. Kleinpeter, A. B. & Freed, E. O. HIV-1 Maturation: Lessons Learned from Inhibitors. *Viruses* **12**, (2020).

24. Hedde, P. N. *et al.* Membrane Remodeling by Arc/Arg3.1. *Front. Mol. Biosci.* **8**, (2021).

25. Goto, T., Nakai, M. & Ikuta, K. The life-cycle of human immunodeficiency virus type 1. *Micron* **29**, 123–138 (1998).

26. Erlendsson, S. *et al.* Structures of virus-like capsids formed by theDrosophila neuronal Arc proteins. *Nat. Neurosci.* **23**, 172 (2020).

27. T=9 ~ ViralZone. https://viralzone.expasy.org/5316.

28. T=4 icosahedral capsid protein ~ ViralZone. https://viralzone.expasy.org/808.

29. CASPAR, D. L. & KLUG, A. Physical principles in the construction of regular viruses. *Cold Spring Harb. Symp. Quant. Biol.* **27**, 1–24 (1962).

30. Barylko, B. *et al.* Palmitoylation and Membrane Binding of Arc/Arg3.1: A Potential Role in Synaptic Depression. *Biochemistry* **57**, 520–524 (2018).

31. Matt, L., Kim, K., Chowdhury, D. & Hell, J. W. Role of Palmitoylation of Postsynaptic Proteins in Promoting Synaptic Plasticity. *Front. Mol. Neurosci.* **12**, (2019).

32. Yang, X., Chatterjee, V., Ma, Y., Zheng, E. & Yuan, S. Y. Protein Palmitoylation in Leukocyte Signaling and Function. *Front. Cell Dev. Biol.* **0**, 1167 (2020).

33. Walczyk-Mooradally, A. *et al.* Phosphorylation-dependent control of Activity-regulated cytoskeleton-associated protein (Arc) protein by TNIK. *J. Neurochem.* **158**, 1058–1073 (2021).

34. Zhang, W. *et al.* Arc oligomerization is regulated by CaMKII phosphorylation of GAG domain; an essential mechanism for plasticity and memory formation. *Mol. Cell* **75**, 13 (2019).

35. Nikolaienko, O., Eriksen, M. S., Patil, S., Bito, H. & Bramham, C. R. Stimulus-evoked ERK-dependent phosphorylation of activity-regulated cytoskeleton-associated protein (Arc) regulates its neuronal subcellular localization. *Neuroscience* **360**, 68–80 (2017).

36. Nikolaienko, O., Patil, S., Eriksen, M. S. & Bramham, C. R. Arc protein: a flexible hub for synaptic plasticity and cognition. *Semin. Cell Dev. Biol.* **77**, 33–42 (2018).

37. Nielsen, L. D., Pedersen, C. P., Erlendsson, S. & Teilum, K. The Capsid Domain of Arc Changes Its Oligomerization Propensity through Direct Interaction with the NMDA Receptor. *Structure* **27**, 1071-1081.e5 (2019).

38. Eriksen, M. S. *et al.* Arc self-association and formation of virus-like capsids are mediated by an N-terminal helical coil motif. *FEBS J.* **288**, 2930–2955 (2021).

39. Rulli, S. J. *et al.* Selective and nonselective packaging of cellular RNAs in retrovirus particles. *J. Virol.* **81**, 6623–6631 (2007).

40. Comas-Garcia, M., Davis, S. R. & Rein, A. On the Selective Packaging of Genomic RNA by HIV-1. *Viruses* **8**, (2016).

41. Nikolaitchik, O. A. *et al.* Unpaired Guanosines in the 5’ Untranslated Region of HIV-1 RNA Act Synergistically To Mediate Genome Packaging. *J. Virol.* **94**, (2020).

42. Nikolaitchik, O. A. *et al.* Selective packaging of HIV-1 RNA genome is guided by the stability of 5’ untranslated region polyA stem. *Proc. Natl. Acad. Sci. U. S. A.* **118**, e2114494118 (2021).

43. Segel, M. *et al.* Mammalian retrovirus-like protein PEG10 packages its own mRNA and can be pseudotyped for mRNA delivery. *Science (80-.).* **373**, 882–889 (2021).

44. Eriksen, M. S. *et al.* Arc self-association and formation of virus-like capsids are mediated by an N-terminal helical coil motif. *FEBS J.* **288**, 2930–2955 (2021).

45. Shepherd, J. D. *et al.* Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. *Neuron* **52**, 475–484 (2006).

46. Steward, O., Farris, S., Pirbhoy, P. S., Darnell, J. & Van Driesche, S. J. Localization and local translation of Arc/Arg3.1 mRNA at synapses: some observations and paradoxes. *Front. Mol. Neurosci.* **7**, 1–15 (2015).

47. Ashley, J. *et al.* Retrovirus-like Gag protein Arc1 binds RNA and traffics across synaptic boutons. *Cell* **172**, 262 (2018).

48. Pegtel, D. M. & Gould, S. J. Annual Review of Biochemistry Exosomes. (2019) doi:10.1146/annurev-biochem-013118.

49. Snead, W. T. *et al.* Membrane fission by protein crowding. *Proc. Natl. Acad. Sci. U. S. A.* **114**, E3258–E3267 (2017).

50. Boucrot, E. *et al.* Membrane fission is promoted by insertion of amphipathic helices and is restricted by crescent BAR domains. *Cell* **149**, 124–136 (2012).

51. Hurley, J. H. & Cada, A. K. Inside Job: How the ESCRTs release HIV-1 from infected cells. *Biochem. Soc. Trans.* **46**, 1029 (2018).

52. McCullough, J., Frost, A. & Sundquist, W. I. Structures, Functions, and Dynamics of ESCRT-III/Vps4 Membrane Remodeling and Fission Complexes. *Annu. Rev. Cell Dev. Biol.* **34**, 85–109 (2018).

53. Dittmar, T., Weiler, J., Luo, T. & Hass, R. Cell-cell fusion mediated by viruses and herv-derived fusogens in cancer initiation and progression. *Cancers (Basel).* **13**, (2021).

54. Blaise, S., De Parseval, N., Bénit, L. & Heidmann, T. Genomewide screening for fusogenic human endogenous retrovirus envelopes identifies syncytin 2, a gene conserved on primate evolution. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 13013–13018 (2003).

55. Lander, E. S. *et al.* Initial sequencing and analysis of the human genome. *Nat. 2001 4096822* **409**, 860–921 (2001).

56. Zurowska, K., Alam, A., Ganser-Pornillos, B. K. & Pornillos, O. Structural evidence that MOAP1 and PEG10 are derived from retrovirus/retrotransposon Gag proteins. *Proteins Struct. Funct. Bioinforma.* **90**, 309–313 (2022).

57. Sha, M. *et al.* Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* **403**, 785–789 (2000).

58. Dupressoir, A. *et al.* Syncytin-A and syncytin-B, two fusogenic placenta-specific murine

envelope genes of retroviral origin conserved in Muridae. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 725–730 (2005).

59. Kremer, D. *et al.* pHERV-W envelope protein fuels microglial cell-dependent damage of myelinated axons in multiple sclerosis. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 15216–15225 (2019).

60. Liu, C. *et al.* Activation of elements in HERV-W family by caffeine and aspirin. *Virus Genes* **47**, 219–227 (2013).

61. Wang, X., Huang, J. & Zhu, F. Human Endogenous Retroviral Envelope Protein Syncytin-1 and Inflammatory Abnormalities in Neuropsychological Diseases. *Front. Psychiatry* **9**, 422 (2018).

62. Küry, P. *et al.* Human Endogenous Retroviruses in Neurological Diseases. *Trends Mol. Med.* **24**, 379 (2018).

63. Evans, T. A. & Erwin, J. A. Retroelement-derived RNA and its role in the brain. *Semin. Cell Dev. Biol.* **114**, 68–80 (2021).

64. Perron, H. *et al.* Molecular characteristics of Human Endogenous Retrovirus type-W in schizophrenia and bipolar disorder. *Transl. Psychiatry* **2**, (2012).

65. Perron, H. *et al.* Human endogenous retrovirus type W envelope expression in blood and brain cells provides new insights into multiple sclerosis disease. *Mult. Scler.* **18**, 1721–1736 (2012).

66. Li, W. *et al.* Human endogenous retrovirus-K contributes to motor neuron disease. *Sci. Transl. Med.* **7**, (2015).

67. Greer, P. L. *et al.* The Angelman Syndrome protein Ube3A regulates synapse development by ubiquitinating arc. *Cell* **140**, 704–716 (2010).

68. Uchil, P. D. & Mothes, W. HIV Entry Revisited. *Cell* **137**, 402–404 (2009).

69. Harrison, S. C. Viral membrane fusion. *Nat. Struct. Mol. Biol.* **15**, 690–698 (2008).

70. Vera-Velasco, N. M., García-Murria, M. J., Sánchez del Pino, M. M., Mingarro, I. & Martinez-Gil, L. Proteomic composition of Nipah virus-like particles. *J. Proteomics* **172**, 190–200 (2018).

71. Alhowikan, A. M. Activity-Regulated Cytoskeleton-Associated Protein Dysfunction May Contribute to Memory Disorder and Earlier Detection of Autism Spectrum Disorders. *Med. Princ. Pract.* **25**, 350 (2016).

72. Fromer, M. *et al.* De novo mutations in schizophrenia implicate synaptic networks. *Nature* **506**, 179–184 (2014).