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Effect of Arc gene on intergenerational memory

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Abstract. Recent studies have revealed that parental exposure to environmental stimuli could induce epigenetic modifications that affect gametes, pass on to offspring, and affect the phenotype of several later generations persistently. This is a phenomenon known as trans-generational memory. The VLPs encoded by Cer1 retrotransposon in C. elegans has been shown to be crucial for trans-generational transmission of learned avoidance behaviour memory, which is propagated and functions via germlines. Arc is a neuronal gene that similar to Cer1, encoding a protein involved in consolidation of animal memory. Here, we hypothesized that Arc and Cer1 shared a similar mechanisms and RNA in Arc VLPs can be spread by germline. GAG is a retroviral protein also encoded by Arc gene, mediating RNA transfer between nerve cells. Therefore, four experiments on mice are designed in this paper in order to confirm the hypothesis above and investigate the functions of GAG protein in trans-generational memory, namely (1) dependency of Arc in intergenerational memory, (2) if there is Arc expressed in sperm, (3) the specific RNA sequences that involved in the process, and (4) the role of the GAG protein.

Keywords: trans-generational inheritance, intergenerational memory, environmental stress.

1. Introduction

It has long been thought that genes are the main templates of hereditary information, and that epigenetic modifications are, to a large extent, reprogrammed between the generations [1]. In recent years, however, it has been discovered that trans-generational inheritance plays an important role in the process, that is, the environmental experiences and physiological stresses of parents can lead to epigenetic changes that result in the persistent traits and adaptability of offspring, which is a phenomenon that has been found in various model organisms [2]. In fact, it has been proved that both chromosomal modifications and non-coding RNAs are involved in this inheritance [1].

Trans-generational memory refers to the transfer of memory from one generation to the next epigenetically and it is a largely unexplored field. For C. elegans, the virus-like particles (VLPs) that encoded by Cerberus 1 (Cer1) retrotransposon has been shown to be necessary for the trans-generational transmission of learned avoidance behaviour memory, which are mainly expressed in germlines and act from germlines to neurons within an individual [3]. Similar to Cer1, the Activity-Regulated Cytoskeletal (Arc) is an activity-dependent immediate early gene that is also known to be involved in animal memory, regulating the strength of synapsis, mediating formation of memory and being associated with a variety of neurological disorders [4]. A protein that encoded by the Arc gene, called Activity Regulated Cytoskeleton Associated Protein, is critical for memory consolidation [5]. Therefore, we can make a hypothesis that Arc have a similar mechanism to Cer1 and RNA in Arc VLPs can be transmitted by

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sperm. Once the hypothesis is confirmed, there is a chance to figure out the actual RNA sequence that involved in the transfer of memory.

Arc gene also encodes a retrotransposon that can mediate RNA transfer between nerve cells called Group-specific antigen (GAG) polyprotein [6]. The GAG protein can forms capsid structures to bind and encapsidate viral RNA during the retroviral replication [7]. However, the functions of GAG protein in trans-generational memory remains unclear.

Here, in this research proposal, the Arc gene in intergenerational memory is the point. A total of four experiments are designed to study: (1) dependency of Arc in intergenerational memory, (2) if there is Arc expressed in sperm, (3) the specific RNA sequences that involved in the process, and (4) the role of the GAG protein. Mice are chosen as the experimental subjects because of their similarity to humans as mammals.

2. Experimental approach

2.1. The dependency of Arc in intergenerational memory

To verify (1) dependency of Arc in intergenerational memory, olfactory fear conditioning will be carried out on mice as such response is more pronounced for olfactory stimulus and olfaction is a dominant sense in rodents [8]. Olfactory molecular have specificity, the trans-generational effects on behaviour and neural structure in progenies are inherited via olfactory experienced parental germline [9]. Olfactory sensory neurons (OSNs) expressing a particular gene are interspersed in a gene-specific epithelial region [10]. M71 is an odor receptor encoded by Olfr151 gene, which is activated by acetophenone and expressed by OSNs [9].

Before the start of the experiment, three groups of male mice (P0) of the same age (2-month-old) and number (15 per group) need to be prepared: trained group, trained + Arc (-) group, naive group (control). It is important to ensure that they are fed with the identical food and live in the same environment. For the training, the two experiment groups of mice will be exposed to acetophenone with mild foot shocks. The mice will be trained 10 minutes per day for three consecutive days so that they will associate acetophenone with the shock. Once the training is done, CRISPR/Cas9 genome editing technology will be used to knock out the Arc gene of the Arc (-) group mice. After that, three groups of mice will mate with naive females to produce three groups of progeny (F1). The avoidance behaviour of the progenies (F1) will be tested with acetophenone, and the density of M71 neurons in olfactory bulbs will be measured by X-gal-label and pictures of dorsal and medial under microscope-mounted digital camera to the progenies (F1).

As for the expected experimental results, the avoidance behaviour should only occurs in the trained group but not in the other two groups, where the M71 neurons in trained group and trained + Arc (-) group will be denser than in the naive group. If the results meet our expectation, it can be proven that intergenerational memory in mice is related to the formation of Arc.

2.2. Verification of Arc expression in sperm

If the hypothesis of involvement of Arc in intergenerational in memory holds, further verification is needed to determine whether it is transmitted through sperm. Firstly, the sperm from trained group mice (P0) is required in order to detect (2) the presence of Arc in sperm. Therefore, the mice (P0) will be sacrificed and the sperm will be collected manually. A centrifugal machine will be used to purify the sperm. Pellet at the bottom of the tube will be taken out, which may contain the Arc VLPs. The Arc VLPs will be injected into eggs that fertilised by sperm from naive male mice, and the embryos will be implanted into the uterus of naive female mice later on. The newborn mice (F1) will be produced subsequently.

For the mature mice (F1) that have never been exposed to acetophenone before, acetophenone will be employed to test avoidance behaviour. The density of M71 neurons in olfactory bulbs will be measured by X-gal-label and pictures of dorsal and medial under microscope-mounted digital camera.

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The results here with those of the naive mice (F1) from experiment (1) will be compared, since whether it have Arc VLPs from trained mice is the only variable. The experimental expectation is that avoidance behaviour will occurs in the mice with Arc VLPs, and M71 neurons will be denser than naive mice. If the results meet our expectation, it can be confirmed that the Arc exists in sperm and can be transmitted to offspring through it.

2.3. The specific sequence of RNA concerned

Once the expression of Arc is verified, the actual RNA sequences that involved in the process need to be determined. For preparation, two groups of male mice (F0) of the same age (2-months-old) and number (15 per group) will be set up with the same food and surroundings. Olfactory fear conditioning will be implemented again as described in experiment (1), but one group with acetophenone and one with another odor (e.g. propanol) this time. Due to the specificity of olfactory molecular, the M71 receptors in mice will be activated in the acetophenone group, but not in the group with the other odor. Therefore, the RNA in Arc VLPs will be the only difference between the two groups of mice. The sperm of each group will be collected and the VLPs will be isolated by centrifuge as described in experiment (2). Phenol will be used to denatured the protein shells of the VLPs, so that the RNAs can be obtained from the buffer. Primers and reverse transcriptase will be added to each group of RNA. After that, a reverse transcription polymerase chain reaction (RT-PCR) will be conducted respectively in order to transcribe the single-stranded RNA into DNA. Then the fragmented DNA will be analysed by High Throughput Sequencing (HTS) and the whole genome of mouse will be obtain. The different parts of the DNA sequence between two group is the objective of this work.

2.4. The functions of the GAG protein

The role of GAG protein can be investigated by replacing the gene with another gene that encoding a different protein. Such pre-determinedly changes of gene can be achieved by homologous recombination and gene targeting [11]. The Embryonic Stem (ES) Cells of mouse are undifferentiated cells in suitable surroundings, which can be derived from the inner cell mass of blastocyst in the mouse. In this experiment, two groups of ES cells will be set up. For the homologous recombination group (experiment group), GAG will be the target gene that need to be altered. The replacement gene-targeting vector use the rest of the chromosome as regions of homology. Antibiotic selectable markers will be demanded as well. After the transfer of vectors to ES cells in the culture dish, the cells containing the targeted construct will be selected by adding drugs. The gene targeted ES cells in vitro will be implanted into the uterus of live female mice. As for the control group, ES cells will be cultured under the same conditions and length of time but without homologous recombination. The cells will be implanted into the uterus of another female mice. Give birth to both groups of mice. Same as described in experiment (1), olfactory fear conditioning test will be carried out and acetophenone will be used to test the avoidance behaviour again after maturation of the mice. After that, the density of M71 neurons in olfactory bulbs will be measured by X-gal-label and pictures of dorsal and medial under microscope-mounted digital camera.

As for expected results, if both the mice of recombination group and control group exhibit avoidance behaviour and have the same density of M71 neurons, it can be inferred that GAG protein may only be involved in transfer of RNA.

On contrary, if the results turn out that the mice of recombination group do not show avoidance behaviour and have thin M71 neurons in olfactory bulbs, it can be suspected that the GAG protein plays a more important role, such as catalyzing the formation of RNA in Arc VLPs.

3. Conclusion

As described above, the four designed experiments regards to (1) the dependency of Arc in intergenerational memory, (2) verification of Arc expression in sperm, (3) the specific sequence of RNA concerned, and (4) the functions of the GAG protein, separately. It is worth noting that the process of inquiry will be progressive and interlocked, since each experiment greatly influences the next. Also, as

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the intergeneration memory is a relatively new area of research, especially on mammals, there may be shortcomings in this study. For instance, the existence of Arc in the sperm is the only concern in experiment (2). However, whether the egg carries the gene is unknown. Besides, this experiment can only be conducted to determine whether sperm contains Arc, but the exact location of its expression originally is still not knowing. In addition, since this study focused more on the relationship between Arc gene and intergenerational memory inheritance, only two generations of mice were included in the experiment, which means trans-generational memory are less involved. Thus the next step may be to conduct experiments on more generations of mice. In a word, more experiments are necessary to verify these aspects above, which could also lead to future research directions as a result.

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