APPLYING MOLECULAR BIOLOGY TECHNIQUES TO
OPHTHALMOLOGIC RESEARCH

APLICACIÓN DE TÉCNICAS DE BIOLOGÍA MOLECULAR A LA
INVESTIGACIÓN OFTALMOLÓGICA

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At present there are over 990 registered hereditary diseases involving only the eye or in the context of a disease involving other organs (1). Aniridia, primary congenital glaucoma, retinoblastoma, Leber’s neuropathy or uveal melanoma are but a few examples. In some cases, the involvement is attributed to a mutation in a specific gene; in others, it is related to changes in different DNA sequences which increase vulnerability against the disease. Sometimes, the hereditary disease is in fact a group of diseases transmitted with different hereditary patterns which involve dozens of loci (for instance, pigmentous retinitis).

Molecular biology offers a high number of applications for medical research and particularly for basic, clinical and applied ophthalmological research. It provides the possibility of studying diseases of genetic origin in order to find genetic variability and proteic expression patterns allowing for a greater knowledge about the disease and to assist a more precise diagnosis, prognosis or enhanced therapeutic approaches. For instance, the DNA sequence analysis in specific regions of the genome and the determination of variants in the arrangement of one or more nucleotides (polymorphisms) constitutes a very useful tool for diagnosing ocular diseases in populations or family groups. By selecting organisms undergoing a spontaneous mutation, or after the external manipulation of DNA (as explained below), animal models have been obtained for analyzing the molecular mechanisms which underlie a nosological entity (2). In addition, this tactic opens the doors for somatic gene therapy which aimed at restoring functions which are absent, weakened or altered in a gene or group of genes.

It is clear that, throughout its development, molecular biology has provided very useful methodological tools for ophthalmologists.

DNA, the molecule in charge of transmitting genetic information, carries out a basically passive role. The proteins derived from its expression are in charge of carrying out the myriad of life-sustaining reactions. Precisely for this reason it is essential to advance the knowledge of proteic functions.

Mutant organisms have provided some keys to unravel the function of proteins. Traditionally, mutations were obtained by treating with mutagenic agents such as specific chemical substances or radiations. In addition, it is possible to produce specific mutants by inserting in a living organism copies of altered or nonfunctional genes to analyze the changes in the behavior or development of said organism. These experiences have been carried out with a variety of organisms. However, taking into account that the mouse shares about 99% of its genes with humans and that it reproduces very quickly, it is a very adequate model for carrying out functional studies. The publication in 1982 of a photograph on the cover of Nature (3) showing a giant transgenic mouse was a landmark for molecular biology.

The transgenic mouse carries in its genome an exogenous gene (transgene), i.e., a gene built in a lab utilizing the recombinant DNA technique and subsequently introduced in the genome of the mouse. Transgenic mice can be obtained by two methods: Either transforming embryo stem cell cul-

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tures with the appropriate DNA (ES cells) or injecting the desired gene in the pro-nucleus of a fertilized mouse egg.

The first of said methods was developed by Mario R. Capecchi, Oliver Smithies and Martin J. Evans (4), who shared the Physiology or Medicine Nobel prize in 2007. The first step is to isolate embryo stem cells starting from early mice embryos. These cells are pluripotent and have the capacity to produce any type of cell of the mature animal including the gametes, and can be cultured in the lab. In this way, it is possible to build the gene of interest to us, joining it to a vector DNA having the purpose of facilitating its insertion in the mouse genome. Sequences are also included to promote and enhance the expression of the gene. When the cultured mouse ES cells get in touch with the construction of exogenous DNA, the latter will become included in its genome. This inclusion can occur randomly, at any place. However, if we include in the vector some sequences of the ends of the gene it is possible to insert it in a directed manner at the place usually occupied by that gene in the chromosome (homologous recombination). The insertion occurs in an essential exon of the endogenous gene, thus causing its deactivation. The ES cells transformed in this manner are reintroduced in blastocyes (through micro-injection or joint culture) to obtain chimera embryos (a part of the cells correspond to the host embryo and another part derived from the transformed ES cells). These embryos are implanted in the uterus of pseudo-pregnant females (i.e., females in which the necessary hormonal changes have been introduced to make their uterus receptive). In some of these females (less than one third) the implant will hold and a mouse will be developed. Then, using a tissue sample, the researchers determine which of the mice (usually under 10%) have the exogenous gene. These mice will be heterozygotic but, by crossing them, homozygotes can be obtained allowing for a full assessment of the transgene effects.

The second methods, demonstrated by several researchers in the early eighties, involves preparing the gene as in the previous method, but the injection is made with a microneedle in the male pronucleus (larger than the female counterpart) of a fertilized egg. Both pronuclei will merge forming a diploid zygote. When the zygote divides originating a 2-cell embryo, it is ready for implanting it in a pseudo-pregnant mouse. The next steps are identical as those of the first method.

When the exogenous gene is a non-functional gene (null allele), both copies of the gene annulled in the line of homozygotic mice. These mice, in which the activity of the gene has been eliminated, are known as knockout mice and their phenotype (appearance, behavior or other observable biochemical characteristics) is of special interest to determine the physiological function of the cancelled gene and, by extrapolation, in humans. The counterpart of the knockout mouse is the knock-in mouse. In the latter, a gene belonging to the mouse is substituted by another one to determine its function. Optionally, specific sequences that block the transcription of a gene are eliminated to obtain its expression in specific tissue or development stages.

The limitations of this technique include for example the fact that some mice do not seem to be affected by the annulment of some of their genes. The mouse genome seems redundant enough to make up for this loss. In addition, the majority of genes are pleiotropic, that is, they cause a number of unrelated phenotypic effects, expressing in different tissues or different stages of development. On the other hand, in 15% of cases it is not possible to obtain an adult individual, which restricts research to the embryo period (or part of it), thus posing an obstacle to the knowledge of the function of a gene in the adult and therefore the extrapolation to humans. Even considering these limitations, the knockout mice are excellent models for research in human diseases related to the loss of a function of the gene. Doubtlessly, all these findings will have an impact on the improvement of strategies for diagnosing, treating and preventing many human diseases.

REFERENCES