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Nei

BACHELOR THESIS

The Activity-Regulated Cytoskeleton Associated Protein (Arc) and its Functions, a Literature Review

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Abstract

The Activity-regulated cytoskeleton associated protein, Arc, is a neuron-specific immediate early gene product that coordinates signaling events in postsynaptic neurons. Several studies have reported that the *Arc* gene and protein is pivotal for synaptic plasticity, long-lasting memory storage, and that it has been implicated in neurodevelopmental and neurodegenerative disorders. This review article is composed using the IMRaD-method and is mainly based on both review- and original articles handed to us by our supervisors and from advanced database-searches. The review describes the molecular and physiological functions of the Arc protein as well as connecting the protein to normal physiology as sleep, disease and diagnostic value. Data presents evidence that Arc self-assembles into oligomers forming virus-like capsids that encapsulates RNA. This further generates RNA-transport between neurons, a previously unknown intercellular communication pathway. Evolutionary analysis show that Arc is derived from an ancient retrotransposon of the Ty3/gypsy family and bears a domain homologous to the retroviral Gag polyproteins. These findings propose that Gag retroelements have been repurposed through evolution to mediate communication within the nervous system. Arc transcription in neuronal circuits is connected to learning. Released Arc protein might function to transfer intercellular cargo through capsids that converts the state of adjacent cells, which is needed for cellular consolidation of memory. This implies that a specific regulation of Arc expression is required for normal cognition. The expression and translation of *Arc* seems to be affected by sleep-related aspects, also suggesting that sleep is a prerequisite for some aspects of memory. Transcripts of Arc are also found in blood serum, indicating a possible diagnostic value of extracellular vesicles containing Arc capsids and mRNA.

Keywords: Arc, sleep, synapse, synaptic plasticity, nervous system, memory, learning, capsids, extracellular vesicles, neurodegenerative disorders, disease, mRNA

Acknowledgement/Preface

Abbreviations and Definitions

ARC	Activity-Regulated Cytoskeleton Associated Protein
AMPA Receptor	Alfa-Amino-3-Hydroxy-5-Metyl-4-Isoxazolepropionic Acid Receptor (a Glutamate Receptor)
ACBARs	Arc Capsids Bearing any RNA
BDNF	Brain-Derived Neurotrophic Factor
CTD	C-terminal Domain
dARC	Drosophila Arc
ERK-Catalyzed Phosphorylation	Extracellular Signal-Regulated Kinases-Catalyzed Phosphorylation
EVs	Extracellular Vesicles
GAG-Protein	Group-Specific Antigen Protein
GSK-Catalyzed Phosphorylation	Glycogen Synthase Kinase-Catalyzed Phosphorylation
HEK293	Human Embryonic Kidney Cells
IRES	Internal Ribosomal Entry Site
LTD	Long Term Depression
LTP	Long Term Potentiation
mARC	Mammalian Arc
miRNA	Micro RNA
mRNA	Messenger RNA
NMDA Receptor	N-Metyl-D-Aspartate Receptor
NMJ	Neuromuscular Junction
NREM	Non-Rapid Eye Movement
NTD	N-Terminal Domain
ORF	Open Reading Frame
REM	Rapid Eye Movement
SD	Sleep Deprivation (sleep loss)
SWR	Sharp Waves/Ripples
3'UTR	3' Untranslated Region

Oligomerization: A chemical process that creates macromolecular complexes bigger than polymers from monomeric proteins.

Open reading frame: Long DNA-sequences lacking stop-codons and therefore is being translated into protein.

Untranslated region: Sequences of an mRNA-strand that is not in the open reading frame and therefore not translated into protein.

Transposon: Mobile genetic elements found virtually in all cells. Their DNA-sequences make up almost half of the human genome. They can insert themselves into any DNA-sequence by a cut-and-paste mechanism, but most of them lack the ability to leave the cell they reside unlike viruses. Transposons are typically classified according to the mechanism by which they move or transpose.

Retrotransposon: A classification of transposons that moves via an RNA intermediate. They appear to be unique to eukaryotes.

Stargazin: A transmembrane AMPA-receptor regulatory protein. It promotes the targeting of AMPARs to the synapses and cell membranes, and it modulates their gating properties by slowing their rates of activation.

Immediate early gene: Genes that responses early after being coordinately activated in response to neuronal activity and neuronal insults/cellular stress.

Non-cap dependent translation: Translation of mRNA that does not require a 5' cap to initiate scanning from the 5' mRNA-end until the start codon. The ribosome will localize and bind directly to the startsite.

Dendritic cell-dependent T-cell activation: The interaction between T-cells and dendritic cells that leads to T-cell activation that affects the progression of the immune response. In an inflammatory environment, autoreactive T-cells are initially activated by dendritic cells.

MicroRNA (miRNA): Micro RNAs are small, highly conserved, single-stranded, non-coding RNA molecules involved in the regulation of gene expression. The molecules bind target mRNA to prevent protein production.

Hippocampus: An extension of the temporal part of cerebral cortex, is a major component of the brain in mammalian and other vertebrates involved with different aspects of memory. It is crucial in the consolidation of information from short-term memory to long-term memory.

Phosphorylation: A post-translational modification (PTM) where an addition of an phosphate-group to a molecule can create docking sites.

Ubiquitination: A post-translational modification (PTM) which adds/attaches ubiquitin, a 76-amino-acid polypeptide, to a protein to target it for degradation.

SUMOylation: A post-translational modification (PTM) where SUMO-proteins (Small Ubiquitin-like Modifier proteins) are attached covalently to the protein to modify its function.

Lysine acetylation: Acetylation is a post-translational modification (PTM) of a protein where an acetyl-group is attached to the epsilon-amino group of lysine residues (a lysine side chain) catalyzed by lysine acetyltransferases.

Palmitoylation: A post-translational modification where an addition of the fatty acid palmitate to a cysteine side chain can drive the protein to associate with cell-membranes.

Ponto-geniculo-occipital waves (PGO waves): PGO waves can be detected under and right before REM sleep. It is waves that propagates activity between the pons, the lateral geniculate nucleus and the occipital lobe in the mammalian brain.

S2 cells: S2 cells is Schneider 2 cells which is one of the most commonly used cell-lines of Drosophila Melanogaster.

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Introduction

Activity-Regulated Cytoskeleton Associated Protein (Arc) and its Evolution

The activity-regulated cytoskeleton associated protein, Arc, is an activity-dependent immediate early gene product (Ashley et al., 2018). It is a hub protein with diverse roles in intracellular neuronal signaling and it is pivotal for long-term synaptic plasticity, memory trace and postnatal cortical development (Eriksen et al., 2020). The expression and translation of *Arc* seems to be affected by changes in sleep. Mutations in the Arc gene are linked to diseases like Alzheimer's disease, Schizophrenia, autism and other neurodevelopmental and neurodegenerative disorders such as fragile X-syndrome and Angelman's syndrome (Pastuzyn et al., 2018).

Two homologues of the Arc protein is expressed in drosophila, dArc1 and dArc2. In mammalians such as humans (homo sapiens), mice (Mus musculus) and rats (Rattus norvegicus), the Arc protein is discussed as mammalian Arc (mArc). Despite some differences in structure, dArc and mArc seems to have some of the same molecular and physiological functions.
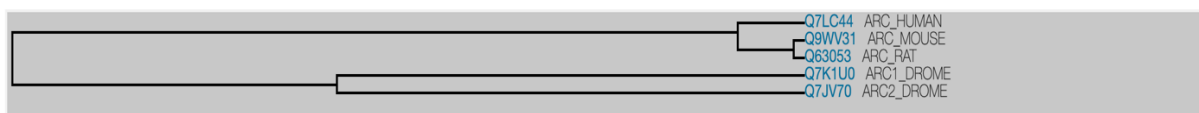
Figure 1. Alignment of Arc orthologues and homologues in mammalians and drosophila from UniProt. *ARC_HUMAN (homo sapiens), ARC_MOUSE (Mus Musculus) and ARC_RAT (Rattus Norvegicus) has differenciated from ARC1_DROME (Drosophila Melanogaster) through evolution. Later in evolution the Arc protein in humans have differentiated from the Arc protein in mouse and rat (Alignment from UniProt, 2021).*

The Arc protein is capable of self-association into oligomers forming virus-like capsids. These capsids are implicated in intracellular RNA transfer (Eriksen et al., 2020). The *Arc* gene bears domains with retroviral/retrotransposon group-specific antigen (Gag)-like sequences, and has therefore homology to retroviruses and other retroviral Gag-proteins such as HIV (Ashley et al., 2018). Evolutionary analysis indicates that Arc is derived from a vertebrate lineage of Ty3/gypsy retrotransposons, likely through an earlier transposon insertion (Pastuzyn et al., 2018; Zhang, 2015). Studies suggests that these retroviral-like sequences and mechanisms has been evolutionary conserved and provided to mediate intercellular communication in the nervous system (Pastuzyn et al., 2018).

Arc in the Nervous System

Arc is expressed in neurons and synapses, and is therefore tightly associated with the nervous system. The nervous system is a complex network of communicating neurons and glial cells sending information between themselves, muscles and endocrine glands in the body/organism (Kandel, 2000, p. 20-21). The central nervous system (CNS) consists of neurons and glial cells in the brain and spinal cord which is protected by the cranium and the vertebral column. Nerves are bundles of axons that connects the CNS to the rest of the body. Neurons are the first cells to differentiate in the nervous system (Kiernan, 2009, p. 3). The fundamental task of a nerve cell, or a neuron, is to receive, integrate, and transmit signals (Alberts, 2014, p. 403).
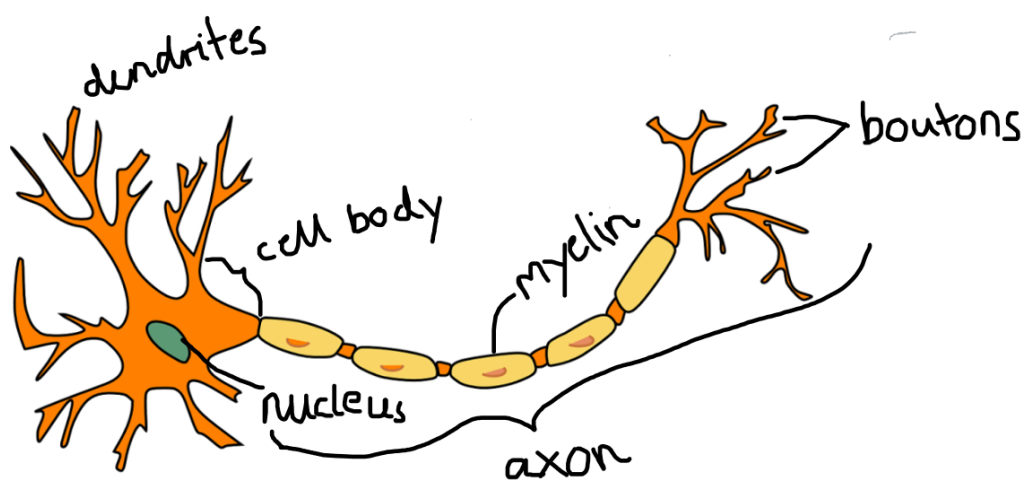
Figure 2. A nerve cell. Neurons have a cell body (soma) with a nucleus. The cell body extends in several short dendrites, and one long tubular axon. Dendrites branch out into tree-like formations and its main function is to receive incoming signal from other nerve cells. The axon extends away from the cell body, and its presynaptic terminals, the so called boutons, is the main conducting unit for carrying signal to other neurons (or glial cells). Some axons are ensheated in myelin, they are myelinated (Kandel, 2000, p.21). Reprinted and modified from https://brainmadesimple.com/neuronal-development/ 13th of April, 2021.

A neuron exhibits two different activities to carry out its communicative function. These functions are coupled and is conduction of a signal from one part of the neuron to another and synaptic transmission. The action potential is an impulsive wave of electrical depolarization due to a quick change in the chemical and electrical potential just in a thin layer on both sides of the nerve cell membrane. When a stimulus is applied to one part of the neuron it initiates an impulse that travels to all other parts of the cell (Kiernan, 2009, p.3).

Arc is Present in Synapses and is Pivotal for Synaptic Plasticity
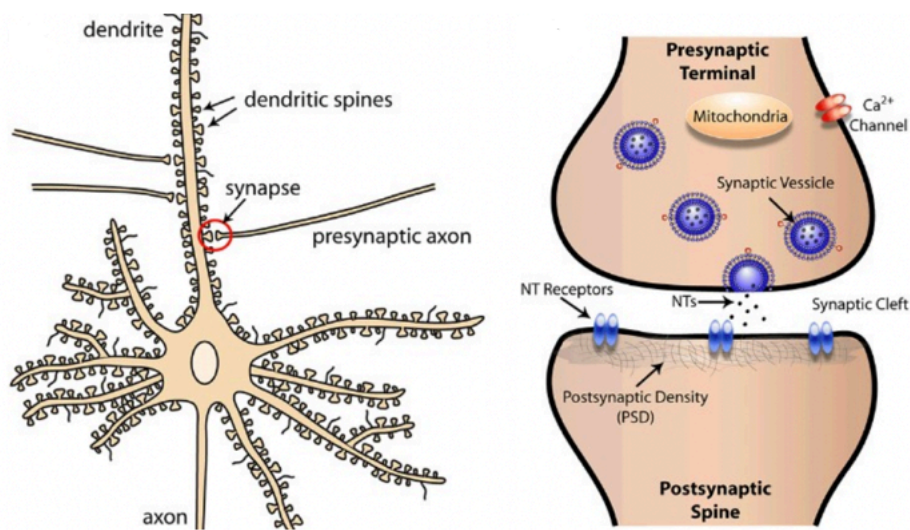
Figure 3. A nerve cell with dendritic spines and a synapse. *Pre- and postsynaptic cells make contacts through so-called synapses. Synapses can be formed after neuronal activity, but also be pruned after less use of the involved neurons. A synapse can transmit a signal from one cell to another, involving synaptic vesicles, neurotransmitters and postsynaptic membrane receptors. Reprinted from* https://dyslectern.info/wp-content/uploads/2015/01/classicneuron.jpeg *12th of April, 2021*

Neurons communicate with each other through synapses. Synapses is known as cell-to-cell contacts between two synaptic partners. Synaptic transmission is therefore communication between adjacent cells, however the postsynaptic cell can be distant from the presynaptic cell due to long-reaching axons. A synapse can also occur between a neuron and other cells, such as muscle cells. A synapse between a motorneuron and a muscle cell is called a neuromuscular junction (NMJ). Synaptic terminals at the presynaptic neuron is at the end of an axon, also known as a bouton. At the postsynaptic neuron, the synaptic terminals can be both dendritic ends and dendritic spines (Kiernan, 2009). Dendritic spines are ramifications of the dendrite (Kandel, 2000, p.224). When an electrical impulse arrive at a synaptic terminal in the presynaptic cell, it triggers the synaptic transmission. Synaptic transmission between neurons normally involves a release of chemical compounds, neurotransmitters, from the presynaptic cytoplasm through synaptic vesicles. These chemical compounds will evoke a response in the postsynaptic cell, either inhibitory or excitatory (stimulated or inhibited). This depends both on which neurotransmitter that is being released and the type of receptor molecule in the membrane of the postsynaptic cell. The two cells in a synapse can also be electrically involved (Kiernan, 2009).

Excitatory synaptic action is mediated by glutamate-gated channels that conduct Na+ and K+ (Kandel, 2000. p.212). Glutamate is the most common excitatory neurotransmitter in all parts

of the CNS. A transmitter of this type will depolarize the postsynaptic cell, most often as a result of Na+ influx through the ligand-gated ion channels. This brings the membrane potential closer to the threshold level which may cause an action potential (Sand, 2016, p. 111). Glutamate can also regulate the physiology of the recipient neurons by inducing oligodendroglial exosome-secretion (Fruhbeis et al., 2013). AMPA- and NMDA-receptors is two major subtypes of ionotropic glutamate-receptors (Kandel, 2000, p.212). m

The ability of a synapse to change in strength in response to use or disuse is referred to as synaptic plasticity. In the mammalian brain, several forms of synaptic plasticity exists at glutamatergic, excitatory synapses. Both long-term potentiation (LTP) and long-term depression (LTD) requires actin cytoskeletal alteration within dendritic spines. Dendritic spine morphology seems to be a result from neuronal activity that leads to strengthening of the synapse because of an increase of active surface volume. An increase of post-synaptic receptors will also influence synaptic plasticity to LTP. A neuron is able to modulate its firing rate by expanding or reducing synaptic efficacy on all entrances of the dendrite. This refers to the homeostatic scaling of synaptic strength (Grønli et al., 2014). Several researches have uncovered diverse forms of synaptic plasticity and different signaling mechanisms that act as positive or negative regulators. cAMP-dependent signaling pathways have shown to be crucially involved in long-lasting synaptic plasticity. This pathway activates enzymes and regulates gene expression (Shetty et al., 2021).

The role of the cytoskeleton of the synaptic cells are important for both increase in size and for synaptogenesis. Dendritic spines are typically mushroom-shaped. The spines exhibit required activity-dependent forms of plasticity that affect both their strength and structural organization. This structural plasticity is mainly controlled by actin filaments, which is the principal cytoskeletal component of the spine. LTP-inducing stimulation leads to a persistently expandation of dendritic spines (Bosch & Hayashi, 2015). The functions of Arc are coupled to many aspects of the scaffold of a synapse and therefore the morphological changes of it. The function of Arc capsids connected to the cytoskeletal scaffold is still unknown.

Extracellular Vesicles (EVs) and Capsids in Intercellular Communication

Multiple studies have shown emerging evidence suggesting that specialized extracellular vesicles (EVs) mediate a pathway for intercellular signaling in the nervous system through enclosing and transferring virus-like capsids containing RNA between cells (Pastuzyn et al., 2018; Turchinovich et al., 2019). Extracellular vesicles are small membranous vesicles that are virtually released from all cells transferring membrane-bound- and cytoplasmic cargos (Hantak et al., 2021). EVs are defined by both size and subcellular origin, and can be broadly divided into microvesicles and exosomes (Pastuzyn et al., 2018). These types of vesicles plays a key role in regulation of various physiological processes in the body, and has been detected in close to all biological human fluids (Turchinovich et al., 2012).

Heinzelman et al., (2016) refers to bloodborne neuron-derived nanoscale EVs (nsEVs) as a substantial potential "window to the brain" enlightening CNS disorder-associated changes in the brain biochemistry and intercellular communication.

EVs can transport cargo that do not readily cross the plasma membrane through endo- and exocytosis, including virus-like capsids containing RNA (Pastuzyn et al., 2018). Exocytosis and endocytosis are special forms of energy-consuming membrane-transports, where water-soluble substances or molecular complexes are transported through the cell membrane by vesicles. In exocytosis, vesicles from inside the cell fuse with the plasma membrane and the cargo can be released from the cell. Through endocytosis, portions of the plasma membrane tuck inward and pinch off to form vesicles that can release the cargo inside the recipient cell (Sand, 2016, p. 67).

Some virus proteins form a spherical shell that encloses the viral genome. Most viral capsids are more or less such spherical protein assemblies. Viral capsids are formed from many copies of a small set of protein subunits, and RNA (or DNA) is packaged inside (Alberts, 2014, p.139-140). The structure of retroviral capsids assemble an immature viral particle through oligomerization of full-length Gag (Dodonova et al., 2019). Arc protein has shown to have Gag-like sequences to form such virus-like capsids.

The Arc Protein and its Possible Function in Long-Term Potentiation (LTP), a Manifestation of Memory

Arc protein is required for transducing experiences into long-lasting changes in visual cortex plasticity (McCurry et al., 2010). Since Arc is pivotal for synaptic plasticity and synaptic plasticity is fundamental for memory and learning (Kyrke-Smith et al., 2021), Arc is too. Learning can be discussed as the process of acquiring knowledge about the world, while memory then will be the process of that knowledge being encoded, stored and then retrieved (Kandel, 2000, p.1227). Learning and memory are often associated with neuronal assemblies and their formation and modification as a result of populations of neurons encoding for what has been learned and therefor mediates memory retrieval upon recall (Holtmaat & Caroni, 2016).

Neurobiological studies has yielded three generalizations when it comes to memory; there are stages of memory, multiple regions in the nervous system represents long-term memory, and the fact that explicit and implicit memory involves different neuronal circuits (Kandel, 2000, p.1245). Memory can either be classified as implicit or explicit based on how the information is stored and recalled. Implicit memory is memory that is being unconsciously recalled and explicit memory is therefore recalled by conscious effort. Explicit memory involves multiple parts of information and is therefore highly flexible, while implicit memory is tightly connected to the original stimulus conditions while the learning occurred (Kandel, 2000, p.1229-1230).

Memory converts from the short-term form to the long-term form by repeated experience. This process is called consolidation and consolidation of long-term memory for simple forms of learning involves gene expression, new protein synthesis and strengthening (or pruning) of synaptic connections (Kandel, 2000, p.1254-1256).

Arc, Sleep and Synaptic Plasticity

Several sources of evidence involve sleep in the consolidation of synaptic plasticity and long-term memory. Sleep is needed for several aspects of good health and has a critical role for human cognitive functions. It has been observed by electroencephalogram (EEG) that sleep is characterized by distinct changes in brain activity (Grønli et al., 2014). Despite this, these

features vary across taxonomic organisms, and some organisms has shown to have various types of sleep. Sleep is a behavioral state characterized by reduced responsiveness to stimulation in relation to both sleep and wakefulness. The fact that some animals spend more time sleeping and have a deeper sleep than humans indicates that sleep is regulated homeostatically (Amlader & Fuller, 2009, p. 11). Sleep homeostasis is a principle of sleep regulation which indicates that sleep deprivation compensatory elicits an increase in the duration and intensity of sleep. Likewise, excessive sleep will reduce the sleep propensity. This homeostatic process also interacts with a circadian process in the regulation of sleep, which is a clocklike mechanism independent of prior wake and sleep. An ultradian process by the alternation of two basic sleep stages also participates in sleep regulation (Amlader & Fuller, 2009; Borbély & Achermann, 1999).

Normally, sleep and wakefulness follows a periodic rhytm as the alternation between day and night occurs, but in addition to this there are different stages of sleep (Kiernan, 2009, p.148). The human sleep process rotates between non-rapid-eye movement (NREM) and rapid-eye movement (REM) in a pattern known as the sleep cycle (Amlader & Fuller, 2009, p.37). NREM is divided into sleep stage 1 (S1/N1), stage 2 (S2/N2) and slow wave sleep (S3+S4/N3) (Amlader & Fuller, 2009, p.36; Moser et al., 2009). Sharp waves/ripples (SWRs) occur often during NREM sleep and are defined as large high-frequency field potentials recorded from the hippocampus. This repeated neuronal activity throughout SWRs has been contemplated to induce synaptic potentiation, but a recent study showed that SWRs instead encouraged synaptic weakening (Tononi & Cirelli, 2020).

SHY is the abbreviation for the synaptic homeostasis hypothesis. This hypothesis proposes that the process of sleep is needed for the brain to maintain the total amount of synaptic strength under control. It predicts that synaptic connections of several neuronal circuits increase the synaptic strength by the end of the waking day due to ongoing learning. Mainly mediated by synaptic potentiation. Synaptic renormalization is needed due to the fact that stronger synapses are in need of more energy and is prone to saturation. This should mainly occur during sleep when the neuronal circuits can undergo specific synaptic down-selection. SHY predicts that sleep is necessary for plasticity during wakefulness, avoiding runaway potentiation, impaired learning due to saturation and decreased ratio of signal-to-noise (Tononi & Cirelli, 2020).
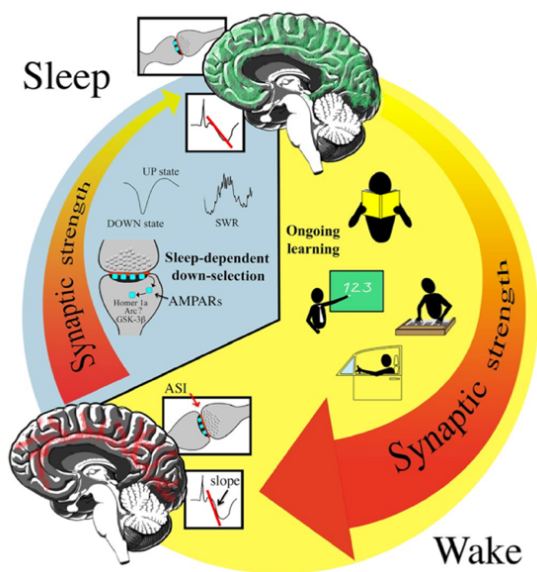
Figure 4. Schematic figure describing SHY, the synaptic homeostatic hypothesis. *Ongoing learning increases synaptic strength during wakefulness in many brain circuits. Brain circuits are here shown as red lines in the brain view. While the brain disconnects from the environment during sleep, most of the brain circuits undergo synaptic down-selection. Here shown as green lines in the brain view. Specific activity patterns during NREM are necessary for sleep-dependent renormalization. This is the UP-state of sharp waves/ripples. Several proteins including Arc accumulates in the neuronal spines at the synaptic level. These proteins seems to control the endocytosis of the glutamatergic receptors, and thus synaptic pruning. Axon-spine interface (ASI) shows AMPA receptors. The cortical evoked responses are presented in a slope used for measuring, the steeper slope, the greater response Reprinted with permission from Tononi & Cirelli, 2020.*

Grønli et al., (2014) describes that sleep loss impairs long-term potentiation (LTP), and that LTP expression takes advantage of non-disrupted periods of sleep (Campbell et al., 2002). This may be partly conflicting with SHY, as SHY predicts that overall synaptic strength should increase during wake and decrease during sleep (Tononi & Cirelli, 2020). Current evidence suggests that loss of REM-sleep impairs the maintenance of LTP and sleep deprivation further impairs cognitive functioning. Studies in rodents suggests a role of total sleep and REM-sleep in cellular mechanisms that are utilized in the formation of stable LTP and thereby long-term memory. Evidence proposes that sleep-stage specific changes in plasticity, synaptic efficacy, firing activity and network synchrony expand during sleep (Grønli et al., 2014).

Noya et al., (2019) revealed that both synaptic size and abundance of total protein are dynamically scaled by sleep and wakefulness. According to SHY the majority of synapses should grow with wake and shrink with sleep since stronger synapses also are bigger in size. Their study established that sleep-dependent synaptic renormalization was found in small and medium size synapses, but not in the largest. In addition, sleep-dependent renormalization appears to protect the neurons and/or synapses that are most active during sleep from down-selection (Tononi & Cirelli, 2020). Synapses must be stable and mutable enough to mediate long-term memory trace. Down-scaling of the synapses is required to generate the rigid and saturated synapse mutable again by decreasing its efficiency. To hold the former traces of excitation in the synaptic structure, rigidity is needed. One of the roles of sleep is to create a

good balance between flexibility and rigidity of synapses (G. Juhász, Personal Communication). Since Arc is involved in synaptogenesis and plasticity, an important goal for the future is to identify how Arc plays a role in this field.

Sleep deprivation is unfavorable as it in humans is a known risk factor for shortened lifespan and disease. WHO among others has presented sufficient evidence for higher risks of ischemic heart disease and stroke amongst people working more than standard hours (Pega et al., 2021). In the long run, lack of sleep due to shift- and night work may lead to persistent and severe disturbances of sleep, psychoneurotic syndromes and chronic fatigue. It is also a risk for other health issues such as type 2 diabetes, weight gain, breast cancer, circulatory related stroke, high blood pressure, immune related and microbiome changes in the gut (Costa, 2015; Kecklund & Axelsson, 2016; Irwin et al, 2016; Irwin & Opp, 2017). In addition to this, it has been shown through animal models that sleep deprivation causes numerous physiological changes in several brain- and body-systems (Amlader & Fuller, 2009, Chapter 20).

Material and Method

Literature Study

A literary study has its purpose in researching a certain topic based on earlier published review articles and original research studies. In this thesis we aimed to write a short systematic literature review on the topic of the molecular and physiological functions of the activity-regulated cytoskeleton associated protein (Arc). Mostly based on literature assigned to us by Alvhild Alette Bjørkum at Faculty of Engineering and Natural Sciences at Western Norway University of Applied Sciences in Bergen, Norway and Gábor Juhász from the Faculty of Science at Eötvös Loránd University in Budapest, Hungary. We have structured the thesis after the IMRaD method with parting it into introduction, materials and method, results and discussion. Due to our situation and our thesis not being a practical study we have chosen to merge results and discussion.

Literature Search

In addition to the literature what was handed out to us by our supervisors, we aimed to do an advanced literature search in the Scientific database PubMed to find more relevant review articles and original research studies of the chosen topic. Database searching like this is a form of online searching, and bibliographic database searches are also described as literature searches (Jankowski, 2008, p.1).

To form our advanced search in PubMed we had to use different combinations of our keywords to achieve a narrowly search relevant for our topic. We also combined our keywords with search words such as "AND" and "OR". In all combinations we used "Arc protein" and "AND" in addition to one of our keywords. We also did this individually with all of our key words and connected the searches with "OR". Doing this we included literature not only containing all of our key words at the same time, but all literature on the Arc protein which was connected to at least one of our preferential key words.

Our search results included articles that was not relevant for our thesis. By reading the abstract of them, we chose to exclude some of them based on their relevance for our topic, even though they involved Arc. We also excluded some articles by just looking at their title. We read through all remaining relevant articles, and included the ones we did not already have in our reference list.

In addition to literature handed out to us by our supervisors in the beginning of the project period, they have tried to do a systematic literature search in the PubMed and Web of Science to supply us with additional relevant literature. We have also used secondary literature such as different subject books about the nervous system etc. to form our thesis.

Results and Discussion

Structure of the Arc Protein

As earlier mentioned, dArc and mArc has some of the same functions despite some differences in structure, especially the ability of oligomerization into virus-like capsids. The molecular basis of Arc self-association and capsid formation is however largely unknown despite a predicted structure of the protein.
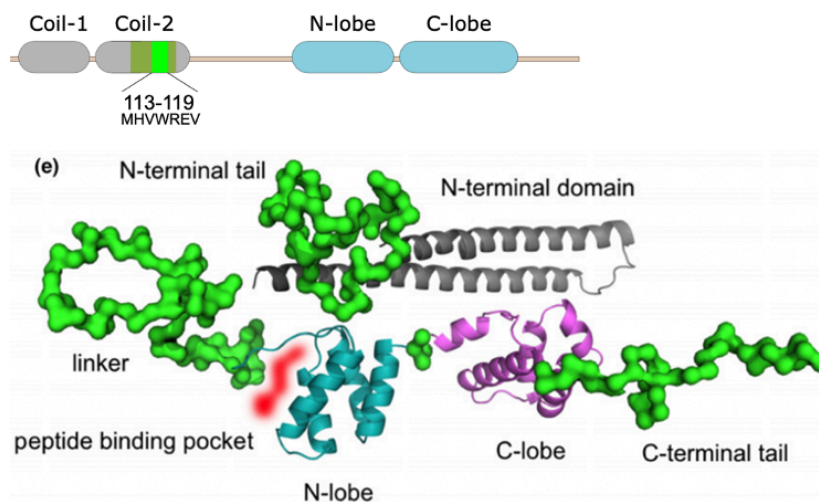
Figure 5. Predicted structure of mammalian Arc protein with N- and C-terminals and corresponding domains, binding pocket and a flexible linker. A simplified model of the mRNA ORF is also shown at the top of the figure. Reprinted and modified with permission from Hallin et al., 2018 and Eriksen et al., 2020.

As all other proteins, Arc consists of a N-terminal and a C-terminal. The structure of monomeric full-length Arc is folded, compact and closed. The C-terminal domain (CTD) of the Arc protein is bilobar with both a N-lobe and a C-lobe. These lobes are globular but separated domains with dumbbell shapes and has shown homology to the HIV Gag capsid (CA) domain. Both mArc and dArc has such CA-like domains with Gag-like/retrovirus-like sequences in the CTD. In mammalians the CTD is negatively charged and the N-terminal domain (NTD) positively charged. The two domains are separated by a not extended flexible linker which is stabilized by the oppositely charged terminals. mArc has a large NTD unique for the tetrapod (mammals, reptiles, birds and amphibians) evolution, located on top of the lobes in the CTD. This NTD in mammalian Arc is predicted to have an anti-parallel coiled-coil structure. The predicted coiled-coil then consists of two alfa-helixes, Coil-1 (20-77) and Coil-2 (78-140). A 28-aminoacid stretch (99-126) in Coil-2 of this domain has been identified as the oligomerization region and is both necessary and sufficient for oligomerization of Arc into virus-like capsids. A 7-residue oligomerization motif (113-119) has also been identified

within this region in Coil-2 of the NTD. This motif is located in the central parts of the helix and has shown to be critical for the formation of virus-like capsids in mammalians. Analysis of the oligomerization region predicts an amphipathic coil where hydrophobic residues are packaged on one side, and polar residues on the other. As already mentioned, such a domain in the N-terminal of the protein lacks in dArc. Despite this, dArc is also predicted to have a short amphipathic alpha helical region at the N-terminus of the N-lobe. This region appears to be involved in forming spikes that extend both inwards and outwards in regular intervals from the capsid shell (Hallin et al., 2018; Eriksen et al., 2020; Erlendsson et al., 2020). The CA-domain of dArc has the ability to oligomerize by itself unlike mArc (Bramham. C, Personal communication). Another difference in structure between mArc and dArc is the fact that mArc has a ligand binding pocket with multiple ligand binding sites that dArc doesn´t have. There are putative internal RNA-binding domains in dArc1 and dArc2 (Hallin et al., 2018; Eriksen et al., 2020; Erlendsson et al., 2020).

Arc has been hypothesized to undergo conformational changes as well as different states of oligomerization during the functional cycle of the protein. It still remains to be determined whether post-translational modifications or binding events can provoke opening of the known compact structure of Arc. Binding to ligand peptides such as Stargazin, GKAP and WAVE1 with the N-lobe of the protein has shown to induce a change in secondary structure, but it is not seen as a major conformational change of the protein (Hallin et al., 2018).

Arc protein and mRNA Compared to Retroviruses and Gag-Proteins

By sequencing the human genome, Lander et al., (2001) revealed that over half of the non-protein-coding sequence had viral or transposable elements. Advances highlight that virus-like intercellular communication might regulate diverse biological processes. This based on the fact that the human genome consists of approximately 100 genes that have homology to Gag and over 1500 Gag open reading frames (ORFs) encoded as endogenous retroviruses (Hantak et al., 2021). Evolutionary analysis illustrates that Arc is derived through a vertebrate lineage of Ty3/gypsy retrotransposons, which indicates that Gag retroelements have been regenerated to mediate an intercellular communication pathway (Pastuzyn et al., 2018). Evidence suggests that the ORFs of mArc and dArc1 largely consist of regions derived of viral-like Gag-sequences (Zhang et al., 2015).

Retroviral Gag proteins consist of three characterized elements which constitute its functions. The CA (capsid), MA (matrix), and NC (nucleocapsid). Arc has structural and sequence-resemblance to the retroviral CA-element that mediates virion assembly. Evolved from sequence analysis is the theory that Arc might also consist of an MA-element (Hantak et al., 2021). The NTD of mArc has both physical and chemical properties similar to retroviral MA- and NC-domains of Gag. The MA-domain is essential for mediating membrane interactions and self-association, as well as RNA interactions that promote oligomerization (Eriksen et al., 2020). Despite that Arc lacks a zinc finger and the NC-element for certain viral RNA interactions, Arc encapsulates RNA which is a critical function for capsid formation (Pastuzyn et al., 2018; Hantak et al., 2021).

The Drosophila Arc homologues appear to result from a genomic duplication event (Ashley et al., 2018) and have CA-like domains that forms capsids. The amphipathic regions that expand from the capsid surface in spikes in dArc capsids are similar to viral amphipathic membrane-penetrating proteins (Spriggs et al., 2019). The dArc1 homologue contains a zinc finger at its CTD that is related to the Gag NC zinc fingers (Eriksen et al., 2020). Although the tetrapod *Arc* genes and the fly *Arc* genes originated independently from distinct lineages, they still share remarkable homology in their retroviral-like regions (Cottee et al., 2020; Hantak et al., 2021).

Life Cycle of Arc and a Comparison to Retrovirus and Gag-proteins Life Cycle

One of the most known and important molecular functions of Arc is the ability it has to self-associate into oligomers that forms virus-like capsids. These capsids are released into extracellular vesicles (EVs) that can transfer RNA between synaptic partners. This transfer indicates a new and previously unknown intra/intercellular pathway for signaling and communication (Eriksen et al., 2020). A predicted "life cycle" of the Arc protein tries to communicate both the release and uptake of these EVs.
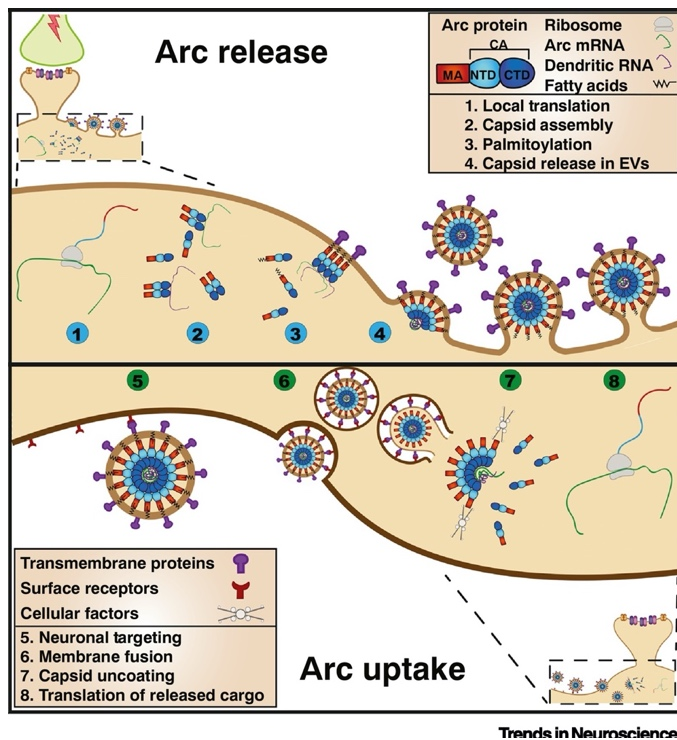
Figure 6. Predicted life cycle of the Arc protein. *Life cycle of Arc illustrating both the release and uptake of Arc capsids/EVs between pre- and postsynaptic terminals including most cellular processes leading to this. Reprinted with from Hantak et al., 2020.*

Arc mRNA travels into active dendritic spines of the neuron due to plasticity-inducing stimulation and therefore becomes enriched at sites of local synaptic activity (Chowdhury et al., 2006; Pastuzyn et al., 2018). The mRNA will here undergo local activity-dependent translation into Arc protein, creating a concentrated pool of the protein (Shepherd and Bear, 2011). This may lead to favoring conditions of capsid formation near the neuronal synapses Hantak et al., 2021). For mArc, the capsid formation is dependent on both the NTD and the CA-domain of the protein, as described earlier. dArc is able to self-assemble into capsids only requiring the CA-domain (Bramham. C, Personal communication). Capsids of dArc consists of 240 monomers of the protein that oligomerizes into icosahedral structural protein shells. The capsids has shown to be 37 nm in diameter. The atomic resolution structure of mArc capsids remains to be determined, but it has shown resemblance to dArc capsids through negative stain electron microscopy (Hantak et al., 2020).

Arc capsids binds RNA, including its own mRNA/transcript, to transfer this across the synapse to synaptic partners (Ashley et al., 2018). *Arc* mRNA is translocated to dendrites via sequences within the 3′ untranslated region (Dynes and Steward, 2007). These sequences are also required for the transfer of the capsids between cells in both mammalians and drosophila (Ashley et al., 2018). In drosophila, some of the N-terminals of the protein are packaged

inside the capsid core, and this may interact with the encapsulated RNA. This also seems to be the case in mArc (Hantak et al., 2021). Binding of RNA to Gag proteins seems to be required for capsid-assembly for viruses, which again might be needed for the loading into EV's. This suggests that Arc binding to its own transcript requiring its 3'UTR is important for proper capsid-assembly, which is also required for the release of Arc into EVs (Pastuzyn et al., 2018). Numerous binding sites in the 3'UTR is predicted in candidate miRNA (Wibrand et al., 2012), but it has not been determined if dArc binds directly to its own transcript. dArc2 capsids does not bind or transfer its own transcript unlike the other homologues and orthologues (Ashley et al., 2018).

Monomeric Arc protein undergoes post-translational modifications (PTM) before possibly forming capsids that is eventually released into EVs. Arc can undergo both phosphorylation, SUMOylation and palmitoylation etc (Hallin et al., 2018). These post-translational modifications can among others both participate in the regulation of Arc and/or mediate the subcellular localization and translation of the protein. In vitro, it interacts with pure phospholipid vesicles and it palmitoylates in neurons (Barylko et al., 2017). Palmitoylation is a modification of the protein that promotes interactions between Arc and the plasma membrane by seeding the formation of Arc capsids at membranes (Hantak et al., 2021). It creates a lipid "anchor" for the NTD which helps promote the association between the protein and cell-membrane (Barylko et al., 2017; Alberts et al., 2014, s.154).

Endogenous Arc protein that has oligomerized into capsids containing RNA is released from neurons in EVs. It remains to be determined how the capsids are enveloped in the EVs. The neuronal release of EVs is quite unknown, but it is principally suggested that Arc capsids can be enveloped by any cellular pathway generating EVs (Hantak et al., 2021). These EVs containing Arc capsids and its mRNA are also termed ACBARs which is short for Arc capsids bearing any RNA (Pastuzyn et al., 2018). The capsids are released into the EVs, or ACBARs, directly at the cell surface, and most likely further exocytosed into the synapse like other chemical compounds (ref neurotransmitters) (Hantak et al., 2021). Accordingly, this molecular mechanism seems to transfer genetic information between neurons (Pastuzyn et al., 2018) and between other synaptic partners.

As already mentioned, the Arc capsids that are loaded into EVs (or ACBARs) can be released from presynaptic sites and taken up by different synaptic partners. The 3'UTR in *Arc* mRNA

is necessary both for the loading into and transfer of the Arc protein and its genetic information. By studying the Drosophila NMJ, a functional role of this mechanism in synaptic development and plasticity has been documented. Unlike this, the importance of this transfer mechanism has yet to be identified in mammalians. dArc1 has shown to be present in both the presynaptic boutons and at the postsynaptic muscle region of the NMJ. On the other hand, it has only been detected that mArc is localized in dendrites postsynaptically (Ashley et al., 2018). However, it is proposed that mArc also serves as s signaling mechanism between dendritic spines as it shares structural and functional similarities to dArc and it has shown to be released into EVs (Pastuzyn et al., 2018). Despite the fact that it has not yet been determined whether Arc capsids transfer locally or long distance, we can assume that dArc capsids has the ability to transfer "long-distance", and mArc only locally due to where it has been detected.

Where the capsid-formation occurs, as well as where it is released will decide the cargo and membrane composition of the purified vesicles released from the presynaptic cell. Their targeting and signaling role is dependent on this (Hantak et al., 2021).

As shown in the figure of the predicted life cycle of Arc, purified Arc capsids lacking membranes are released into the postsynaptic cell directly at the cell surface. The EVs (or ACBARSs) seems to be targeted to the postsynaptic cell through specific surface receptors and other transmembrane proteins, but it has not yet been determined if there is any spatial/temporal cell-type specificity of the intercellular signaling in the brain (Hantak et al., 2021; Pastuzyn et al., 2018). The N-terminal of Arc is required for and mediates binding to the phospholipid membrane of the postsynaptic partner. Studies have indicated some specificity of transfer or uptake, due to the fact that it has been discovered that the transfer of *Arc* mRNA is much more efficient in neurons than in other cells such as HEK293 (Pastuzyn et al., 2018). Both purified Arc capsids and other endogenous EVs are taken up by endocytosis. Non-enveloped (no lipid bilayer) viruses have the ability for RNA-transfer across the endosomal membrane and studies suggest that Arc protein can do the same. Despite this, it is not clear how these non-enveloped capsids transfer the RNA across the membrane into the cytoplasm without membrane fusion and how the capsids escapes the endosome in advance of the degradation (Hantak et al., 2021; Pastuzyn et al., 2018). We can speculate on Arc taking advantage of membrane-penetrating instead of membrane fusion for membrane interaction to deliver cargo in the recipient cell (Hantak et al., 2020). dArc has spikes

extending from the cell surface that might have the ability for this penetrating mechanism (Erlendsson et al., 2020). Mammalian Arc undergoes conformational change due to exposure of low pH-conditions (Hallin et al., 2018), and the structural changes can lead to either pore formation or lytic degradation of the membrane, allowing Arc capsids to discharge its cargo (Tsai, 2007). The uptake of *Arc* mRNA requires properly formed and intact Arc capsids (Pastuzyn et al., 2018).

The Arc capsids containing its mRNA need to be uncoated and disassembled in the target cell to carry out its function. Without disassembly, no translation of the mRNA into protein is to happen. Disassembly of virus capsids is a complicated process dependent on several cellular host factors. Viral RNA needs to be reverse transcribed to DNA and then trafficked to the nucleus after it is delivered to the cytoplasm (Hantak et al., 2021). By interacting with the microtubule machinery, HIV-capsids promotes postentry trafficking either by disassembling while trafficked or when docking at the nuclear pore complex (Ambrose & Aiken, 2014). After disassembly the viral DNA can be released into the nucleus through the pore complex. Arc seems to not highjack the cellular translation machinery in the same manner, but it is predicted that the Arc protein has an internal ribosomal entry site (IRES) that allows preferential non-cap-dependent translation directly after disassembly in the dendritic cytoplasm (Pinkstaff et al., 2001; Hantak et al., 2021). RNA Polymerase II is stalling at startsite mediating and allowing rapid transcription of the immediate early *Arc* gene when disassembled (Bramham et al., 2010).

Taken together, all data suggests that Arc capsids in EVs can transfer its own transcript (expect dArc2) between neurons. This mRNA will after uptake and assembly be available in the cytoplasm of dendrites for activity-dependent translation (Pastuzyn et al., 2018). Arc is therefore rapidly synthesized in dendrites. Such high local concentrations of the protein may promote assemblance of capsids in the dendrites where the encapsulation of mRNA localized in dendrites can occur (Pastuzyn et al., 2018). In this manner, the life cycle of Arc continues as long as there is neuronal activity.

This possible and earlier unknown pathway for inter- and intracellular signaling reveals new mechanisms for information-transfer between synaptic partners, but big parts of the cell biology and function of these processes remains unknown (Hantak et al., 2021).
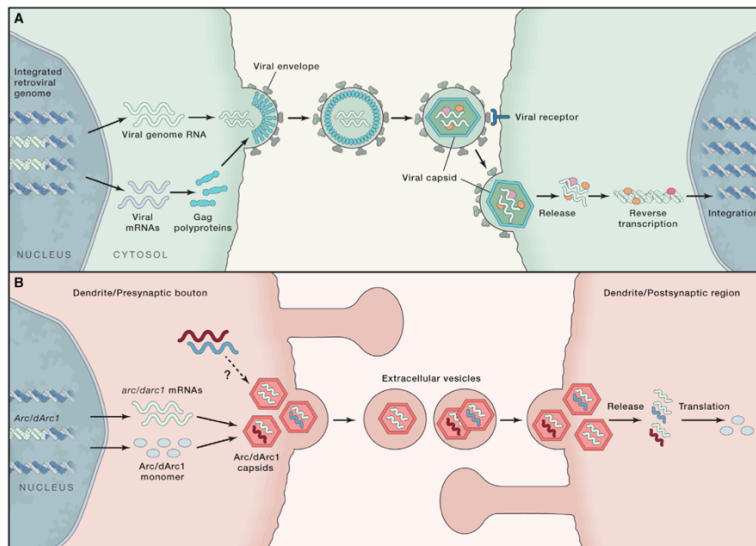
Figure 7. Arc as an endogenous retrovirus. *Release and uptake of Arc capsids (B) compared to similar processes proceeded by retroviruses (A). Reprinted from Parrish & Tomonaga, 2018.*

The homology between retroviruses/Gag-proteins and Arc and their capsids seems to be essential for the functions of Arc. As shown in figure 7, the compared life cycle of the two shows a lot of similarities including capsids and RNA transfer at the synapse. Despite this, they differ after assembly in the postsynaptic cell as viral RNA is reverse transcribed and integrated into the nucleus and *Arc* mRNA is directly translated in an activity-dependent manner (Eriksen et al., 2020). Retroviral Gags encapsulates viral RNA using the psi domain binding zink-finger motifs as a packaging signal (Comas-Garcia et al., 2017). dArc1 also has this zinc finger that plays a role in the binding of its mRNA (Eriksen et al., 2020). In addition mArc and dArc2 does not seem to have this zink-finger domain, but seems to use ionic interactions in the N-terminal to bind RNA (Pastuzyn et al., 2018). This indicates an earlier unknown, non-cell autonomous function for Arc as an endogenous neuronal retrovirus (Eriksen et al., 2020).

mRNA Specificity of Arc Capsids

It is an interesting topic whether the binding of Arc capsids to RNA is specific to *Arc* mRNA or not. The binding of both mArc and dArc capsids seems to be ionic interactions between the encapsulated RNA and the N-terminals of the protein packaged inside the capsid core (Hantak et al., 2021). This interaction requires the 3' UTR of the mRNA, and the binding of RNA seems to be necessary for normal capsid-assembly (Pastuzyn et al., 2018), except for dArc2 that does not bind its own mRNA (Ashley et al., 2018).

Retroviral Gag proteins has the ability for specific binding to viral RNA. Neither mArc or dArc contain the canonical NC element in their ORF for these specific viral RNA interactions. This proposes that RNA interactions and packaging of RNA into capsids probably isn't sequence specific, which means that both mArc and dArc capsids may not only bind to its own transcript, but genomic information from other genes as well. Despite this, the function of this other mRNA being transported is still unknown (Hantak et al., 2020),

In a study by Pastuzyn et al., (2018) performed on purified preparations of rat Arc (prArc), both *Arc* mRNA and mRNA from highly abundant bacterias, asnA, was determined in purified protein and total bacteria lysate. The results showed higher levels of *Arc* mRNA than asnA in both concentrates. This study supports the theory of Arc capsids not having any mRNA specificity as it shows that prArc capsids has little specificity of a particular mRNA, but it abundantly encapsulate RNA with similar stoichiometry.

Because of the fact that Arc has shown the ability to carry highly abundant mRNAs and it is hypothesized that Arc capsids doesn't show specificity *in vitro*, it is predicted that precise spatial and temporal expression of Arc in neurons is a prerequisite for the specificity of ACBAR cargo (Pastuzyn et al., 2018). Furthermore, packaging specificity may also be mediated by where the self-association into capsids and capsid release takes place, which again arises from different interactions between Arc and RNA (Pastuzyn et al., 2018).

We also know that if only one chain of *Arc* mRNA is loaded into Arc capsids, 2.7 kilobases of space remains available for other RNAs also supporting the suggestion that Arc EVs/capsids have the ability to contain other cargo than its own mRNA (Juhász. G, Personal Communication).

Ashley et al., (2018) on the other hand suggests some type of specificity *in vivo* between dArc1 and its own mRNA as no co-precipitation was observed with other RNAs based on a mass-spectrometry-based proteomic analysis of EVs compared to total cell proteins. The study was performed on S2 EVs.

Arc has a Functional Role in Synaptic Plasticity

As previously mentioned, the adaptive capacity of the mammalian brain depends on synaptic plasticity. Convergent lines of evidence support a role for Arc as a cell-autonomous organizer of synaptic plasticity and its function like a signaling hub protein (Eriksen et al., 2020). Plasticity in neuronal circuits forms cognitive mobility, emotional reactions and the basis of memory formation (Nikolaienko et al., 2018). Arc has been introduced to intersperse between oligomeric and monomeric forms (Byers et al., 2015; Myrum et al., 2015). Knowledge of the structure of monomeric full-length Arc is a crucial point to understand its function and has been studied in the plasticity investigations. The present *in vitro* work implies a potential physiological role for the mRNA at distinct stages of oligomerization (Eriksen et al., 2020). It will be critical to determine the specific functions of capsids versus monomeric Arc, due to functions in different aspects of synaptic plasticity. Intercellular signaling through extracellular vesicles (EVs) is thought to play important roles in synaptic plasticity, and previous studies have found that EVs can include AMPARs as cargo (Pastuzyn et al., 2018; Fauré et al., 2006).

Arc is involved in all aspects of synaptic regulation and plasticity (Bramham et al., 2010). An excitatory synapse displays a great diversity of plasticity. It is known that the abundance of AMPA-type glutamate receptors at the postsynaptic membrane is a major determinate of synaptic strength and long-term morphological changes such as spine growth and spine shrinkage. This depends on active cytoskeleton dynamics and *de novo* protein synthesis. Monomeric Arc interacts with dendritic spines and nuclear domains to regulate synaptic strength through well-defined molecular and cellular mechanisms (Nikolaienko et al., 2018). In the nucleus, Arc regulates gene transcription fundamental for homeostatic synaptic plasticity (Hallin et al., 2018). To consolidate activity-induced synaptic changes, Arc may stabilize the actin cytoskeleton and regulate the traffic of AMPARs both from and to the postsynaptic membrane (Grønli et al., 2014).

Brain derived neurotrophic factor (BDNF) may act like a trigger for stable synaptic change in regulation of dendritic protein synthesis with Arc as an effector. Therefore, blocking Arc synthesis may block this plasticity response to the tumor necrosis factor (TNF) in the brain *in vivo* (Kung et al., 2014). Additionally, it occurs that palmitoylation adjusts a part of Arc's functions in synaptic plasticity (Barylko et al., 2018).

Through nuclear import of Arc that leads to downregulation of GluA1 transcription, dendrite-wide down-scaling might be achieved (Korb et al., 2013). This mechanism may also be mediated by selective targeting of Arc to weakly activated inactive synapses as it results in Arc-dependent endocytosis of AMPARs (Shepherd et al., 2006; Béïque et al., 2011). Arc moves into dendritic spines as a result of neuronal activity and synaptic activation. In this process the mRNA encoding the protein is locally storaged, translated or decayed. Local synthesis of Arc may consolidate LTP through regulating the dynamics of the cytoskeleton and morphological enhancement of the dendritic spines (Fakuzawa et al., 2003; Messaoudi et al., 2007).

Neuronal circuits are adjusted by changes in neurotransmitter release. Previous studies suggest that a significant function of Arc is a process coupled to LTP (Barylko et al., 2018). It is shown that *Arc* mRNA transfers into active dendritic spines when being exposed to neuronal activity. Here it gets translated and then contributes to regulating trafficking of AMPARs by engaging the endocytic machinery (Chowdhury et al., 2006). Experience and synaptic activity affects the AMPARs (Shepherd, 2012), and AMPARs is responsible for the fast, immediate postsynaptic response to glutamate release. The monomeric Arc N-lobe regulates surface mobility of the receptors, probably by regulating a postsynaptic protein, Stargazin, interacting with Postsynaptic Density Protein 95 (PSD-95) (Bats et al., 2007). In LTD and scaling, Arc has the ability to interact with Dynamin and Endophilin, elements of the endocytic machinery, and it has been observed that enhancement of Dynamin polymerization and GTPase activation might explain the Arc-mediated endocytosis of synaptic AMPARs (Byers et al., 2015). In LTP, Arc allows stabilization of dendritic spines. Unlike this, in LTD Arc reduces the spine number and communicate with parts of the endocytic machinery to increase endocytosis of AMPA receptor trafficking (Hallin et al., 2018; Suzuki et al., 2020).

Previous studies provide evidence that Arc also mediates homeostatic scaling of AMPARs (Shepherd et al., 2006), and cross-modal plasticity through different brain regions (Kraft et al., 2017). This implies regulation at the circuit level in a non-cell autonomous way (Pastuzyn et al., 2018). High levels of Arc shut off the homeostatic expansion in AMPAR's function, caused by chronic neuronal inactivity. In contrast, loss of Arc leads to strengthening of AMPAR's function and eliminates homeostatic scaling of the receptors. Therefore, Arc

provides a precise and continuous control over cellular excitability and synaptic strength. These observations highlight the importance of Arc's dynamic expression (Shepherd et al., 2006).

As a contrast to what has just been presented, a recent study took a comprehensive approach to determine if Arc actually is required for hippocampal LTP by using multiple LTP-inducing protocols in a variety of Arc knock-out (KO) lines. Surprisingly, they concluded that Arc is not needed for hippocampal LTP, indicating that Arc's role in memory consolidation is likely mediated through mechanisms that do not affect the maintenance of synaptic potentiation (Kyrke-Smith et al., 2021). This is partly in line with SHY due to the fact that there may exist other mechanisms important for LTP that are still unknown. It is also uncertain even if Arc participate in this mechanism at all. In addition, SHY claims that one not only gets an increase in synapses in the different stages of sleep, but probably rather just a selection of some synapses that are amplified. Thus, it is possible that reinforcement also can happen during sleep with increased activity in some selected, spared and then amplified synapses that reactivates by LTP during both REM and NREM sleep, however still not dependent of Arc necessarily (Tononi & Cirelli, 2020).

A lot of the cellular physiology connected to the Arc capsids in EVs is not well known yet, but evidence from the fruit fly (Drosophila) suggests that the transmission of RNA in Arc capsids are necessary for plasticity at the neuromuscular junction (NMJ) (Erlendsson et al., 2020). Ashley et al., (2018) suggest that a reduction of dArc1 in motorneurons inhibits synaptic extension throughout larval development. Previous studies indicate that numerous mutations in genes required for NMJ development result in a reduction in bouton numbers (Ataman et al., 2006; Harris et al., 2016). In addition, Ashley et al (2018) found a downregulation of dArc1 in neurons which resulted in a collection of immature synaptic boutons. Thus, a functional role in synaptic plasticity and development is documented at the NMJ. The transfer of dArc1 is essential for synaptic bouton maturation and the formation of activity-dependent synaptic boutons. Therefore, dArc1, like mArc, is needed for both acute and developmental forms of synaptic plasticity (Ashley et al., 2018).

Present work implies a potential physiological role for *Arc* mRNA at distinct stages of oligomerization. Therefore it will be critical to determine the specific functions of Arc capsids versus monomeric Arc (Eriksen et al., 2020). Arc is a regulator of synaptic plasticity as it

regulates trafficking of AMPARs through the endocytic machinery, and maturation of dendritic spines. Both dArc and mArc plays a role in diverse aspects of synaptic plasticity (Ashley et al., 2018). The expression of Arc is highly dynamic thus it mediates homeostatic scaling of the AMPARs. However, Arc may not be needed for hippocampal LTP but is mediating memory consolidation through other mechanisms (Kyrke-Smith et al., 2021).

Additional Molecular Functions of Arc

In addition to capsid formation and participating in the regulation of synaptic plasticity, Arc also has several other molecular functions. Some of them applicable only for mArc, and some only mediated by the monomeric form of the protein.

The full-length recombinant monomeric mArc protein in humans seems to be pyramid-shaped and not only capable of self-association into oligomers, but also competent of reversible self-association. That means that the oligomers formed by this variant of the protein is soluble. The oligomeric species are dependent on the ionic strength available, and at low ionic strength it will reverse into monomers or dimers. This molecular mechanism addresses the possibility that the function of the Arc protein is determined by the oligomeric state (Myrum et al., 2015).

Arc mediates a highly dynamic system with a rapid turnover and is expressed in the excitatory projections of neurons in the mammalian brain. Arc also has rapid proteosomal degradation of the protein and mRNA degradation by translation-dependent decay (Bramham et al., 2010).

Studies has shown that monomeric mArc controls dendritic cell-dependent T-cell activation, and is therefore implicated in the mammalian immune system. Arc is activating T-cells by specifically being expressed in skin-migratory dendritic cells regulating fast inflammatory migration of dendritic cells from the skin. This shows the possible potential and importance of Arc intercellular signaling outside the nervous system (Ufer, 2016; Pastuzyn et al., 2018; Uniprot, mArc). By harnessing the pathway of RNA transfer that the Arc protein seems to regulate, it is believed that new aspects of genetic engineering of/or RNA delivery into cells by using Arc capsids bearing any RNA (ACBARs) may avoid the hurdle of immune activation (Pastuzyn et al., 2018).

Through searches at the UniProt-database, we found that mammalian Arc capsids, including rat, mice and human, may participate in the elimination of synaptic material as the protein is a key mediator of activity-dependent synapse elimination in the developing cerebellum in the brain. Arc accumulates at weaker synapses, most likely to prevent unwanted enhancement as the elimination occurs at surplus climbing fiber synapses (Uniprot, mArc).

Arc also regulates an endosomal pathway essential for activity-dependent β-amyloid generation (Wu et al., 2011).

Mammalian Arc (mArc) and Drosophila Arc (dArc1 and dArc2) – a Comparison

Arc structures has already been discussed, but to make a summary there are some differences between mArc and dArc. The most apparent difference is the fact that dArc is lacking a large N-terminal domain (NTD) called the oligomerization region that mArc has. dArc is able to self-associate into oligomers forming virus-like capsids only requiring the capsid-domain (CA-domain). The CA-domain with virus/Gag-like sequences is present in both orthologues. Another similarity in structure is the bilobar CTD. At last, mArc has a ligand binding pocket that dArc is missing (Hallin et al., 2018).

Despite some differences in structure mArc and dArc (dArc1 and dArc2) carry out a lot of the same molecular functions. mArc is predominantly localized at postsynaptic sites in the mammalian brain. Unlike this, it remains to be determined whether dArc is mostly expressed at the pre- or postsynaptic cell in the fly brain (Hantak et al., 2021). Both mammalian and drosophila Arc forms virus-like capsids, quite similar in structure. Thus, dArc capsids also has its so-called spikes (Erlendsson et al., 2020). The monomeric form of the proteins in all species regulates homeostatic forms of synaptic plasticity, such as AMPAR scaling (Pastuzyn et al., 2018). Another similarity between the two orthologues is the fact that the retrotransposon-like sequences in the 3'UTR of the *Arc* mRNA is important for both the loading and transfer of its mRNA in the virus-like capsids. dArc2 does not bind its own transcript. 40% of the DNA of the protein is considered as transposable elements, also referred to as "junk DNA" (Ashley et al., 2018). The translation of *Arc* mRNA is activity-dependent in both mammalians and drosophila. mArc plays a role in T-cell activation and also provides elimination of synaptic material which dArc doesn't.

The open reading frame (ORF) of dArc1 and dArc2 is highly conserved through evolution. Despite this, the two homologues have vastly differences in the 3' UTR of the mRNA. The 3' UTR of dArc2 is much shorter. Supported by this, the absence of *dArc2* mRNA in EVs might be explained. It seems like the 3' UTR of dArc2 is lacking some functions required for loading its mRNA into EVs. The ORF of dArc2 has a similarity of 71,6% and identity of 52,2% compared for the ORF of dArc1. The main difference between the two is the putative zink-finger domain at the C-terminal that is lacking in dArc 2 (Ashley et al., 2018).

As previously discussed dArc has been detected at both sides of the synaptic cleft at the drosophila NMJ, while mArc only has been localized postsynaptically in dendrites (Ashley et al., 2018). Differences between the different homologues of mArc in mice, rats and humans remains to be determined.

Regulation of Arc Expression and Capsid Assembly

It is to be expected that the oligomeric state of the Arc protein is highly controlled. The local concentration of monomeric Arc in the cells may be one part of the regulation. This statement is supported by the fact that such virus/Gag-like capsids that Arc forms requires the local concentration of the monomeric form of the protein (Hantak et al., 2021). Local concentrations of the protein are "seeded by" interactions between proteins and membrane or proteins and nucleic acid (Lingappa et al., 2014). *Arc* is being translated into protein due to synaptic activity where it is trafficked into active synapses, which means that Arc expression is induced by synaptic activity. By being activity-dependent translated into protein, a highly concentrated pool of the monomeric protein will be created (Shepherd and Bear, 2011). This may lead to favoring conditions of capsid forming nearby neuronal synapses. It seems to be essential with a precise regulation of Arc expression, capsid formation and activity in the nervous system for normal cognition (Pastuzyn et al., 2018).

As already discussed, the Arc monomer can undergo post-translational modifications (PTMs) which might be a critical determinate for switching the functional modality of the protein. Some of these modifications, such as SUMOylation (Craig et al., 2012) and ERK-catalyzed phosphorylation (Nikolaienko et al., 2017), plays a role in the regulation of interactions between proteins, the capsid formation, and the subcellular localization of them. Therefore SUMOylation are a link between Arc and action cytoskeleton regulation in LTP (Craig et al.,

2012) as it helps to carry out Arc's function. Other PTMs regulates Arc turnover. Both ubiquitination, GSK-catalyzed phosphorylation and lysine acetylation are involved in this process (Greer et al., 2010; Mabb et al., 2014; Nikolaienko et al., 2017; Lalonde et al., 2017). Ubiquination is targeting Arc for rapid proteosomal degradation (Greer et al., 2010; Soulé et al., 2012; Mabb et al., 2014). The difference between ERK-catalyzed and GSK-catalyzed phosphorylation is which enzyme that catalyzes the process but they both lead to nucleocytoplasmic localization of the Arc protein (Nikolaienko et al., 2017). Calsium-calmodulin kinase II phosphorylation of Arc inhibits oligomerization into virus-like capsid (Zhang et al., 2019). Palmitoylation as mentioned earlier is also a prime candidate of capsid-regulation as it allows insertion directly into the hydrophobic core of the lipid bilayer (Barylko et al., 2018). This is a dynamic process is neurons, which indicates that the assembly can be controlled by neuronal activity (Hantak et al., 2021). Post-translational modifications might have a role in the dictating of Arc´s localization and function (Craig et al., 2012).

Another factor that also contributes in the regulation of capsid formation is the interactions between Arc and other host cellular factors, such as proteins. An example is that through direct binding of the capsid domain of Arc, interactions with NMDA-receptors will inhibit the oligomerization (Nielsen et al., 2019). Similarly to other host cellular proteins, Staufen regulates Gag-RNA interactions. In addition, Staufen is also critical for the regulation of dendritic trafficking of mRNA in neurons, including *Arc* mRNA. This regulates the expression of Arc as it can promote higher level of local concentration of *Arc* mRNA and therefore induce the translation of it into protein due to neuronal activity. Strikingly, the parallels between virus-RNA interactions and dendritic mRNA regulation suggests an important role of cellular host factors in the biogenesis of ACBARs and RNA packaging (Herauds-Farlow and Kiebler, 2014; Pastuzyn et al., 2018).

Some aspects of the regulation of *Arc* mRNA resemble viral RNA. This is supported by the fact that Arc has an internal ribosomal entry site, also mentioned as IRES, which allows cap-independent translation of the mRNA (Pinkstaff et al., 2001).

It is predicted through bioinformatic analysis that the 3'UTR of Arc has several miRNA binding sites. The conclusion of the study was that the expression of Arc is partly regulated by multiple such miRNA bindings (Wibrand et al., 2012). We can also say that the 3'UTR of *Arc* mRNA in general is participating in regulation of Arc as it required for both loading and

transfer of the mRNA into capsids and therefor the translation into protein in the postsynaptic cell.

The presence of and interaction with different ligand binding proteins may also affect and regulate the capsid-formation of mArc. For example, through direct binding of the capsid domain of Arc, interaction with NMDA-type glutamate receptor will inhibit the oligomerization of monomeric Arc (Hallin et al., 2018; Hantak et al., 2021).

Physiological Functions of Arc

The Arc-dependent intercellular communication may participate in a variety of cell types and physiological functions. The translation and expression of Arc are affected by sleep-related changes in the human brain. The monomeric Arc is functioning in many aspects in the scaffold of the synapses and is a master regulator of synaptic plasticity. In conjunction with this, Arc also plays a role in long-term memory trace formation and postnatal cortical development (Eriksen et al., 2020).

Arc gene expression and translation is connected to sleep-related aspects and is implicated in LTP, LTD and homeostatic scaling

Most brain circuits undergo synaptic down-selection during sleep according to SHY (Tononi & Cirelli, 2020). Arc is a neuronal immediate early gene (IEG) product that are involved in such synaptic downscaling. Since Arc is involved in memory trace formation at a synaptic level and consolidation of memory trace is performed under sleep, we can speculate on sleep-related changes in Arc expression (Juhász. G, Personal Communication). The Arc gene has shown to be induced by prolonged wakefulness (SD) in the rodent brain (Suzuki et al., 2020; Honjoh et al., 2017; Soulé et al., 2012; Taishi et al., 2001). It is a previously known fact that Arc as a multifunctional dendritically translated protein is implicated in LTP, LTD and homeostatic scaling, but the function of Arc related to LTD and LTP during sleep remains to be determined (Shepherd and Bear., 2011; Grønli et al., 2014; Suzuki et al., 2020).

Sleep promotes the cortical translation of mRNA related to plasticity, such as Arc and BDNF, but not the transcription. If this process is interrupted, it will lead to consequences in the proteins' function as it will stop the cortical consolidation of experience (Seibt et al., 2012).

After long periods of spontaneous nocturnal wakefulness, Arc moves into the nucleus of cells in the cerebral cortex (Honjoh et al., 2017). This also occurs due to sleep deprivation (SD) and/or only a few hours of sleep after long spontaneous wakefulness (Suzuki et al., 2020). A study performed by Honjoh et al., (2017) on mice (mArc) revealed that 2 hours of sleep lead to an increase in the ratio between nuclear Arc and cytoplasmic Arc when compared to 2 hours of SD. The study found a 15-30% increase of this ratio after sleep than in waking and sleep deprived mice. The effect of these two hours of sleep then revealed a small and restricted effect on the superficial layers of several cortical areas in the brain. The functional effect remains to be established, but it is suggested that Arc here inhibits GluA1 transcription, a subunit of AMPARs (Honjoh et al., 2017). This agrees with SHY that suggests that most brain circuits undergo sleep-dependent down-regulation.

Suzuki et al., 2020 also found that the expression of the *Arc* gene was dramatically induced both in the nucleus and cytoplasm of the neurons, as well as in the synaptic clefts of the cerebral cortex. It still remains unclear if and how the Arc protein is participating in sleep regulation, but the study performed by Suzuki et al., (2020) aimed to find the functional role of Arc in homeostatic sleep regulation. The results found was that Arc was important for inducing several homeostatic responses at behavioral and molecular level, induced by SD. Arc was firstly shown to induce rebound of sleep, expression of a subset of SD-induced genes and expression of AMPARs especially the GluA1 subunit. Multiple roles of Arc are suggested to be dependent on the subcellular localization of the protein which seems to correlate with Honjoh et al., 2017.

Sleep-deprivation affects translation initiation and elongation activity in a region-specific manner. *Arc* mRNA has shown to be increased in the prefrontal cortex in the rat brain without having an effect on the protein expression (Grønli et al., 2014). Ubiquitination of Arc and the proteosomal degradation of Arc that it causes might be an explanation for this (Soulé et al., 2012). During sharp waves/ripples (SWRs), rapid rates of *Arc* mRNA and degradation of the protein can burst the expression of the Arc protein (Grønli et al., 2014).

In contrast to other studies presented, Taishi et al., (2001) study on rats presented results showing that *Arc* mRNA not only increased in the cerebral cortex of the brain after SD, but also in the hippocampus. Conversely, the study also showed that the expression of *Arc* mRNA decreased with enhanced ambient temperature during sleep, suggesting that such manipulation

in addition to different aspects of the sleep also affects synaptic plasticity and memory consolidation. In contrast to this Delorme et al., (2019) claims to see evidence that Arc is affected minimally across the hippocampus during SD due to a decrease in the cells expressing Arc.

It is already mentioned that the Arc gene and the translation into protein is dramatically induced in sleep-deprived brains, both by forced and spontaneous wake (Suzuki et al., 2020; Honjoh et al., 2017; Soulé et al., 2012; Taishi et al., 2001). During sleep on the other hand, Arc seems to be down-regulated in the brain (Suzuki et al., 2020). Surprisingly, Arc has shown to be is induced during REM-sleep (Grønli et al., 2014). The gene is triggered by PGO-waves during REM sleep (Ribeiro et al., 2002). An episode of REM sleep provides an opportunity window reactivation of expression of several immediate early genes including Arc (Mendes et al., 2019). Another study contrastingly presents that the expression of various immediate early genes throughout the cerebral cortex, including *Arc*, is decreased following a few hours of sleep (Cirelli & Tononi, 1999; Cirelli et al., 2004).

Looking forward, it will be important to clarify time-dependent functions of Arc and whether Arc entrance into specific synapses is gated by sleep as the role of Arc in sleep is still unknown (Bramham et al., 2010; Craig et al., 2012; Suzuki et al., 2020; Honjoh et al., 2017). Also its function in sleep architecture, sleep-related gene-expression and synaptic scaling, or sleep homeostasis need to be investigated due to the lack of known molecular mechanisms shown until now related to this (Suzuki et al., 2020). Convergent lines of evidence suggest that sleep reduces, and wakefulness potentiates synaptic strengthening (Suzuki et al., 2018) which again suggest that Arc has a potential role in such sleep-related processes supported by the findings in presented studies. This supported by the fact that Arc is a key mediator of synaptic plasticity and synaptic strengthening, and that the gene and the translation of it has shown to be induced after sleep deprivation and only a few hours of sleep (Suzuki et al., 2020, Honjoh et al., 2017; Soulé et al., 2012; Taishi et al., 2001). In this manner sleep-related aspects of Arc expression also is connected to memory and memory consolidation. Sleep-related studies on Arc expression has mostly been performed on rodents, but because mammalian Arc (mArc) shows similarity in both mice, human and rats, we can suggest that the results also applies for human brains.

Acute sleep deprivation leads to decrements in spatial memory and cognitive impairments (Grønli et al., 2014), in addition to worsening of neuropsychiatric and mood disorders (Short & Louca, 2015; Bishir et al., 2020). The hippocampus is vulnerable to changes in gene expression, cell signaling and protein synthesis, which are outcomes after acute sleep deprivation. Moreover, sleep deprivation also has long lasting effects on memory that continue after recovery sleep (Gaine et al., 2021). Studies from humans indicates that acute sleep deprivation impairs episodic memory and hippocampus dependent memory associations for more than two days, despite recovery sleep (Chai et al., 2020). A recent study investigated the effects of acute sleep deprivation on gene expression in the hippocampus. The genes that were significantly upregulated after SD were related to RNA splicing and the nucleus. On the other hand, cell adhesion, dendritic localization, the synapse and the postsynaptic membrane were the cellular compartments most significantly associated with downregulated genes after SD. The study validated the effects of sleep deprivation on *Arc* and other genes of interest that act in the postsynaptic dendrites. All these genes were significantly changed after SD, and the results establish that SD differentially regulates gene expression on various transcriptomic levels to effect hippocampal function. Further research is vital to understand the effects of acute sleep deprivation on long-term memory (Gaine et al., 2021).

Arc Mediates Memory Formation Through Synaptic Plasticity Which Again is Tightly Connected to Learning and Normal Cognition

It has been demonstrated that Arc is an effector in dendritic plasticity and is associated with a function in learning and memory (Li et al., 2015). Mammalian brains process and store information from the outside world through synaptic connections within networks of neurons (Pastuzyn et al., 2018). Studies provide persuasive evidence for the existence of engrams, which is defined as a physical trace of a memory that are encoded in a neuronal circuit. To create the memory engram, certain memories are thought to be encoded via specific neurons that are active during learning. This through forming a particular circuit between neurons by synaptic plasticity mechanisms. Thus, there must exist specific physical-chemical changes in the nervous tissue between neurons that correlate to the engram or the storage of information. These changes represent the required circumstances of remembering (Josselyn & Tonegawa, 2020). Arc-dependent homeostatic scaling might also be essential for stabilizing memory engrams (Shepherd et al., 2006).

Arc capsids released in EVs functions to transfer intercellular cargo that converts the state of adjacent cells, which is needed for cellular consolidation of information. This implies that a specific regulation of Arc expression is required for normal cognition (Pastuzyn et al., 2018).

A well-established view of how experience are stabilized over time suggests that *de novo* protein synthesis must occur to obtain substrates for the functional and structural modifications of synapses which support memory consolidation (Hernandez & Abel, 2008; Yap & Greenberg, 2018). Synapse-specific plasticity is required for creating memory circuits, but it remains unclear how the gene program leads to consolidation and memory storage. The Arc transcription in neuronal circuits is connected to learning *in vivo* (Hantak et al., 2021). Due to ongoing learning, it is assumed that synaptic strength expands during wake in many brain circuits, and then synaptic strengthening generates the need for synaptic renormalization. Despite this, pruning might rather happen during sleep, however where some or main elected synaptic contacts still are getting strengthened (Tononi & Cirelli, 2020).

Monomeric Arc is critical for experience-dependent plasticity in the visual cortex in vivo (McCurry et al., 2010). Thus, it is one of the main effector proteins localized at the synapse and it works as a key component for transducing experience into long-lasting synaptic changes in the brain (Day & Shepherd, 2015). It remains unclear how Arc mediates this process. Kyrke-Smith et al., (2021) have presented the surprising observation that Arc is not required for hippocampal LTP and may regulate memory consolidation through alternative mechanisms. The observations posit that Arc expression driven by LTP-induction might contribute to non-LTP plasticity. This further serves homeostatic functions such as heterosynaptic LTP or synaptic downscaling to maintain network stability. This idea is also compatible with the finding that Arc interacts with calcium/calmodulin-dependent protein kinase (CaMKIIβ) at inactive synapses to decrease synaptic strength by removing AMPARs (El-Boustani et al., 2018). Prolonged fear memories are associated with a specific delayed elimination of dendritic spines and the reactivation of neuronal ensembles formed in fear experience. Late Arc expression is required in both of these processes. Arc may also eliminate small mushroom spines after learning, but it is not clear if this occurs by heterosynaptic LTD (Nakayama et al., 2015).

Many possibilities exist to explain how Arc function as a key component in long-term memory without being necessary for LTP maintenance. Arc's possibility to form virus-like capsids that can transfer RNA cell-to-cell is another mechanism that may underlie long-term memory that is independent of LTP (Kyrke-Smith et al., 2021).

All the processes from the turnover of spines to memory are highly dynamic and change constantly due to ongoing stimulation. According to SHY, a specific process of down-selection can explain how sleep promotes memory consolidation and new learning. Neurons that are co-active during learning will more likely co-fire during subsequent NREM and REM sleep and therefore be protected from down-selection. After sleep, these neurons maintain or slightly expand the overall level of activity that they had after learning, but it is unknown what happens to their synapses (Tononi & Cirelli, 2020). A competing hypothesis to SHY suggests that the active upscaling and downscaling of synaptic weights during sleep have an effect on memories in activated or deactivated circuits during waking experience. This is then leading to memory changes beyond rescaling. This agrees with the fact that memory have been shown to depend on both synaptic upscaling and downscaling. It has been observed that Arc also is reinduced during REM, which provide evidence that synaptic upscaling and downscaling may occur concomitantly during sleep (Calais et al., 2015).

The memory consolidation process seems to rely on the complementary process during the diverse sleep stages. REM sleep constitutes a privileged window for hippocampus-driven cortical activation, which further may be vital in the communication of memory traces from the hippocampus to the vertebral cortex (Ribeiro et al., 2002). Given the importance of REM sleep for memory, it is possible that neural coordination during this sleep phase can contribute to cognitive deficits. Memory impairment is one of the most usual cognitive deficits in patients with epilepsy. Therefore, circuits responsible for memory consolidation during sleep appear to be gradually degraded in epilepsy (Mendes et al., 2019).

Put into context, sleep is well documented to strengthen memory and memory consolidation which again is tightly connected to learning. The protein is also critical for the previously mentioned behavioral and multiple molecular responses to SD. It is known that inhibition of Arc expression crucially impairs long-term memory, and sleep deprivation have shown to be a prerequisite to increase the level of Arc in neurons in published studies so far. This further provides a multifunctional role for Arc in sleep homeostasis, that also might be attributed by

sleep/wake-associated changes in the subcellular location of Arc (Suzuki et al., 2020). However, selectively and even strengthening specific synapses may implicate Arc, but the detailed cellular mechanisms implicating LTD and LTD, synaptic strengthening and synaptic pruning remains to be elucidated. Arc may regulate memory consolidation through other mechanisms than LTP as previously discussed. Arc functions necessary for memory consolidation implies that regulation of the expression of Arc is needed for learning and normal cognition. Arc functions necessary for memory consolidation implies that regulation of the expression of Arc is needed for learning and normal cognition.

Arc is Connected to Disease Through Gene Mutations and Misregulation

Due to the fact that the nervous system is such a complex system important for all parts of the body, it is vulnerable to various diseases. Mutations in the *Arc* gene is associated to diseases like Alzheimer´s disease, Schizophrenia and autism. The expression of Arc protein is also associated with various disorders of human cognition as Gordon Holmes syndrome and fragile X-syndrome. Arc protein expression is also associated with the neurodevelopmental disorder Angelman syndrome (Ashley et al., 2018; Pastuzyn et al., 2018; Nikolaienko et al., 2018). Also, the action of Arc is thought to contribute to decreased synaptic strength and genetic or pharmacological inhibition of Arc expression that critically impairs long-term memory (Grønli et al., 2014; Zhang et al., 2015).

Alzheimer´s disease is linked to cytoskeletal abnormalities in neurons (Kandel, 2000, p.1154), and both increased and reduced expression of Arc are reported in this disease (Nikolaienko et al., 2018). Arc also regulates an endosomal pathway essential for activity-dependent β-amyloid generation. β-amyloid peptides are pathological mediatoros of Alzheimer´s disease, suggesting that Arc participates in the pathogenesis of Alzheimers´s disease (Wu et al., 2011). Other findings indicates that Arc capsids bearing any RNA (ACBARs) also might be connected to Alzheimer pathogenesis as results suggests that EVs may be providing a significant source of extracellular Aβ peptide (Pastuzyn et al., 2018).

Gordon Holmes syndrome is characterized by cognitive decline and dementia. The TRIAD3A protein in neurons regulates synaptic transmission and plasticity through acting as a ubiquitin ligase of Arc. Loss-of-function dementia-related mutations in the *TRIAD3A* gene or reduced TRIAD3A protein levels may participate to cognitive deficits in dementia through misregulation of Arc degradation in neurons (Mabb et al., 2014; Husain et al., 2017). In

fragile X-syndrome, inhibitory control of monomeric Arc translation is disturbed. Mardirossian et al (2009) propose that aspects of plasticity, including Arc, are affected by deletion of Ube3A protein and may contribute to the hippocampal dysfunction observed in Angelman syndrome in mice. Several studies suggest that the Angelman Syndrome protein Ube3A regulates synapse development through ubiquitinating monomeric Arc, and reduced ubiquitination of Arc might result in an expansion of Arc-dependent synaptic down-regulation (Greer et al., 2010; Zhang et al., 2015). Disruption of Ube3A function in neurons leads to an increase in Arc expression and a decrease in the number of AMPARs at excitatory synapses. They propose that this deregulation of AMPARs expression at synapses might commit to the cognitive dysfunction that occurs in Angelman Syndrome (Greer et al., 2010). Although Arc is not the predominantly cause of any of the mentioned disorders, it is clearly affected in this spectrum of neurodegenerative disorders (Nikolaienko et al., 2018).

Additionally to Arc misregulation and mutations, Gag proteins expressed by endogenous retroviruses may contribute to the spread of pathology via atypical intercellular communication or interference with the signaling of endogenous Gag proteins (Hantak et al., 2021).

Different types of sleep deprivation, such as night- and shift work is a known risk factor for various neurodegenerative diseases (Pega et al., 2021; Kecklund & Axelsson, 2016). Since Arc protein is affected after sleep-deprivation and prolonged waking as shiftwork especially at night, the protein may also be associated with dementia. Continuous short sleep duration between the age of 50 and 70 compared to continuous normal sleep duration have been found to associate with a 30 % increased dementia risk independently of sociodemographic, behavioural, cardiometabolic, and mental health factors. These data indicate that short sleep duration in midlife is connected to an increased risk of late-onset dementia (Sabia et al., 2021). Changes in the level and compartmentalization of Arc after sleep deprivation or lack of sleep then also likely play a role in development of some of these neurodegenerative disorders. Marti et al (2017) investigated the effects of stimulated shift work on protein synthesis markers. The results indicated time-of-day variation of protein synthesis markers in the prefrontal cortex, together with a significant reduction in Arc protein. Simulated night shift work in rats disrupted the pathways regulating translation of mRNA in the prefrontal cortex, which might explain the impaired waking function in night shift work.

In addition to this, transcription of synaptic plasticity-related genes in patients with abnormal sleep patterns may be combined with type 2 diabetes. It has been shown that sleep disorders can regulate gene expression in the mouse brain by DNA methylation and hydroxymethylation, and it is well accepted that DNA methylation and hydroxymethylation are closely connected to the prevalence of type 2 diabetes. Hydroxymethylation levels of Arc in patients with abnormal sleep patterns combined with type 2 diabetes have been reported to be significantly higher than in patients without abnormal sleep patterns (Zhang et al., 2017).

Possible Diagnostic Value of EVs Containing Arc Capsids and mRNA

Accumulating evidence suggests that monomeric Arc is involved in the spread of pathology during neurodegeneration. This spread may occur through synaptic connections and it is therefore regulated by neuronal activity. In various experiments, EVs are thought to bypass the blood-brain barrier and have shown promise as a pool of enriched biomarkers in several diseases (Thompson et al., 2016). Thus, the EVs collect their cargo from the content of the cell that constructs them (O´Brien et al., 2020). These studies have resulted in an enthusiasm for EVs as a trans-cellular communication strategy in the healthy and diseased brain (Ashley et al., 2018). Elevated levels of specific miRNAs in EVs have been observed in cerebrospinal fluid from patients with Alzheimer´s disease, Parkinson disease, glioblastoma and breast cancer. Furthermore, the reproduction of neurodegenerative disorders in mammals, such as Amyloid Lateral Sclerosis (ALS), emerge to be partly mediated by the transfer of prion-like proteins through exosomes across cells (Basso & Bonetto, 2016). Transcripts of Arc are also found in blood serum (Sanders et al., 2019), here suggesting a connection with a bloodbourne neuronal signaling pathway.

What kind of biological impact the extracellular vesicle (EV)-associated transcriptomes have remains to be validated. A crucial aspect is that exosomal miRNA can mediate communication between immune cells (Turchinovich et al., 2019). The Arc transcripts expressed in dendritic immune cells contribute to immune response. Since Arc controls inflammatory dendritic cell migration from the skin, it thereby controls T-cell activation (Ufer et al., 2016). A recent study established that Arc is released from peripheral dorsal root ganglion neurons that possibly is significant for controlling neuroinflammation in the skin (Barragan-Iglesias et al., 2020).

In addition to being expressed in the nervous system, Arc is also expressed in the periphery whi raises the suggestion that Arc capsids may not be immunogenic (Ufer et al., 2016; Sanders et al., 2019). In fact, the Arc capsids may contain anti-inflammatory cellular products as additive signaling proteins in addition to the protein. Based on this data a suggestion of Arc playing a role as a mediator of neuron to non-neuronal signaling in the periphery has been presented. The same study also revealed a novel mechanism of Arc-dependent regulation of neuroinflammation (Barragan-Iglesias et al., 2020).

Conclusion

The activity-regulated cytoskeleton associated protein, Arc, is an activity-dependent immediate early gene product. It is a hub protein with diverse roles in intracellular neuronal signaling. Evolutionary analysis indicates that Arc is derived from a vertebrate lineage of Ty3/gypsy retrotransposons, likely through an earlier transposon insertion. Arc shows homology to retroviruses/Gag-proteins with its retroviral/retrotransposon Gag-like sequences (Ashely et al., 2018). Drosophila Arc (dArc) and mammalian Arc (mArc) has some of the same functions despite some differences in structure.

Arc has the ability to self-associate into oligomers forming virus-like capsids that seems to transfer mRNA across synapses through being released into EVs. However, the function that this earlier intra/intercellular communication pathway with capsids bearing mRNA mediates is largely unknown. The mRNA specificity of Arc capsids has not yet been fully determined, but they do not seem to show mRNA specificity in vitro which mean they may have the ability to transfer other cargo.

Unlike the functional role of the oligomeric form of Arc, the functional role of monomeric Arc has been determined on a much larger scale. Arc is pivotal for both long-term synaptic plasticity, memory trace and postnatal cortical development which are all tightly connected to each other (Eriksen et al., 2020). Earlier studies have proposed that Arc leads to maintenance of LTP which is a manifestation for memory formation. However, a recent study hypothesize that Arc is not required for hippocampal LTP, and therefore mediates long-term memory by other functions. The Arc protein has several additional molecular functions beyond capsid formation and mediating synaptic plasticity. The protein is tightly controlled and regulated, among others by different post-translational modifications.

The expression and translation of the Arc protein are affected by sleep-related aspects. Prolonged wakefulness, SD, induces Arc mRNA expression in the rodent brain, but its functional role in LTD, LTP and homeostatic scaling during sleep remains to be determined. Mutations in the Arc gene is related to several neurodegenerative/neurodevelopmental disorders similarly to misregulation of the protein. EVs containing Arc capsids and mRNA might have a diagnostic value.

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