REVIEWS IN MEDICINE

PAUL LICHTER, EDITOR

The Genetic Basis of Ophthalmological Disease

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Abstract. The genes of a fertilized ova contain all of the information needed to construct an eye, regulate its function, maintain it in working order, decipher its signals and store the vision it gathers. Analyzing genes in informational and physical terms, the author discusses the genetic basis of eye structure and function. Current knowledge and techniques for genetic study are described, as are specific abnormalities which have a familial or genetic basis. (**Surv Ophthalmol 25**:37–48, 1980)

Key words. cellular disorders • DNA • genetics • heredity • metabolic disorders

Many eye diseases are familial and follow a Mendelian pattern of inheritance. Until recently, the gene was a theoretical unit which did not permit fruitful speculation of the biological mechanisms. The appreciation of the biological basis for genetic disease has only been realized since the establishment of a molecular model of genetic inheritance in microorganisms. The intensive study of microorganisms permitted a sound framework which has led to the present era of dissection of the genes of higher organisms. The recent insights into the organization and structure of human genes permits understanding of the normal and abnormal genetic function and development — including that of the eye.

It is difficult to understand the genetic basis of eye structure and function unless we analyze genes in informational and physical terms. The genes of a fertilized ova contain all of the information needed to construct an eye, regulate its function, maintain it in working order, decipher its signals, and store the visions it gathers. When we consider the volumes of books needed to understand some of the simpler aspects of ophthalmology, the economy and efficiency of genes is astounding. The period at the end of this sentence could easily accommodate 700 sets of genes. Within a single set of genes there is the capability to organize 5×10^{12} bits of information. In computer terms, 5×10^{12} bits of information would be equal to about 250,000 pages or 500 volumes of printed data.

The information of genetics is written with a four-letter alphabet in a straight line just as a sentence is written.[43] The letters of genetic sentences are represented by the four nucleic acid bases — adenine, thymidine, cytosine, and guanine (A, T, C, G). It takes three letters to form a translatable word. The

letters are linked by ribose phosphate bonds to form a long strand of DNA in the form of a chromosome. Each chromosomal strand contains about 100,000,000 letters and averages three centimeters in length. In terms of our written language, each chromosome would be a separate volume of information. There are 46 chromosomes in each cell for a combined length of about 1½ meters containing 4.6 billion letters. The packaging of chromosome strands is a complex cellular problem since strands total 1.5 million microns in length and are packaged in the nucleus which is less than 10 microns in diameter.

The genes are transcribed in the nucleus from DNA to RNA; in the cytoplasm they are translated to functioning proteins. Often these proteins are enzymes which perform a specific task in the cell. Fig. 1 shows this transfer of information.

Defects which can occur in this information can be schematically represented by a sentence composed of English three-letter words. Since all genetic words are exactly three letters long, no spacing is required as long as there is a cue for beginning. For instance, in the following sentence, the capital letter is a cue for the sentence to begin.

"ThebigreddogranoutHerpupdidtoo"
becomes:
"The big red dog ran out. Her pup did too."

If a letter, as in a sentence (or a base as in a cell) were replaced, the meaning of the sentence would change. Changing the letter g to h the sequence would be:

"ThebigredhogranoutHerpupdidtoo"
becomes:
"The big red hog ran out. Her pup did too."

If a letter were missing, the result would be chaotic:

"The bireddogranoutHerpupdidtoo"
would read
"The bir edd ogr ano ut. Her pup did too."

The spurious insertion of a letter would bring about the same result. The change of any part of the code will change the rest of the sentence until the translation mechanism finds another beginning cue to reorient itself. Sometimes there are large deletions which remove the entire sentence or most of the sentence.

Extensive work with bacteria has established that a gene is a code for an enzyme which performs a specific function. Three bases specify an amino acid by transcription through an intermediate message. When the base is changed, the amino acid of the enzyme changes. Some changes cause disease, others are inconsequential.

Mammalian Genes

In the few mammalian genes which have been directly visualized, the structure of a gene is more complicated than this simple code since there are two further aspects of higher genes. The gene is surrounded by sequences which are not transcribed into messages and are called *spacers;* and within the code for the gene are several insertions or *introns*. In order for the final enzyme to be made, the sequence within the gene (or intron) must be clipped out. Figs. 2 & 3 illustrate the way that this is probably done. Such an elaborate system was never imagined until it became possible to directly visualize genes. Now that we know that these inser-

(a)	GATACGATTGATCG CTATGCTAACTAGC			DNA in nucleus
(b)	CUAUGCUAACUAGC initiation signal			RNA in cytoplasm with initiation initiation signal
(c)	leucine—threonine—serine			amino acid sequence of an enzyme
	CUA	ACU	AGC -	codons for specific amino sequence of enzyme

FIG. 1. (a) Bases represented as G, C, A, or T are linked together by a phosphodiester deoxyribose backbone. Two strands are twisted together in a double helix as strategy for replication. (b) One of the two strands is transcribed in the nucleus to form a single stranded ribose form or messenger RNA. The mRNA is used as an informational tape to program the protein machinery of the ribosome. The mRNA specifies the beginning of the program and the exact order of amino acids. (c) The order of the amino acids determined the three dimensional shape and catalytic activity of the enzyme.

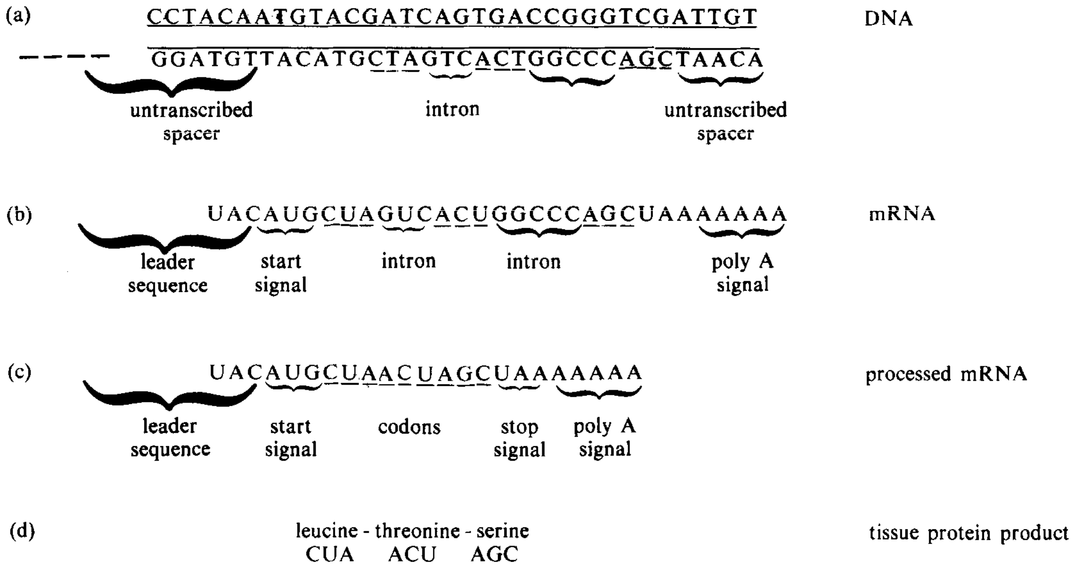

FIG. 2. (a) In the mammalian gene, the transcribed information is preceded by a string of bases which are not transcribed and therefore are not part of the coding system. This DNA is termed *Untranscribed spacer* and comprises about ½ of the DNA sequences of the genes isolated. (b) The DNA is transcribed and a series of A's are added to the right hand side of the message, the poly A signal. The series of A's may be added so that the message is recognized as such and to insure its proper handling and delivery to the proper site in the cytoplasm. (c) The sequences which intervene in the code (the introns) are then excised and a message is presented to the ribosome which permits translation to the correct protein sequence. In comparison to the system in Figure 1, there is much more DNA required for a given protein sequence. The greater complexity of the processing permits more steps for modification or regulation and also allows for more errors.

tions or *introns* are present, it is thought that they are important regulatory devices which assure that the information is used only at the proper time and place. For instance, hemoglobin is to be made in the red blood cell and not in other places. Designated cells use certain genes in the same way that only television sets with a decoder can unscramble signals sent on the pay-television channel. All of the homes receive the same signal but only those homes with a decoder can utilize the information sent to it.

Spacers may be related to a higher level of regulation which is not yet understood. Or they may be related to the packaging — the spacers may be needed to package the enormous amount of DNA. Or the spacers may be related to another feature of mammalian genes which had not been expected, that is, the large number of *repetitious DNA sequences*. Our cells contain some sequences which are repeated several thousand times. Since it requires only one copy of a gene to make an enzyme, it is apparent that much of our informational system is not used for coding proteins. Spacers may be used to form

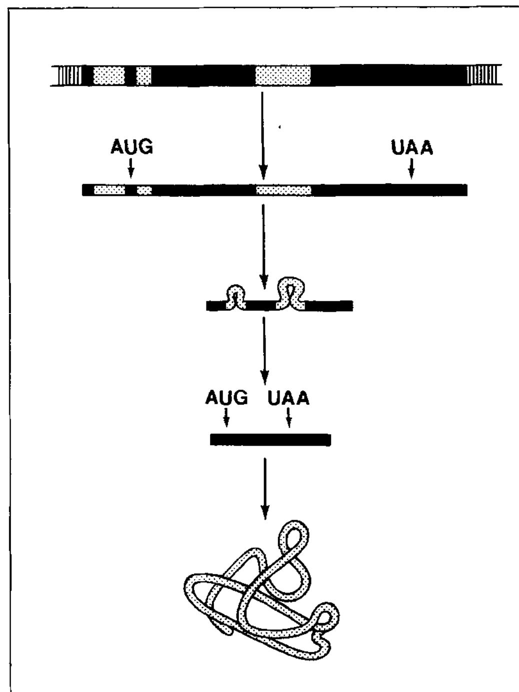

FIG. 3. An illustration of the processing steps which begin with a gene and end with a functional protein. The steps are those given in Figure 2.

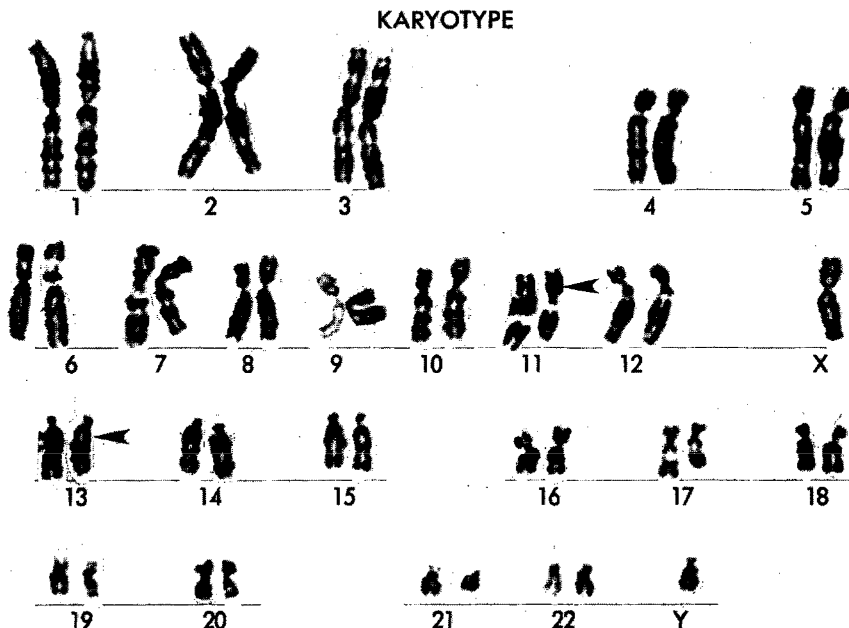

Fig. 4. Karyotype of the human. The banded chromosomes of the human permit the identification of each chromosome and the subdivision of each. Arrow at chromosome 11 and 13 indicate the positions of deletions which lead to AGR and retinoblastoma respectively.

signals to the machinery of the cells so that the information can be cataloged, as we might use titles to a chapter.

Recombinant DNA

Since specific genes have been directly visualized, it may be important to explain how this advance was made. The ability to see the structure of a single gene and relate it to a single activity has been made possible by the ability to fractionate the entire set of genes. This is done with recombinant DNA technology.

A single human cell contains enough DNA for six million genes which are connected on 23 pairs of chromosomes. Therefore, any given gene is only one part in six million. Until recombinant techniques were developed, it was technically impossible to chemically locate a specific single gene or to do biochemical analysis of a single gene. With recombinant DNA technology, the genes are broken up from long strands into pieces about the size of two or three genes. These small fragments are inserted into the much simpler genetic strands of plasmids. These organisms themselves contain only two or three genes and are so simple that they exist only within other organisms such as bacteria. The human genes within the simple organisms are introduced into a bacterial cell which will replicate and enormously amplify the gene.

Once the billions of copies of the gene are made, the genes can be viewed by electron microscope to detect their shape (Fig. 4). It is possible to see genes in which there has been a major change. For instance, it is possible to see when parts of a gene are missing or rearranged or inverted. In alpha Thalassemia a large section of the alpha chain is missing. Also, at this purity it is possible to determine the exact base sequence. Knowing the base sequence, one can predict the protein which is coded by the gene. For instance, if a particular gene is isolated in the manner illustrated above, the ability to sequence the isolate permits one to detect which gene is its neighbor and has been isolated with it. This is a direct means of identifying gene organization.

Chromosome Structure

One further level of gene organization can be studied by the light microscope, the

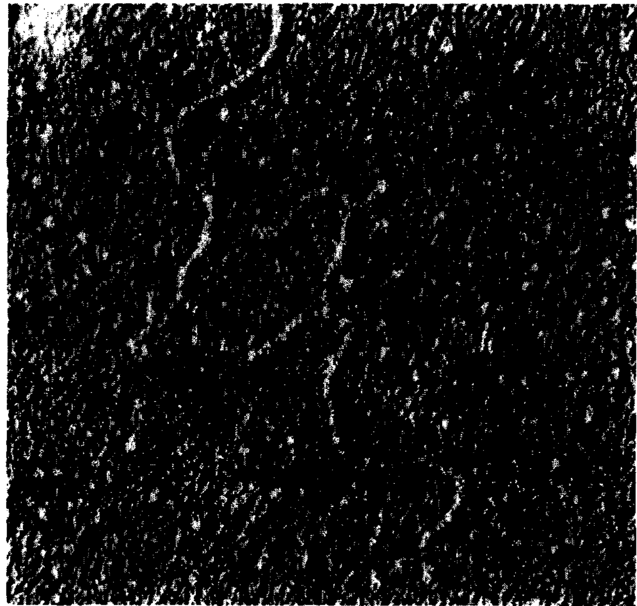

FIG. 5. A purified human ribosomal gene. This gene is inserted into a bacteriophage. It is seen at 100,000 × magnification. The human gene begins and ends at the bifurcation indicated by the arrows. This gene forms a Y shaped structure which may be important for its function.

chromosomal level. Single genes are much too small to visualize at the level of the light microscope. However, the light microscope permits us to see a complete chromosome. Each chromosome appears to consist of bands when treated with mild denaturing agents (Fig. 5). It is likely that any visible chromosomal change is a rearrangement of several hundred genes. The genes coding for enzymes may be very unevenly distributed over the chromosome since large parts of some chromosomes can be deleted with no effect while small changes in other areas of the chromosome cause serious and widespread damage. Presently, it is not known exactly how a chromosome is given its particular shape, but the DNA of the chromosome appears to be built on a protein scaffold.

There have been two major means of studying the function of chromosomes. The first has been to clinically correlate chromosome changes with both disease and hereditary patterns. The second method has been to isolate individual chromosomes from their original cell and transfer them to a different cell where their action can be observed independent of the other chromosomes.

The general lessons learned from clinical correlations tell us about the nature of gene organization at the chromosome level. Chromosome defects cause widespread mental retardation. Chromosome changes do not affect the metabolic reactions in a person so that we can surmise that the chromosome organization is not an important unit in regulating metabolic activity, but is important in determining the final form of anatomical structure and brain development.

The second method of studying chromosomes is called *somatic cell hybridization.* If cells from a mouse are grown in culture with cells from a human, the two cells can be fused together. A Sendai virus added to the culture will cause the nuclei of the cells to fuse and form a new cell with chromosomes from both organisms. The new cell then tends to lose the human chromosomes and as they divide they retain relatively few human chromosomes. The cells can then be tested for specific enzyme activity. Correlations are then made between chromosomes present and enzyme activity. For instance, if whenever the human enzyme thymidine kinase is present in the cell, only the human chromosome #17 is present; it can be concluded that chromosome #17 contains the gene for thymidine kinase. This relationship can be shown even more clearly if the mouse cell does not have a thymidine kinase gene and the hybrids are grown in a culture medium which requires the cellular production of thymidine kinase for the cell to survive. The mouse cell depends on a thymidine kinase molecule and can only grow when it has received a human #17 chromosome. Under these culture conditions

the mouse cell tends to lose all of the human chromosomes except the essential one.

The techniques of somatic cell hybridization combined with correlating inheritance of disease with familial inheritance of chromosomes has resulted in the chromosomal location of over 300 genes. These include the immunity genes on chromosome 6, hemoglobin alpha on chromosome 4 and the beta and gamma hemoglobin chains on chromosome 11. About 1300 diseases are established as genetic and 17% of these can be assigned to chromosomal location. Because better mapping makes future mapping easier, we would expect the chromosome map to continue to rapidly increase in detail.

The biological basis of genetic eye disease is based on the facts briefly outlined above. The number of genes present in the human determine the number of genetic diseases possible. The more components there are to a system, the more things that can go wrong. The number of genes required for a human to function normally is not known. Although the cell contains enough DNA for 6,000,000 genes, we do not know how much of the DNA is essential. Many genetic abnormalities are inviable and are never seen. Therefore, the number of genetic diseases which allow the formation of a fetus and are recognized are only a small proportion of all of the possible defects. According to the recent catalog of human genetic disease, there were about 2811 diseases which can be recognized and one-half of these have well documented mendelian inheritance. The other half are strongly suspected of having a genetic basis. It is interesting that the Birth Defects Compendium lists 361 different diseases that affect the eye out of a total of 1005 birth defects listed.

The best understood form of genetic disease is that which results in a defective enzyme which we can recognize. The structure of an enzyme is determined by the amino acid sequence of its enzyme. In turn, the amino acid sequence is determined by the base sequence of the DNA. Any of the possible changes in the base sequence discussed above can cause a defective enzyme. In metabolic diseases, there are two major consequences of enzyme defects: 1) The accumulation of polymers which build up in the cell and interfere with cell function; or 2) the accumulation of small molecules that inhibit normal metabolic pathways. Table 1 lists some examples of ophthalmologic diseases where the specific enzyme defect is known which fall into each of these categories. Characteristically, the polymer storage diseases are associated with cataracts, corneal clouding, cherry red macula, and optic atrophy. The storage of mucopolysaccharides leads to corneal clouding. The storage of cerebrosides and gangliosides leads to a cherry red macula and optic atrophy. In the storage diseases, specific enzymes are responsible for the degradation of each polymer in steps. It is not known why these polymers need to be replaced, but it is obvious that degradation must balance production or the product will accumulate and destroy the structures. These diseases become progressively worse and the deterioration of function is related to the amount of abnormal storage.

Accumulation of small molecules results in chemicals which inhibit other metabolic pathways. In each instance the abnormal metabolite can be identified in the blood or urine. The symptoms most associated with defects in metabolism are cataracts, pigmentary changes, neurological dysfunction, and retinitis. The discovery of these signs should lead to a screening of the urine and blood, especially if they are associated with other neurological symptoms such as seizures or mental retardation. Because the polymers or small molecules rarely accumulate prior to birth, most children with severe metabolic diseases are normal at birth and eye signs may be the first signal of a metabolic disease.

Chromosome Disease

The molecular basis of chromosomal disease is more complicated. The chromosome is a large collection of genes which are active for varying durations during the genetic lifetime. In the life of a genotype the most critical time span is during embryogenesis. It is necessary that the chromosome contains the information for the timing of expression. An abnormality of a chromosome will do three things: 1) it will affect a specific set of genes; 2) it will interfere with information about the timing of expression since chromosomes control the time interval of expression (e.g., 2nd to 4th day of embryogenesis); and 3) a deletion or a duplication will affect the amount of a gene product produced (i.e., genes are present in two doses so that deletion of a single

TABLE 1

Enzyme Disorders and Corresponding Ocular Signs (Examples of Ophthalmic Genetic Diseases in which the Specific Enzyme Defect is Known)

STORAGE DISEASES

Disease	Defective Enzyme	Eye Sign	Reference
Fabry disease	Ceramide trihexosidase (α-galactosidase)	Corneal dystrophic fundi	39
Krabbe	cerebroside galactosidase	cherry red macula; optic atrophy	1
Mannosidosis	α-mannosidase	Lenticular opacities	9
Metachromatic leukodystrophy	arylsulfatase A	retinal discoloration; degeneration	27
Mucopolysaccharidosis			
Hurler IH	iduronidase	Corneal opacity	25
Scheie IS	iduronidase	Corneal opacity	25
Morquio IV	N-acetyl hexosamine 6 sulfate sulfatase	Corneal opacity	25
Maroteaux — Lamy VI	arylsulfatase B	Corneal opacity	25
Sandhoff disease	Hexosidase A & B	Cherry red macula	29
Tay Sach	Hexosaminidase A	Cherry red macula	29

METABOLIC DISORDERS

Disease	Defective Enzyme	Eye Sign	Reference
Alkaptonuria	Homogentisic acid oxidase	Dark sclera	30
Albinism	Tyrosinase	Photophobia, nystagmus	45
Intermittent Ataxia	Pyruvate dicarboxylase	Nystagmus	6
Criegler Najar	Glucuronyl transferase	Extraocular movement	8
Ehlers Danlos	VI lysyl hydroxylase	Retinal detachment	25
Galactokinase	galactokinase	Cataracts	20
Galactosemia	galactose uridyl transferase	Cataracts	20
Homocystinuria	Cystathionine synthetase	Dislocated lens	32
Hyperglycinemia	Glycine Cell transport	Optic atrophy	3
Leigh necrotizing encephalopathy	pyruvate carboxylase	Optic atrophy	15
Maple syrup urine disease	branch chain decarboxylase	Ophthalmoplegia; nystagmus	13
Nieman Pick	sphingomyelinase	Cherry red spot	41
Refsum syndrome	phytanic acid oxidase	Retinitis pigmentosa	38
Tyrosinosis	tyrosine amino transferase	Corneal dystrophy	16
Sulfite oxidase def.	sulfite oxidase	Extopia lentes	37
Tyrosinemia	tyrosine amino transferase	lens opacity	17

CELLULAR DISORDERS

Disease	Cell Defect	Eye Sign	Reference
Ataxia telangectasia	sensitivity to X-ray	Conjunctival telangectasia	24
Bloom's syndrome	chromosome exchange	Telangectasia of lids	18
Cockayne syndrome	sensitive to UV light	Retinitis pigmentosa	31
Cystinosis	Lysosomal cystine	Corneal crystals	35
Fish eye disease	serum triglycerides; very low density lipoproteins; high density lipoprotein	massive corneal opacities	11
Lowe's syndrome	abnormal amino acid transport	cataracts	23
Menkes Syndrome	copper retention	Tortuous retinal vesels; Blindness	5
Porphyria, Gunther type	uroporphyrin I	photosensitivity	42

TABLE 2

Examples of Chromosomal Defects with Ocular Signs

Name of Syndrome	Chromosome Defect	Abnormalities of the Eye
4p⁻—Wolf	deletion of chromosome #4	Epicanthus, strabismus, ptosis, oblique palpebral fissure, hypertelorism, iris and retinal coloboma.
5p⁻—Cri du Chat	deletion of chromosome #5	Oblique palpebral fissures, hypertelorism, epicanthal folds.
18q⁻—de Grouchy	deletion of chromosome #18	Deeply set eyes, strabismus, glaucoma, nystagmus, optic atrophy, tapetoretinal degeneration.
+13 Patau	47 chromosomes, extra #13 trisomy 13	Epicanthal folds, iris colobomas, absent eyebrows, micropthalmos.
+18 Edwards	47 chromosomes, extra #18 trisomy 18	Ptosis, epicanthal folds, micro-ophthalmus, corneal opacities.
+21 Down	47 chromosomes, extra #21 trisomy 21	Brushfield spots, oblique palpebral fissures, cataracts, strabismus.

chromosome will decrease the dosage by 50%). Different chromosomal defects could lead to the same abnormal increase of gene activity. An increase in the gene product would result if the number of genes were increased and also an increase in gene product would result if the time interval of activity were increased. The interval of activity for a specific gene may be critical. For instance, if it takes four weeks for the optic cup to fuse, the genes responsible for optic cup growth must be active for that amount of time. If ½ of the genes for optic cup growth are missing, the growth may be insufficient during that time interval for completion of optic cup development. This decrease in gene dosage would lead to coloboma formation. The same effect would result if the gene dosage were normal but the interval were shortened by interference with information about timing. Table 2 lists examples of the most common chromosomal syndromes and the eye abnormalities associated with each of these.

As we might expect, the chromosomal abnormalities result in *multiple defects* since many genes are affected. If we agree that there are no less than 100,000 active genes, a change in 1% of the chromosomes would affect at least 1000 genes. The types of defects seen in chromosomal diseases can generally be classified as *dysmorphic*. There is an abnormal shape or configuration to the structure. Defects such as oblique palpebral fissures or hypertelorism reflect the relative growth of the bones surrounding and comprising the orbit. The presence of epicanthal folds is seen when the relative growth of the nasal bridge is less than the growth of the skin of the medial portion of the eyelid. Some dysmorphic syndromes result when the *relative* growth of adjoining structures is abnormal. This changed growth pattern may reflect a change of relative dosage of the genes which affect the adjoining structures. Hypertelorism could result if the genes for frontal bone growth were increased relative to those for the parietal and temporal bones. This disproportion would also result if the interval of growth for the frontal bone were increased in relation to the other facial bones.

With advanced techniques in cytogenetics, it is possible to correlate small chromosomal changes with major genetic disease. The AGR triad of *a*niridia, ambiguous *g*enitalia, and mental *r*etardation is associated with an interstitial deletion of the short arm of chromosome 11. This syndrome also is associated with Wilms' tumor. The segment of this chromosome which is associated with the triad is no more than 3% of the number 11 chromosome. This chromosome comprises only 2.3% of the DNA of a cell. Therefore, only .03 (2.3%) or .07% of the genes are lost in the deletion which caused the AGR triad. If there are 100,000 structural genes, this means that we can see a chromosome change which represents only 70 genes. It should soon be possible to relate specific genes to syndromes and thereby provide a molecular explanation for syndromes such as this.

Retinoblastoma is associated with a small deletion of chromosome 13. This represents

no more than .3% of the chromosomal complement. The vast majority of patients with retinoblastoma do not have a chromosomal deletion. Those persons with a chromosomal deletion and retinoblastoma have mental retardation. Those with a large deletion often have physical defects as well. It is not surprising to find chromosomal defects associated with a malignancy if we consider that the chromosome contains genetic information about gene regulation. Certainly, the control of cell growth or lack of control is genetically determined.

The fine mapping obtainable by cytogenetics might be compared to the fine structure analysis obtainable by recombinant DNA. The largest structure observed by EM and recombinant techniques is the phage — which has a size of 30 million Daltons or roughly 30 genes in size. The smallest band observable by light microscopy is about 1,200,000,000 Daltons or 1,200 gene-size. Although the two methods are looking at quite different sizes in terms of gene organization, it is certainly predictable that the two lines of investigation will converge. Technique of chromosome analysis will continue to be refined and the recombinant DNA technology will demonstrate ways to piece together small units of information.

The ophthalmologic abnormalities which have a familial or genetic basis fall into two categories. The first includes those diseases that are determined by a single gene and can be explained by an altered enzyme or protein function. The second category includes those diseases that are associated with interactions between genes and result in disordered embryonic growth and cell control. With the second type, the relative activity and dosage of genes is important. The understanding of the first category will continue to increase as we learn more about the structure of mammalian genes. The understanding of the second category will be advanced when we better understand the interactions of the many genes on a chromosome. Although the human chromosome is undergoing continual dissection, there is still much to be learned about the ways genes work together to form a normal fetus.

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