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Molecular-Genetic Analysis in an Animal Model, Genetic Engineering

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ABSTRACT

This article describes the influence of many genetic and environmental factors on the development of alcoholism, the assessment of the contribution of individual genes to the development of alcoholism in living organisms through an animal model.

The use of animal models to elucidate the causes and mechanisms of human diseases and to develop new treatment approaches is a major focus of modern biological research, including alcohol research. Laboratory animals, such as rats and monkeys, are used to model alcohol drinking behavior, study alcohol-related damage to various organs, and analyze the brain chemistry that mediates alcohol's effects.

An important focus of current alcohol research is the identification of genes that contribute to alcohol consumption behavior and its consequences. It is difficult to study the functions of these genes, that is, the mechanisms by which genes exert their influence. Genetic studies with isolated cells and tissues, which have been successful in analyzing the causes and consequences of other diseases, cannot capture the behavioral responses that occur in, for example, alcoholism. Conversely, genetic studies in whole organisms, whether in laboratory animals or humans, are difficult to conduct and interpret because scientists and researchers believe that both genetic and environmental factors influence alcoholism. In addition, alcoholism is a polygenic disease (that is, many genes play a role in its development), which makes it difficult to determine the contribution of each individual gene.

Recent advances in the use of genetic engineering technologies to develop new animal models allow researchers to overcome some of these challenges and study the role of individual genes and their products in the development of alcoholism. These technologies allow the introduction of foreign genes, the permanent inactivation of certain genes, and the temporary destruction of individual gene products in a living organism. Using these approaches, researchers can assess the

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influence of individual genes on the development of a disease such as alcoholism.[1]

This article describes three new technologies: transgenic mice, knockout mice, and antisense ribonucleic acid (RNA) treatment. Although these approaches have not been widely used in alcohol research, their use in other research areas illustrates their potential applications and limitations in the alcohol field.

In transgenic animals, a foreign gene is permanently integrated into the animal's genetic material, i.e., DNA, in reproductive (i.e., sex) cells and non-reproductive (i.e., somatic) cells, resulting in gene expression and propagation. This method is primarily used to evaluate the role of specific genes in fetal development or to apply and study human diseases in animals. In the second case, scientists insert a human gene known to cause a disease into an animal and then study how the disease develops in the animal.[2]

Similarly, genes known or suspected to contribute to alcoholism can be introduced into transgenic animals. However, measuring the effect of a gene on behavior, such as alcohol consumption, can be more difficult than on a specific bodily function or biochemical process. Thus, alcohol researchers are still evaluating the potential of transgenic animals for their research.

Although researchers can use several mammalian species to create transgenic animals, they primarily use mice. Mice are easy to breed, have a short generation period, their embryos can be easily manipulated during experiments, and their genes have been extensively studied.

Before it can be introduced into a mouse or other animal, the foreign gene must first be identified and isolated from its host organism (for example, from the DNA of human cells). Next, many identical copies of the gene are chemically synthesized and then injected into mouse embryos.

To create mouse embryos, laboratory mouse eggs are fertilized in a test tube with mouse sperm. A fertilized egg or embryo contains two sets of DNA, each located in a separate structure called a pronucleus. One set of DNA comes from the mother (ie, the female pronucleus); the other set comes from the father (ie, the male pronucleus). In this step, a foreign gene is inserted by injecting the DNA directly into the male pronucleus with a very fine glass needle (Figure 1). Although this procedure seems simple, its drawbacks become apparent when considering that the entire embryo is only 0.1 mm in diameter.

In 50-90% of injected embryos that survive this procedure (the success rate depends on the skill of the scientist), the foreign gene is integrated into the embryo's DNA, and the embryo continues to develop normally.[3]

Nucleotides, the building blocks of DNA, are sugar molecules linked to organic bases. DNA contains four different organic bases: adenine (represented by the letter A), cytosine (represented by the letter C), guanine (represented by the letter G), and thymine (represented by the letter T). Their arrangement determines which amino acids are linked to form a protein. Because there are more than four amino acids and they are needed to make a protein, a triplet of three nucleotides represents (i.e. codes for) one specific amino acid in the final protein. For example, the nucleotide triplet ATG encodes the amino acid methionine, and the triplet TGG encodes the amino acid tryptophan. A section of a DNA molecule that contains the information needed to make a specific protein is called a gene.

DNA is a double-stranded molecule: two strands of nucleotides are opposite each other and connected by specific bonds (see Figure 3 of the main article). Because of the nature of these bonds, each nucleotide can only bond with one other nucleotide. For example, a nucleotide containing A always pairs with a nucleotide containing T, and a nucleotide containing C always pairs with a nucleotide containing G. Therefore, the composition of the second chain depends on the composition of the first chain. Accordingly, threads are called fillers. This means that if one

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knows the nucleotide sequence of one strand, the sequence of the second strand can be automatically deduced.[4]

Transcription. The first step in converting the information encoded in a gene's DNA into a protein is to copy or transcribe one of the DNA strands into another nucleic acid molecule called messenger ribonucleic acid (mRNA). This process is carried out by special enzymes in the cell nucleus.

A cell has different types of RNA with different functions but different chemical structures. RNA molecules are chemically similar to DNA molecules. The main differences are that the sugar component is different between DNA and RNA, and the organic base thymine in DNA is replaced by the base uracil (represented by the letter U) in RNA. Also, RNA molecules are single-stranded; Unlike DNA, they do not have a complementary strand.

During transcription, the DNA sequence that represents a gene is translated into mRNA. Only one strand of a double-stranded DNA molecule serves as a template for mRNA synthesis. RNA nucleotides are directed to and transiently bind to the DNA sequence being transcribed. However, only one specific RNA nucleotide can bind to each DNA nucleotide (for example, an RNA nucleotide containing A binds to a DNA nucleotide containing T and an RNA nucleotide containing C A DNA containing G pairs with a nucleotide). This specificity ensures that genetic information contained in DNA is accurately translated into mRNA. As with a DNA template, a triplet sequence of nucleotides in RNA codes for a single amino acid in the final protein.

After all the information for one gene is transferred to the mRNA molecule, the DNA and mRNA molecules separate. The mRNA then undergoes some additional changes in the nucleus of the cell before being transported to the cytoplasm for the next step, the protein product.

Broadcasting. In the cytoplasm of the cell, macromolecules called ribosomes attach to the mRNA and move it. In this way, ribosomes "read" the sequence of nucleotide triplets of mRNA. According to this sequence, ribosomes produce a second type of RNA, called transfer RNA (tRNA) molecules, which direct the amino acids needed for protein synthesis to the mRNA-ribosome complex. At one end of each tRNA molecule there is a region that recognizes one specific nucleotide triplet in mRNA. A different region of each tRNA molecule is attached to a specific amino acid. Thus, by recruiting tRNA molecules that recognize the nucleotide sequence of the mRNA, ribosomes also store the correct amino acids in the correct order to make the protein encoded by the gene expressed in the mRNA. Then specific enzymes connect amino acids until the complete protein is synthesized. Because each mRNA molecule can be read sequentially by multiple ribosomes, many protein molecules can be produced from just one mRNA template.[5]

A foreign gene product can usually be detected in 10-35 percent of puppies. These numbers suggest that creating transgenic mice is a somewhat inefficient process. Out of 100 embryos injected with a foreign gene, only a few will develop into viable pups that can become adult transgenic animals.

Although biochemical tests can determine whether mice developing from injected embryos (known as first-generation, or F1, animals) express the foreign gene, the tests cannot determine whether all of the animal's cells, particularly germ cells, have incorporated the foreign gene. . F1 animals are mated to each other to ensure the presence of the gene in the germ cells. Only F1 animals with the foreign gene in their gametes can pass it on to their offspring (ie, second generation, or F2, animals). F2 animals with a foreign gene carry it in all their cells. These animals are used to study gene function.[6]

As mentioned above, transgenic animals have not yet been used specifically for alcohol research. A study of transgenic mice created for unrelated research projects, however, yielded unexpected results relevant to alcohol research that show the technology's potential.

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Another available transgenic mouse line that can be used in alcohol research contains the rat gene for corticotropin-releasing factor (CRF). CRF is a hormone involved in the body's response to stress. One of the body's physiological responses to stress is the synthesis of hormones called glucocorticoids. CRF synthesis is the first step in the chain of events leading to glucocorticoid production. Thus, CRF is studied in many animal experiments as a physiological measure of the behavioral or emotional state of stress.

Alcohol changes the body's response to stress. For example, alcohol has been shown to increase glucocorticoid levels, which are released in response to stress. Therefore, CRF mice may provide insight into the mechanisms by which alcohol affects the level of glucocorticoid production under stressful conditions.

Both examples illustrate how transgenic animals created for research unrelated to alcohol research can be used to study phenomena such as physiological sensitivity to alcohol or the effects of alcohol on stress. Other available transgenic mouse strains may be equally valuable tools for studying the effects and mechanisms of action of alcohol on various organs.[7]

While transgenic mice carry an extra foreign gene in their DNA, knockout mice are characterized by the targeted deletion of one of their own genes, resulting in the destruction of the gene products. This approach makes it possible to infer the function of the deleted gene by comparing the phenotype (ie, appearance or behavior) of knockout mice to that of normal mice. In extreme cases, when the deleted gene has an important function in embryonic development, knockout mice that lack the gene do not develop beyond a certain embryonic stage. In more desirable scenarios, only one specific aspect of metabolism or behavior is eliminated or altered.

The creation of transgenic and gene-knockout mice is a powerful tool that will ultimately help scientists better understand human drinking behavior and the effects of alcohol on the brain and other organs, but there are some limitations to the usefulness and validity of such experiments. These limitations are not unique to alcohol research, but apply to all areas of research that analyze bodily functions and disorders that depend on the cooperation of multiple genes or that manifest through behavior rather than biochemical reactions.

- First, the individual gene being studied must have a sufficiently large effect on the development of the disease (e.g., alcoholism) so that the effect of overexpression or deletion of the gene is reliably outweighed by all other factors contributing to the disorder. can be determined. In addition, other genes sometimes naturally compensate for the function of the gene that is deleted in knockout mice, thereby masking the effects of the deletion. Accordingly, not all genes and their products can be analyzed using these technologies; the functions of some genes can be more easily determined than the functions of others.
- Secondly, the studied gene product should not be important for embryo development; otherwise, too much or too little of it will prevent normal development. In this case, transgenic or knockout mouse embryos do not develop to term and the desired effects (such as those caused by alcohol) cannot be studied.
- Thirdly, integration of foreign DNA into mouse DNA in transgenic mice cannot be directed to a specific region of mouse DNA so far. As a result, foreign DNA can integrate into the middle of another gene, disrupting the function of that gene. Researchers should be aware of this possibility when interpreting their findings.
- Fourthly, similar to the site of foreign DNA integration, the amount of foreign gene and its product in each cell cannot be predicted. The effect of a foreign gene on a transgenic animal can vary, depending on how much of the gene product is produced. Similarly, in many cases, a foreign gene is expressed in all tissues, in contrast to the normal physiological condition where the gene may be expressed only in selected cells. To circumvent this problem, scientists are now developing ways to express foreign genes in cells where they are normally

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active. This process uses special regulators that allow genes to be expressed only in certain cells or tissues.

- Fifth, the choice of mouse lines used to create transgenic or knockout mice can affect the results of the experiments. Just as two people may have the same genes that mediate the effects of alcohol, but react differently to alcohol, two lines of mice may react differently to the addition or deletion of a gene in their genetic material.

Nevertheless, these caveats do not diminish the potential of transgenic and knockout mice for alcohol research; however, they suggest that results obtained with genetically engineered animals should be interpreted with caution, as they may not be as straightforward as they first appear.[8]

Antisense RNA strategies. Antisense RNA technology is another way to study the role of specific genes in mediating the effects of alcohol on living organisms. Similar to the approach of using knockout mice, antisense RNA technology reduces or prevents the expression of a specific gene. Unlike knockout mice, this modification is usually not complete or permanent, as antisense RNA can be transiently administered to the animals.

Antisense RNA technology has several potential advantages over transgenic and knockout mice. For example, antisense RNA therapy is faster and cheaper than creating and breeding genetically modified mice. Because this is not a permanent modification, antisense RNA treatment also avoids some of the limitations of knockout and transgenic mice, such as the difficulty of modifying genes that are important during development. With antisense RNA technology, animals can develop normally before gene expression is manipulated. Finally, antisense RNA may have some therapeutic potential in humans, for example by targeting receptors for certain neurotransmitters in the brain, provided sufficient RNA is delivered to brain cells.[9]

What is antisense RNA therapy? Converting the genetic information encoded in DNA into a protein product is a complex process. Briefly, through a process called transcription, DNA information in the cell nucleus is transferred to an intermediary molecule, messenger RNA (mRNA). The mRNA is transferred to the cytoplasm of the cell, where it serves as a template for protein synthesis in a process called transcription.

Antisense RNA technology aims to inhibit the translation of mRNA into the corresponding protein. As with transgenic and knockout mice, the technique requires that the gene under study be isolated and copied, and that its exact DNA sequence be known. During antisense RNA therapy, the gene itself is not the target of the procedure. Instead, the target is the mRNA transcribed from the gene.

To block the translation of an mRNA, scientists create a short synthetic DNA molecule called an oligonucleotide, which can bind to the end of the mRNA molecule where translation begins. This oligonucleotide is called an "antisense" molecule because it complements (ie, as an inverted image) part of the "sense" information encoded in the mRNA. When a DNA oligonucleotide is introduced into a cell, it can bind to the complementary region of mRNA, forming a DNA-RNA hybrid region. This DNA-RNA hybrid region interferes with the translation of mRNA, because the proteins necessary for this process bind only to the free RNA molecule, not to the DNA-RNA hybrid. In addition, DNA-RNA molecules are rapidly degraded in the cell. Consequently, protein cannot be synthesized from mRNA.

This mechanism works in isolated cells, tissues, and whole animals. Oligonucleotide, which is easily absorbed by individual cells, can be delivered to cells and tissues in various ways. It can be given to animals by a single injection or by chronic blood transfusion over a longer period of time. Cells in specific target organs or regions of the body contain the oligonucleotide. By changing the length or frequency of administration of the oligonucleotides, researchers can alter the length of time that the target protein is not synthesized. They can also examine the effects of this manipulation at different developmental stages and under different experimental conditions

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(eg, in the presence or absence of alcohol). Thus, antisense RNA technology provides flexibility in studying the effects of individual genes that cannot be achieved with transgenic or knockout mice in which the genetic material is permanently altered.[10]

Application of antisense RNA technology. Antisense RNA strategies have not yet been specifically applied to alcohol research. However, the functions of several neurotransmitters and their receptors that may contribute to alcohol's effects on the brain have been studied under experimental conditions unrelated to alcohol.

N-methyl-D-aspartate receptors One important neurotransmitter in the brain is the amino acid glutamate, which binds to a receptor on the surface of nerve cells called the N-methyl-D-aspartate (NMDA) receptor. Alcohol researchers study this receptor because its response to glutamate binding is thought to contribute to alcohol withdrawal seizures.

NMDA receptors have other effects on the brain that are not related to alcohol. For example, its response to binding to glutamate can lead to cell death after an ischemic stroke in the brain. Ischemic stroke occurs when obstruction of blood vessels disrupts blood flow to the brain. Such a stroke can lead to the death of brain cells in that area.

When isolated brain cells are treated with antisense oligonucleotides that can bind to NMDA receptor mRNA, the number of NMDA receptors in the cells is reduced and cell death can occur to a lesser extent than in untreated cells. Similarly, in animals treated with antisense oligonucleotides, the number of dead cells after cerebral artery obstruction was significantly reduced. These findings suggest that NMDA receptors contribute to cell death after ischemic stroke. The example also demonstrates that antisense RNA technology can affect gene expression in both isolated cells and whole animals, allowing researchers to study the function of these genes in both experimental systems.

Limitations of antisense RNA technology. One of the major advantages of antisense RNA technology—a transient, easy-to-manipulate pharmacological approach—also contributes to the technology's limitations. Some of these restrictions are:

Delivering adequate amounts of antisense oligonucleotides to living animal tissues, especially the brain, can be difficult. As a result, inhibition of gene function is rarely complete in an animal or even in a specific tissue.

Antisense oligonucleotides have a limited lifetime in the body before being degraded. Researchers are trying to modify the chemical structure of oligonucleotides to extend this lifespan, but a specific treatment protocol for each gene or gene product to be studied must be determined empirically.[11]

The lifetime of the mRNA and protein under study should be determined for each experiment, as they determine the duration of antisense treatment required to obtain meaningful results.

Researchers must ensure that oligonucleotides bind only to the desired mRNA and do not interfere with the translation of other mRNAs in the cell. Such unwanted interaction can distort results and lead to misinterpretation of gene function.

In some cases, oligonucleotide treatment can be toxic to cells or living animals.[12]

Summary. This paper introduces three exciting new technologies that allow scientists to study the function of single genes in the context of a living organism. Because these methods study the functions of individual genes, they are well suited for analyzing genes involved in the development of polygenic diseases such as alcoholism.

Until now, these technologies have not been systematically applied to study the causes and consequences of alcoholism. This is partly because researchers have identified several candidate genes that may mediate the development of alcoholism or the effects of alcohol. However, some

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of the examples in this article show that even unrelated experiments can provide relevant results for alcohol research. At the very least, the examples show the potential of new genetic engineering technologies in animal models to help scientists answer pressing questions in many areas of research.

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