Molecular biology in ophthalmology

Biologia molecular em oftalmologia

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The term molecular biology has been used loosely to define certain techniques and an aggrandizing field in biology. Historically, what is now called molecular biology began its development with the classical experiments by Avery, MacLeod and McCarthy demonstrating that DNA carries the genetic information in an organism. The next step in the development of the field was the isolation of the first restriction enzyme by Kornenberg and finally the definition of the DNA structure by Watson and Crick (for a review see [1]). In the early 1970's it became clear to scientists in the field that the techniques available would expand significantly and that their use would have a significant impact in biology in general and in medicine in particular. In 1977 the Asilomar Conference tried to bring together the foremost experts in Biochemistry and Molecular Biology and to achieve consensus towards the ethics of DNA research. We are still today dealing with these issues.

The impact of molecular biology has being felt in all branches of medicine and ophthalmology is no exception. For the purposes of this review we will divide these advances generated by developments in Molecular Biology into three categories: disease biology, diagnosis and therapy.

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DISEASE BIOLOGY

Molecular biology has revolucionized the study of disease biology and opened up new avenues in both basic and applied research in ophthalmology. The ability to locate genes that are fault or missing in inherited diseases as well as the ability to understand gene variants that are altered and contribute to the establishment of acquired disease have allow us a better understanding of the mechanisms behind the development of numerous ocular diseases. Also, the possibility to dissect the functions of a given gene have immensely the understanding of vision physiology and the pathologic conditions that follow when the normal physiological pathways are detoured.

The conservation of vision-related gene sequences among species is remarkable [2, 3] and due to the homology between humans and other species researchers were able to isolate genes of interest starting from genes cloned in lower species. For instance, the human counterparts of genes cloned originally in *Drosophila melanogaster* and mice have been identified as the cause of aniridia (PAX-6) and Retinitis pigmentosa *(Rds)*, respectively [4, 5]. This greatly highlights the importance of animal research in the field of vision and ophthalmology. The value of animal models of human disease has been greatly enhanced by advances in molecular biology. Our ability to knockout genes or insert genes of interest has greatly implemented our ability to investigate diseases of specific interest. Genetically engineered mice by the incorporation of foreign DNA into their genome, enable *in vivo* analysis of the expression of specific genes. Thus, transgenic models of retinitis pigmentosa [6, 7], microophthalmia [8], several eye malformations [9] and sickle cell disease [10] have been generated and contribute immensely to our understanding of the pathologies they represent.

One of the greatest impacts of molecular biology has been in the elucidation of mutations responsible for many forms of hereditary eye disease. Molecular biology techniques have helped mapping diseases to specific chromosomal *loci* and the cloning and isolation of genes involved has allowed the study of specific mutations that contribute for the development of pathology. These include, for instance, more that 70 mutations on the rhodopsin gene (see table 1 for a reference on ocular disorders whose genes have been mapped using molecular biology techniques). Furthermore, the insertion of such genes in mice permits the study of the abnormal functions of mutated genes *in vivo* that will eventually lead to better therapeutic approaches to such diseases. Genetic maps are also important because they allow the localization of a gene and provide insights into its expression that lend the basis for future interventions by gene therapy.

Another area in which molecular biology has aided both in the understanding of disease biology and as a direct consequence also provides a tool for diagnosis is the case of diseases of unknown etiology that may be caused by undetected microorga-

nisms. The availability of the Polymerase Chain Reaction (see description bellow) has allowed for instance Alvarado and colleagues [11] to identify herpes simplex virus DNA sequences from corneal specimens from patients with iridocorneal endothelial syndrome. This result suggests viral participation in the pathogenesis of disease.

DIAGNOSIS

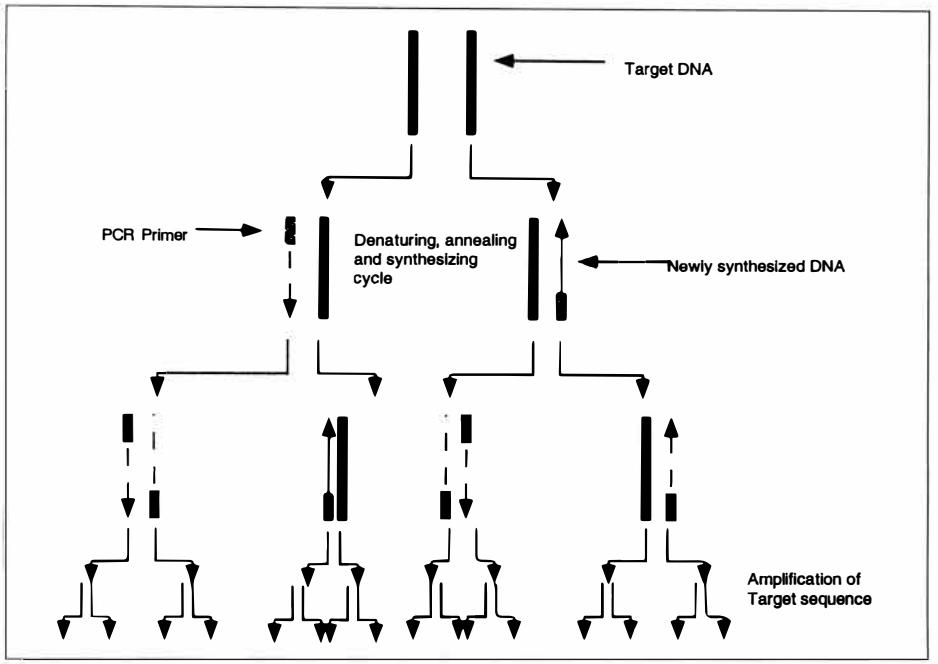
Molecular Biology techniques have already made important contributions to the betterment of disease diagnosis. Among all techniques, none has been more important to laboratory medicine than the Polymerase Chain Reaction (PCR). PCR is a rapid procedure for *in vitro* enzymatic amplification of a specific segment of DNA or cDNA. The power of this technique lies on its ability to amplify a sequence of DNA many times over allowing its detection by Southern Hybridization. For instance, the technique is so powerful that as little as 10 copies of viral DNA (or cDNA reverse transcribed from viral RNA) can be detected. The theoretical basis for PCR is depicted on Figure 1. Briefly, there are three nucleic acid segments: the target double-stranded DNA to be amplified and two single stranded oligonucleotide primers flanking this segment. Primers are added in great excess compared to the target DNA. They hybridize to opposite strands of the DNA be amplified and are oriented with their 3' ends facing each other so that synthesis by the DNA polymerase (which occurs in a 5' to 3' direction) extends across the segment of DNA between then. The first round of synthesis result in new strands of indeterminate length that, like parental strands, can hybridize to each of the primers upon denaturation and annealing. These products accumulate with each subsequent cycle of denaturation, annealing to the primers, and synthesis. However, the following cycle results in two single-stranded products that together compose a discrete double-stranded product which is exactly the length between the primers. Each strand of this discrete product is complimentary to one of the two primers and can therefore participate as a template in subsequent cycles. The amount of this product doubles with every cycle accumulating exponentially so that 30 cycles should theoretically result in a 270 million fold amplification of the target sequence. The number of applications of PCR seems infinite and it is still growing. They include direct cloning from genomic DNA or cDNA, assays for the presence of infectious agents, prenatal diagnosis of genetic diseases, analysis of allelic sequence variations, genomic footprinting and direct nucleotide sequencing. It is note worthy however, that due to the extreme sensitivity of this technique it is prone to false-positive results and data must always be analyzed considering the appropriate negative controls.

Another area of diagnostics in ophthalmology that has been aided immensely by the advances in molecular biology, notably the PCR, has been that of infectious diseases. PCR has been performed on vitreous or aqueous samples to confirm a presumptive diagnosis or to establish a diagnosis in patients with atypical clinical findings cytomegalovirus retinitis [12], ocular tuberculosis [13], acute retinal necrosis due to herpes simplex virus (HSP) type 2 [14] or herpetic disease in general [15] and uveitis due to *Toxoplasma gondii* infection [16]. Recently, the detection of adenoviruses in ocular swabs by PCR has been described [17]. Besides these, commercially available testing by PCR or by dot-blot exists for *Streptococcus pneumonia, Hemophilus influenza, B. burgdorferi, Legionella sp., Chlamydia trachomatis,* cytomegalovirus (CMV), human immunodeficiency virus (HIV -1 and -2), Epstein-Barr virus (EBV) and hepatitis virus [18].

TABLE 1
Ocular disorders where the causative gene(s) was identified by molecular biology techniques.

Ocular Condition	Gene or Chromosomal Change	Reference
Aniridia	PAX6	(19)
Peter's anomaly	PAX6	(20)
Waardenburg (WS1)	PAX3	(21-28)
Waardenburg (WS3)	PAX3	(25)
X-linked Alport Syndrome	COL4A5	(29)
Lowe disease	$InsP^5$-ase	(30, 31)
Stickler disease	COL2A1	(32-35)
X-linked FEVR	Norrin	(36)
Norrie's disease	Norrin	(36-38)
Retinoblastoma	$p110^{rb1}$	(39)
ARRP*	RHO/PDEB	(40, 41)
ADRP**	RHO/Peripherin	(42-45)
Gyrate Atrophy	OAT	(46)
OCAI-A	tyrosinase	(47-49)
OCAI-B	tyrosinase	(50)
Choroideremia	GG transferase	(51-54)
Neurofibromatosis (NF1)	neurofibromin	(55, 56)
Myotonic dystrophy	myotonin protein kinase	(57-63)
AVMD***	Peripherin	(64)
Congenital complete nyctalopia	Rhodopsin	(65)
Congenital stationary night blindness	Rhodopsin	(66)
Macular distrophy	Peripherin	(64)
Marfan's syndrome	Fibrillin	(67, 68)
Retinitis punctata albescens	Peripherin	(69)

*ARRP - autossomal recessive retinitis pigmentosa
**ADRP - autossomal dominant retinitis pigmentosa
***AVMD - adult vitelliform macular dystrophy



The advantages of PCR over many of the conventional methods are linked to its sensitivity that allows the diagnosis of incipient infections and or the analysis of samples of extremely small sizes. However, as discussed above, due to its nature this technique is prone to false positives and in several cases a negative PCR result may be more informative than a positive one. Another draw back of the technique is the sophistication of the equipment and personnel to perform such studies. Nevertheless, a PCR-based diagnostic system that eliminates most of the sophisticated training required to perform this technique has been developed and in spite of its initial limited menu it is projected to make a great impact in the generalization of the use of PCR to detect infectious agents.

THERAPY

Finally, advances in molecular biology are already shapping the way patients will be treated in the future. Our ability to insert genes in any cell and have the product of such gene expressed while maintaining the viability of the host cell has sprawled a new field in medicine, gene therapy. In ophthalmology the use of gene therapy is still in its infancy and is confined at the moment to in vitro experiments or animal models. However, important advances have been made and one can foresee the day when diseases such as gyrate atrophy of the retina will be treated not by some empirical prescription of vitamin B but by the reinsertion of a functional ornithine amino-transferase gene into the patients. We can also foresee the day in which uveits will be treated by inserting specific cytokine genes into T lymphocytes selected by their ability to migrate to the eye. At the turn of the century physicians worldwide are faced with a whole new world the terms of diagnosis and specially treatment of diseases and ophthalmology is no exception.

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XXI CONGRESSO PANAMERICANO DE OFTALMOLOGIA

Cancún, Q. Roo, México, 1º a 6 de maio de 1997

A Associação Panamericana de Oftalmologia fará realizar entre 1 a 6 de maio de 1997 o XXI Congresso Panamericano de Oftalmologia na cidade de Cancun, México. Esta será mais uma grande festa de congrassamento e de ciência oftalmológica entre os oftalmologistas das Américas.

A comissão organizadora preparou um programa científico com o que há de melhor na oftalmologia americana e latino-americana. Teremos conferências magistrais, simpósios, cursos e temas livres que abrangerão desde cirurgia refrativa até doenças do nervo óptico. Enfim, os temas mais atuais da oftalmologia moderna estarão sendo abordados pelos maiores especialistas da oftalmologia nas Américas.

Além disso, Cancun oferece um sem número de atrações turísticas a serem aproveitadas pelos congressistas e familiares. A programação social do congresso também será do seu maior agrado.

Para maiores informações sobre o programa do Congresso favor contactar a Sra. Claudete Moral no CBO. Para informações sobre pacotes de viagem e hotéis, contactar a agência oficial de turismo para o congresso no Brasil. Agência Mello Faro de São Paulo.

Esperamos por uma grande delegação brasileira no congresso.

Carlos Moreira Jr.
Secretário Língua Portuguesa da APAO.