International Core Journal of Engineering Volume 7 Issue 6, 2021
ISSN: 2414-1895 DOI: 10.6919/ICJE.202106_7(6).0051

The Potentials of Arc on Delivering Gene Therapies

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Abstract

The special virus-like behavior of Arc (Activity-regulated cytoskeleton-associated protein) has provoked a wide range of discussion for scientists about its possible application in gene therapy delivery. Research reveals that Arc messenger RNA might be one of the first examples that is able to carry the instructions of making protein across synapses. Thus, we suggest the capsid that Arc produce could be a very promising transmitter for medical drugs if it's carefully planned. This paper mainly explores arc's potentials on delivering gene therapies by conducting several hypothetical experiments around this main point. Different results will lead to different conclusions in the end. With possible future expansion of experiments on Arc, more surprising potentials of arc can be discovered and hence unlock a brand new world to help with the drug delivery.

Keywords

Arc; Retrovirus; GFP; Capsid; Genetic Engineering; Intercellular RNA Transfer.

1. Introduction

The neuronal gene Arc (Activity-regulated cytoskeleton-associated protein) [1] is playing a quite essential role in our long-lasting information storage in brain [2]. Arc mRNA could be transported to dendrites and enriched at sites of local synaptic activity, where Arc mRNA is locally translated into protein [3]. Thus, it is a significant regulator of synaptic plasticity in mammals; activities like long-term potentiation (LTP) and depression (LTD) all require Arc's involvement [4]. Indeed, mice that lack Arc tend to exhibit great deficits in the process of memory consolidation, despite short-term memory and learning acquisition remain intact [5]. Though much work and researches had been conducted to understand the role of Arc in synaptic plasticity, the underlying molecular mechanisms of Arc's biochemical function still stay obscure.

Back to the mid-90's when Arc was first discovered by two independent research groups, one of which that led by Paul Worley and Dietmar Kuhl was looking for genes that could be switched on by learning; meanwhile the other team led by Oswald Steward were investigating whether protein synthesis could occur at the synapse, instead of the cell body like in most cells. According to the result, on the one hand Arc was uniquely switched on by synaptic activity, and on the other hand, Arc messenger RNA which carries the instructions to make protein becomes one of the first examples of an RNA molecule that gets transported across synapses.

As shown in figure 1, this finding of Arc had triggered a wide range of discussion on, for example, how it specifically works or is translated from RNA into protein at the synapses. The finding had suggested one quite significant and stunning feature of Arc that opened up a completely new world for Arc applications.

Arc, unlike other normal human protein, acts more like a virus and is able to form protein shells that package RNA together and are transported across synapses. More recently, arc has motivated researchers to suggest that it can actually be a highly beneficial tool in human's medical therapies for a number of brain disorders. Dr. Shepherd (2018) had pointed out that it is probably possible for Arc-like capsids to be harnessed to conduct medical cure for humans like removing toxic proteins without

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causing an immune response. The goal in this paper is try to take a primal step towards the question that whether Arc can be harnessed to do drug delivery job so that it can carry out more gene therapies for humans.

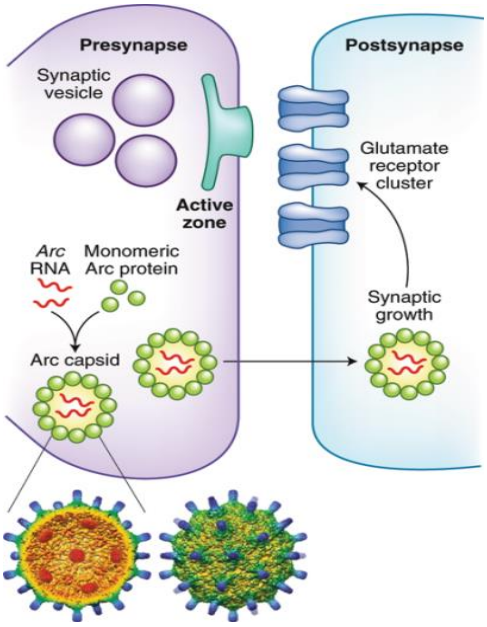

Figure 1. A cell that behaves like a virus and transport through synapses. [6]

2. Experiment

To verify whether Arc can be harnessed to deliver gene therapies, some brief experiments are designed as follow. We will be mainly using GFP (Green Fluorescent Protein) RNA, Arc RNA and two groups of experimental rats.

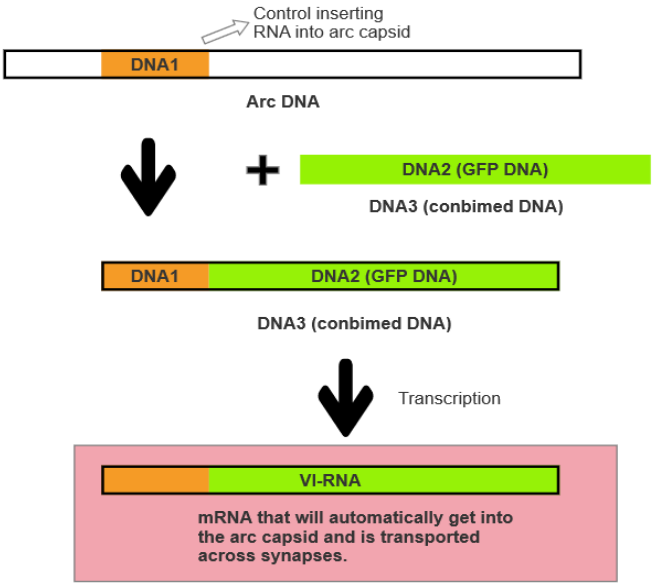

Figure 2. How to Get One Processed RNA (VI-RNA) Processes are elaborated in the following text.

2.1 Get the specific segment of Arc DNA labeled as DNA1 that control inserting RNA into the capsid.

At the very beginning of the experiment, extract some Arc RNA first from the rat and do the sequencing.

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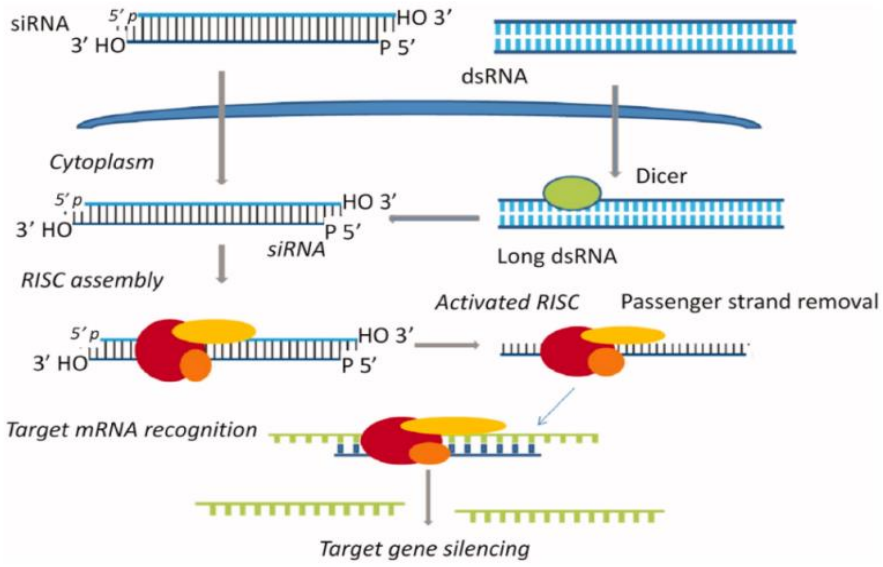

Figure 3. RNA silencing model

Then apply RNA silencing technology to find the specific RNA segment that controls inserting RNA into the Arc protein shell (label it as segment A). Shown as figure 3 above which indicates the normal process of RNA silencing. After long dsRNA is introduced into the cytoplasm, it is cleaved into siRNA by Dicer. The siRNA incorporated into RISC assembly, and the sense (passenger) strand is degraded by the protein Argo-2 in the RISC. The activated RISC–siRNA complex then binds to and degrades the target mRNA, which leads to the target gene silencing [7]. Along the process, to determine whether the Arc RNA has gotten into the capsid, use isotope labeling [8] to check for the staining in the capsid, which if can be achieved then the RNA had successfully gotten into the capsid, as well as the knock-out fragment in that case can be leaved out since it has no control on the inserting of RNA into the capsid. Repeating this process until we find the correct segment of Arc RNA that we need (label it as VI-segment, very important segment). After that, find out the sequence of correspondent DNA expression of the VI-segment based on its sequence we get from the step above. Label the obtained DNA as DNA1.

2.2 Get GFP DNA and combine the two.

According to the gene bank, find out the sequence of GFP DNA (labeled as DNA2), which produce bases for PCR production. After getting DNA2, combine DNA1 and DNA2 together through a gene engineering measure that can help to get one single DNA named DNA3. Then get our final processed RNA labeled as VI-RNA through the transcription of DNA3, which plays a vital role throughout the experiment later on.

2.3 Insert into the environment and test.

2.3.1 Experiment first between cells

First, for the experimental group, extract one neuron A out in a petri dish and add it with VI-RNA, where it will then assemble with Arc protein inside the neuron and finally get into the capsid. Then insert another neuron named B. Wait for some hours and then check for green light spots in neuron B. If we did detect GFP in neuron B, which means VI-RNA had been successfully transported through neurons in this case. Take down the picture at a rate of every 1 hour. Then experiment in a larger scale. But if it fails and remain the same after trying several times, it may either disprove the hypothesis or just calling for more improvements in the experiment methods.

2.3.2 Experiment in a larger scale to further ascertain the possibility.

Insert the VI-RNA into the neurons in the hippocampus of a mice. Wait for some hours, and using Western blot - which is an important laboratory technique that allows for specific identification and

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characterization of proteins [9] -- to observe the final distribution of VI-RNA across neurons. Take down pictures at a rate of 1 per hour. If we can find green light spot also outside the neuron which is packaging VI-RNA, it proves that Arc do possess certain available potentials to help with gene therapy delivery for human. If it fails and remain the same after trying more several times, it may either disprove my hypothesis or just calling for more improvements in the experiment methods.

As for the control group, use Western blot with red fluorescent dye to locate the Arc protein. Take down the picture at a rate of 1 every 1 hour. Then analyze the normal activity of Arc protein across the neurons and synapses and compare it with the experimental group to make sure Arc is working normally as usual.

3. Conclusion

Using RNA delivery to conduct medical therapies has actually been put into applications in several previous pharmaceutical factories. For instance, pharmaceutical factories like Alnylam and Moderna are making coronavirus vaccines in terms of RNA delivering as therapy [10]. In this case, if any more studies are needed, we can consider applying that magical piece of Arc DNA and attach it with any other medical DNA we may want to experiment with. To see whether they make a difference, use the graphs from Western Blot to locate the timely situation in vivo. If we could luckily figure the secret out, this additional feasible treatment could be provided to help patients with better efficiency, availability and flexibility. Personally speaking, while it is possible for ways like conditioning converted viruses to face recombination problem in which viruses pick up their expression in our cells again, or some side effects take place as our immune system is being triggered, I think Arc can somehow reduce those risks as it is part of our human cell after all. Via conducting more scientific research on it as well as further improving Arc gene usage, it would no longer be a fantasy for humans to dig out the infinite possibilities behind this tricky human-owned retrovirus.

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