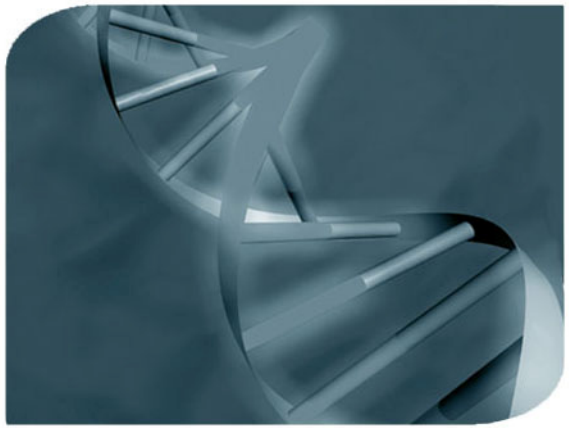
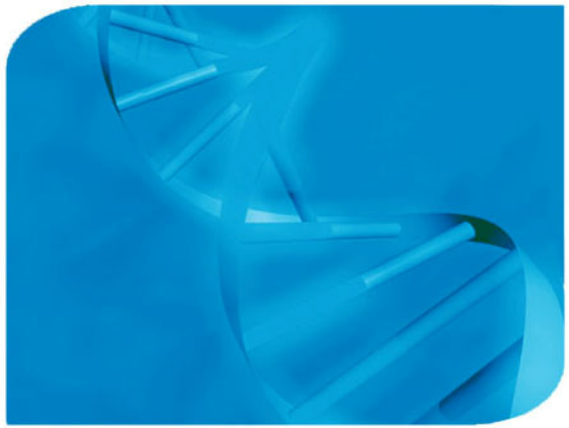
eshre
SCIENCE MOVING PEOPLE MOVING SCIENCE

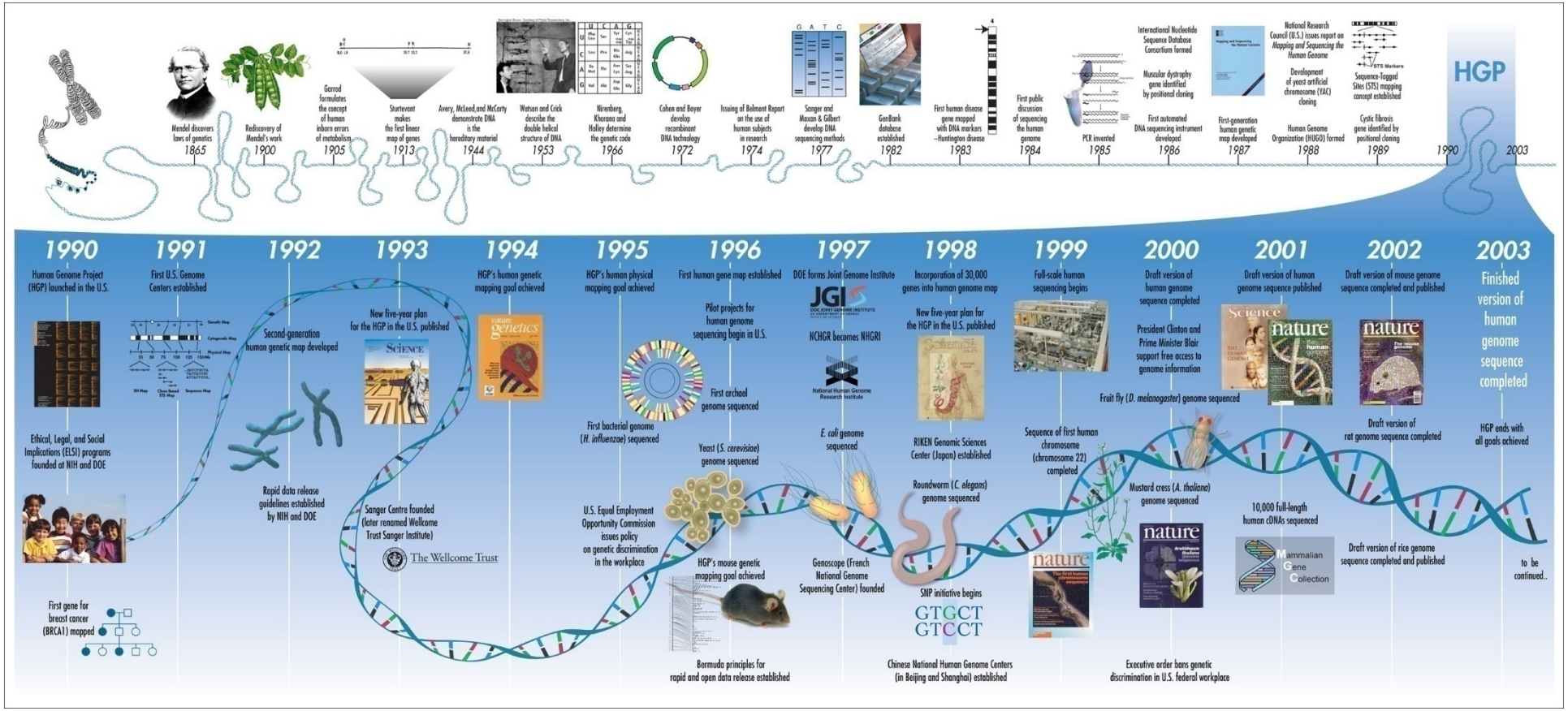
European Society of
Human Reproduction and Embryology

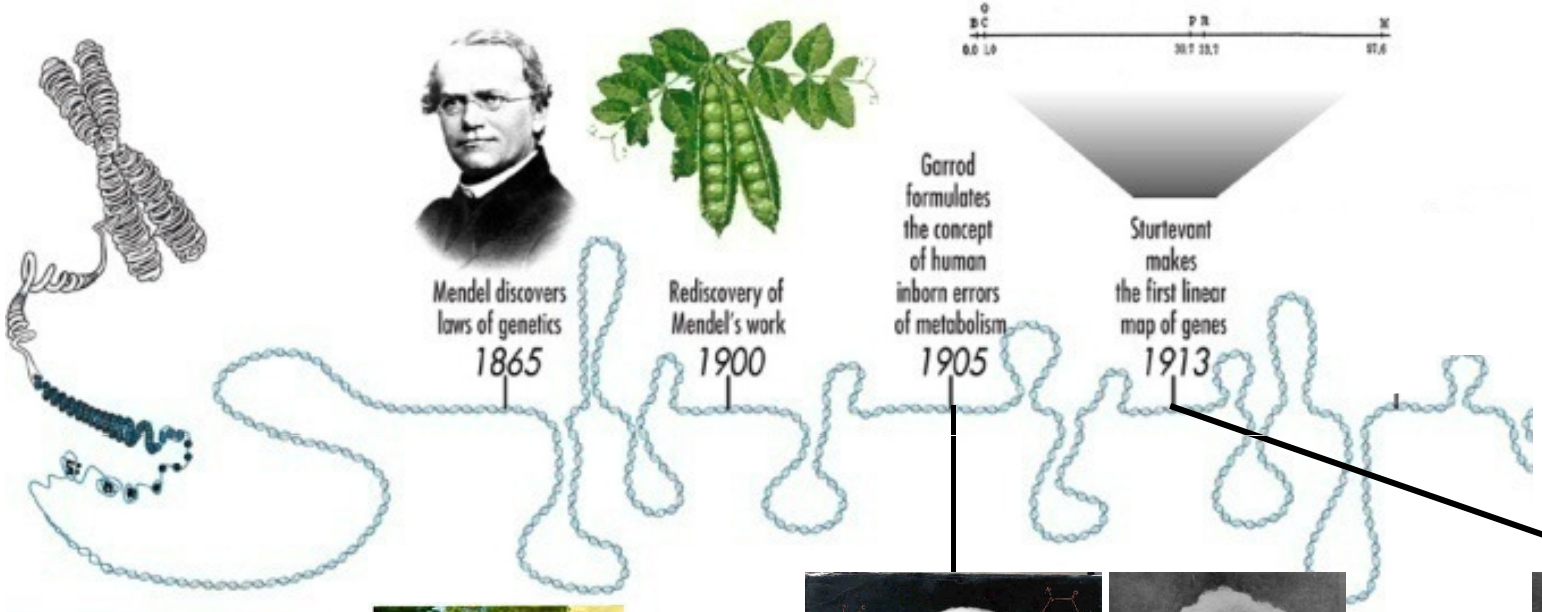
Genética básica para enfermeras

Dr. Xavier Vendrell
Unidad de Genética Reproductiva



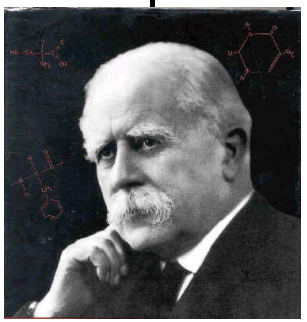



Mendel discovers laws of genetics 1865
Rediscovery of Mendel's work 1900
Garrod formulates the concept of human inborn errors of metabolism 1905
Sturtevant makes the first linear map of genes 1913
Avery, McLeod, and McCarty demonstrate DNA is the hereditary material 1944
Watson and Crick describe the double helical structure of DNA 1953
Nirenberg, Khorana and Holley determine the genetic code 1966
Cohen and Boyer develop recombinant DNA technology 1972
Issuing of Belmont Report on the use of human subjects in research 1974
Sanger and Maxam & Gilbert develop DNA sequencing methods 1977
GenBank database established 1982
First human disease gene mapped with DNA markers –Huntington disease 1983
First public discussion of sequencing the human genome 1984
PCR invented 1985
International Nucleotide Sequence Database Consortium formed
Muscular dystrophy gene identified by positional cloning
First automated DNA sequencing instrument developed 1986
First-generation human genetic map developed 1987
National Research Council (U.S.) issues report on Mapping and Sequencing the Human Genome
Development of yeast artificial chromosome (YAC) cloning
Human Genome Organization (HUGO) formed 1988
Sequence-Tagged Sites (STS) mapping concept established
Cystic fibrosis gene identified by positional cloning 1989
1990
2003
HGP
1990
Human Genome Project (HGP) launched in the U.S.
Ethical, Legal, and Social Implications (ELSI) programs founded at NIH and DOE
First gene for breast cancer (BRCA1) mapped
1991
First U.S. Genome Centers established
1992
Second-generation human genetic map developed
Rapid data release guidelines established by NIH and DOE
1993
New five-year plan for the HGP in the U.S. published
Sanger Centre founded (later renamed Wellcome Trust Sanger Institute)
The Wellcome Trust
1994
HGP's human genetic mapping goal achieved
1995
HGP's human physical mapping goal achieved
First bacterial genome (H. influenzae) sequenced
U.S. Equal Employment Opportunity Commission issues policy on genetic discrimination in the workplace
1996
First human gene map established
Pilot projects for human genome sequencing begin in U.S.
First archael genome sequenced
Yeast (S. cerevisiae) genome sequenced
HGP's mouse genetic mapping goal achieved
Bermuda principles for rapid and open data release established
1997
DOE forms Joint Genome Institute
NCHGR becomes NHGRI
E. coli genome sequenced
Genoscope (French National Genome Sequencing Center) founded
1998
Incorporation of 30,000 genes into human genome map
New five-year plan for the HGP in the U.S. published
RIKEN Genomic Sciences Center (Japan) established
Roundworm (C. elegans) genome sequenced
SNP initiative begins
Chinese National Human Genome Centers (in Beijing and Shanghai) established
1999
Full-scale human sequencing begins
Sequence of first human chromosome (chromosome 22) completed
2000
Draft version of human genome sequence completed
President Clinton and Prime Minister Blair support free access to genome information
Fruit fly (D. melanogaster) genome sequenced
Mustard cress (A. thaliana) genome sequenced
Executive order bans genetic discrimination in U.S. federal workplace
2001
Draft version of human genome sequence published
10,000 full-length human cDNAs sequenced
2002
Draft version of mouse genome sequence completed and published
Draft version of rat genome sequence completed
Draft version of rice genome sequence completed and published
2003
Finished version of human genome sequence completed
HGP ends with all goals achieved
to be continued..



Gregor Mendel

Proclamación de "Las leyes de Mendel"



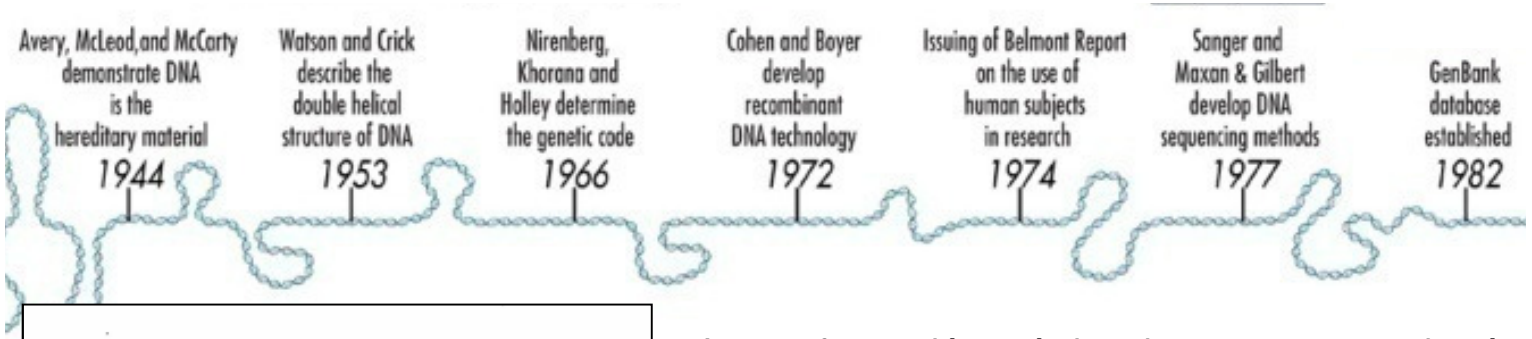
Archibald Garrod *William Bateson*

"Errores congénitos del metabolismo"

Alcaptonuria

Alfred Henry Sturtevant

"Mapa ligamiento genético"



STUDIES ON THE CHEMICAL NATURE OF THE SUBSTANCE INDUCING TRANSFORMATION OF PNEUMOCOCCAL TYPES

INDUCTION OF TRANSFORMATION BY A DESOXYRIBONUCLEIC ACID FRACTION ISOLATED FROM PNEUMOCOCCUS TYPE III

BY OSWALD T. AVERY, M.D., COLIN M. MACLEOD, M.D., AND MACLYN MCCARTY,* M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

PLATE 1

(Received for publication, November 1, 1943)

Biologists have long attempted by chemical means to induce in higher organisms predictable and specific changes which thereafter could be transmitted in series as hereditary characters. Among microörganisms the most striking example of inheritable and specific alterations in cell structure and function that can be experimentally induced and are reproducible under well defined and adequately controlled conditions is the transformation of specific types of Pneumococcus. This phenomenon was first described by Griffith (1) who succeeded in transforming an attenuated and non-encapsulated (R) variant derived from one specific type into fully encapsulated and virulent (S) cells of a heterologous specific type. A typical instance will suffice to illustrate the techniques originally used and serve to indicate the wide variety of transformations that are possible within the limits of this bacterial species.

Griffith found that mice injected subcutaneously with a small amount of a living R culture derived from Pneumococcus Type II together with a large inoculum of heat-killed Type III (S) cells frequently succumbed to infection, and that the heart's blood of these animals yielded Type III pneumococci in pure culture. The fact that the R strain was avirulent and incapable by itself of causing fatal bacteremia and the additional fact that the heated suspension of Type III cells contained no viable organisms brought convincing evidence that the R forms growing under these conditions had newly acquired the capsular structure and biological specificity of Type III pneumococci.

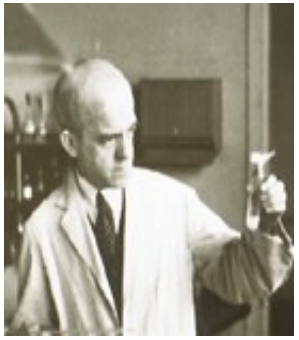
The original observations of Griffith were later confirmed by Neufeld and Levinthal (2), and by Baurhenn (3) abroad, and by Dawson (4) in this laboratory. Subsequently Dawson and Sia (5) succeeded in inducing transformation *in vitro*. This they accomplished by growing R cells in a fluid medium containing anti-R serum and heat-killed encapsulated S cells. They showed that in the test tube as in the animal body transformation can be selectively induced, depending on the type specificity of the S cells used in the reaction system. Later, Alloway (6) was able to cause

* Work done in part as Fellow in the Medical Sciences of the National Research Council.

137

La trasformación química de *pneumococos* hacía que cambiaran sus características de una generación a otra.

1944



Oswald Avery

Colin McLeod

Maclyn McCarty

CONCLUSION

The evidence presented supports the belief that a nucleic acid of the desoxyribose type is the fundamental unit of the transforming principle of Pneumococcus Type III.

sistemas genómicos
BIOMÉDICA

Avery, McLeod, and McCarty demonstrate DNA is the hereditary material — 1944

Watson and Crick describe the double helical structure of DNA — 1953

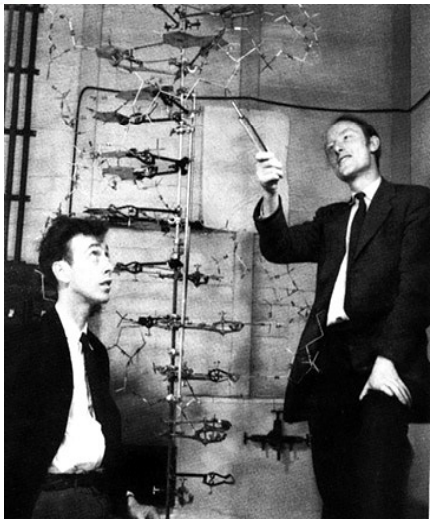
Nirenberg, Khorana and Holley determine the genetic code — 1966

Cohen and Boyer develop recombinant DNA technology — 1972

Issuing of Belmont Report on the use of human subjects in research — 1974

Sanger and Maxan & Gilbert develop DNA sequencing methods — 1977

database established — 1982



No. 4356 April 25, 1953 NATURE 737

equipment, and to Dr. G. E. R. Deacon and the captain and officers of R.R.S. *Discovery II* for their part in making the observations.

[1] Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 149 (1920).
[2] Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
[3] Von Arx, W. S., Woods Hole Papers in Phys. Oceanog., Meteor., **11** (3) (1950).
[4] Ekman, V. W., *Arkiv. Mat. Astron. Fysik. (Stockholm)*, **2** (11) (1905).

MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

We wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

This figure is purely diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining β-D-deoxyribofuranose residues with 3′,5′ linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyrimidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

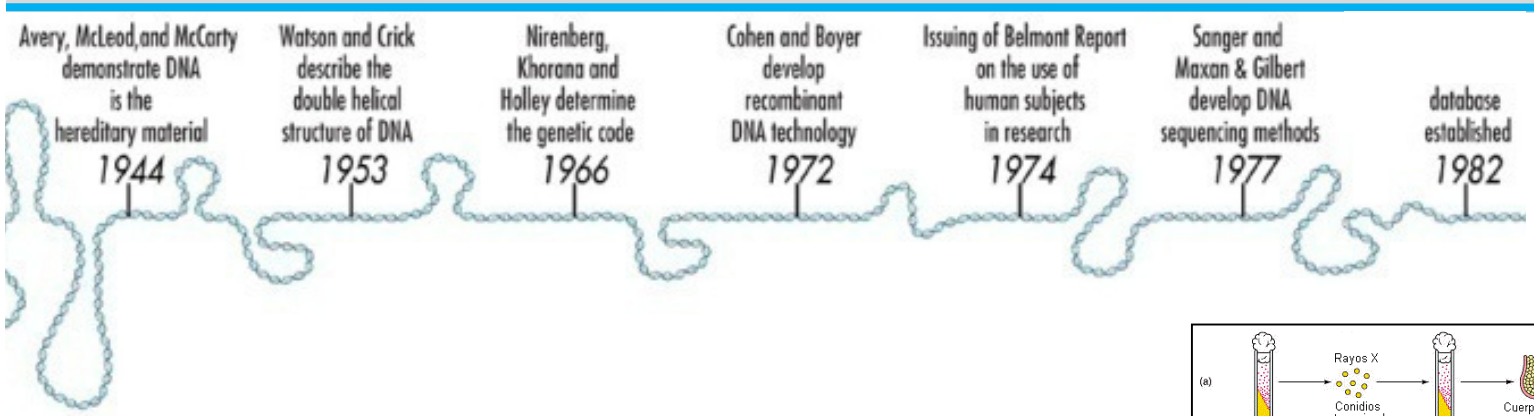
The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

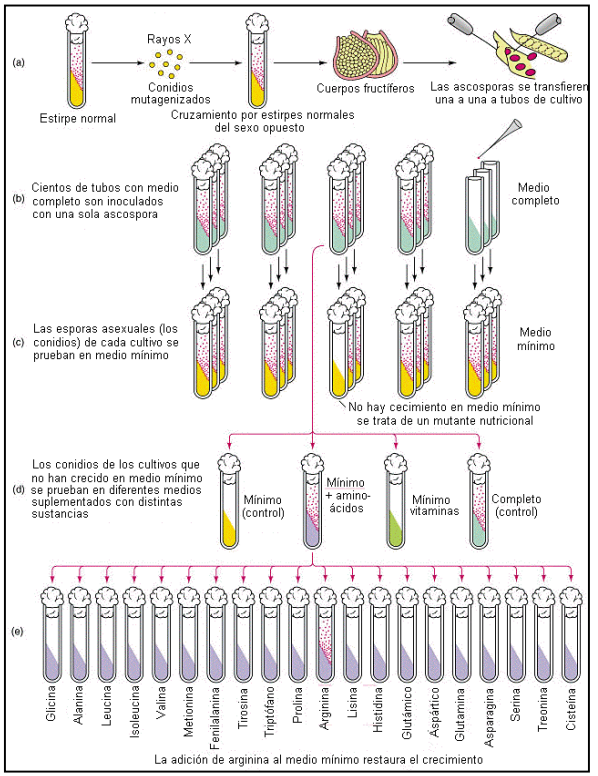
James D. Watson *Francis H.C.Crick*



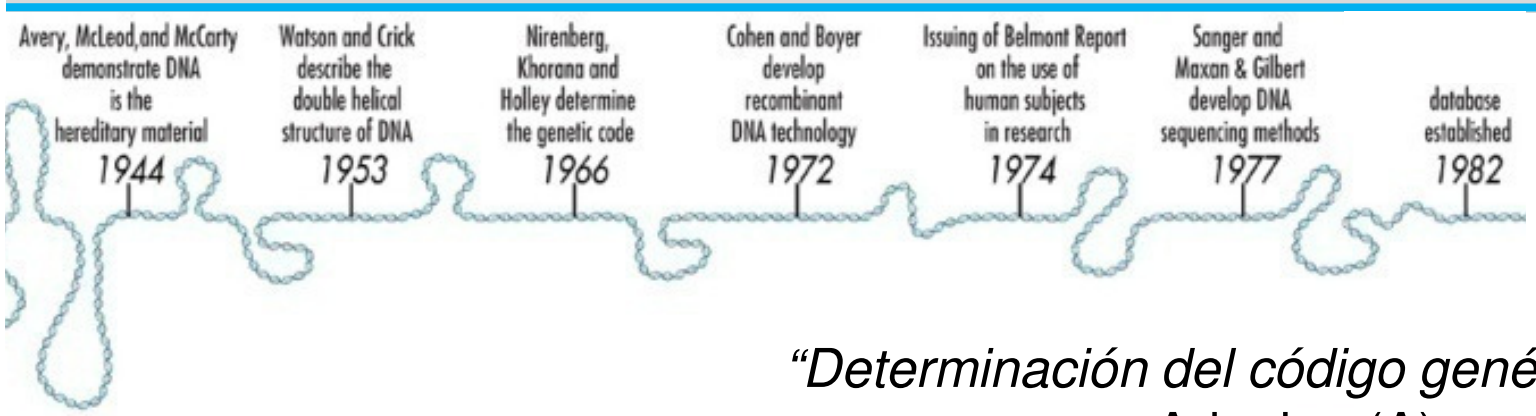
"Un gen, una proteína" (1958)
Paradigma de la Biología Molecular

George W. Beadle

Edward L. Tatum



(Neuroespora crassa)



"Determinación del código genético" (1966)

Adenina (A)

Timina (T)

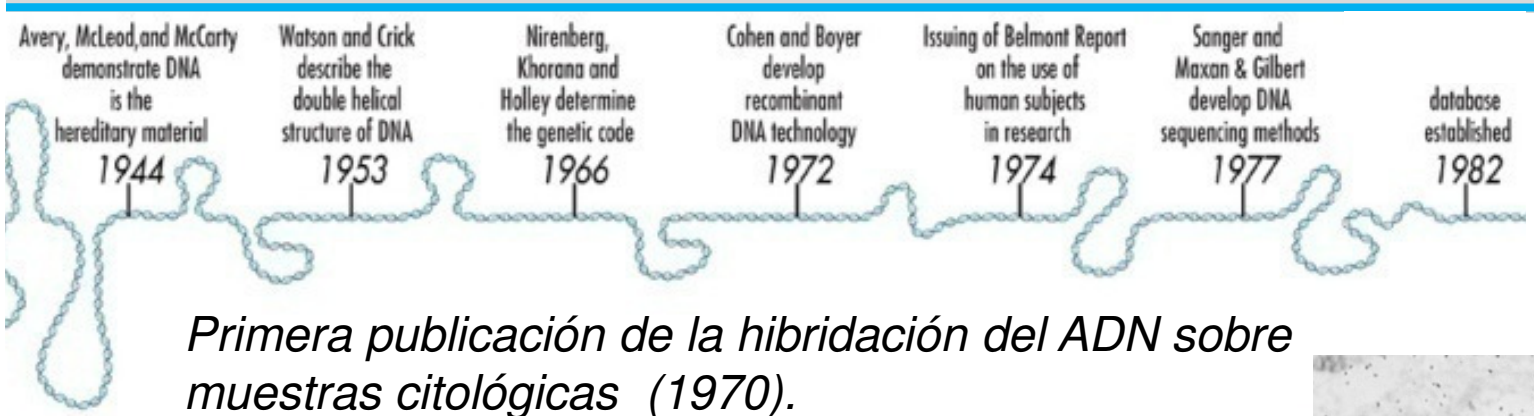
Guanina (G)

Citosina (C)

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Robert W. Holley | Har Gobind Khorana | Marshall W. Nirenberg

sistemas genómicos
BIOMÉDICA



Primera publicación de la hibridación del ADN sobre muestras citológicas (1970).

MOLECULAR HYBRIDIZATION OF RADIOACTIVE DNA TO THE DNA OF CYTOLOGICAL PREPARATIONS

By Mary Lou Pardue and Joseph G. Gall

Kline Biology Tower, Yale University

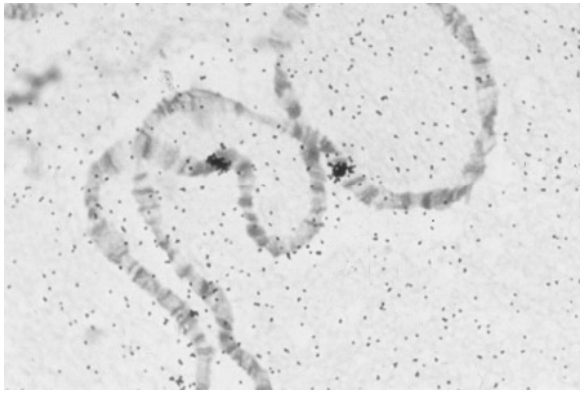
Communicated by Norman H. Giles, August 13, 1969

Abstract.—A method is presented for detecting the cellular location of specific DNA fractions. The technique involves the hybridization of a radioactive test DNA in solution to the stationary DNA of a cytological preparation. Sites of DNA binding are then detected by autoradiography. Experiments with DNA of the toad *Xenopus* are described.

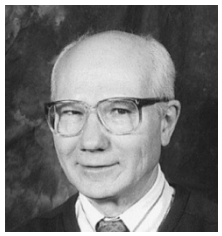
The technique of DNA-DNA hybridization has been applied to a variety of genetic problems since its introduction by Schildkraut, Marmur, and Doty.[1] Hybridization of purified DNA has been used to investigate homologies between the DNA of phage and the DNA of the host bacterium,[2] to study genetic relationships among higher organisms,[3, 4] and to examine the relation of particular DNA fractions to the rest of the genome.[5] Reannealing kinetics have been used as a measure of genome complexity.[6, 7] Recently, substitutions and deletions in phage λ DNA have been mapped from electron micrographs of hybrid molecules.[8]

We have now developed a technique which permits the localization of DNA-DNA hybrids in cytological preparations. In this method the DNA of the cytological preparation is denatured *in situ* and then hybridized with a radioactive test DNA in solution. The binding of the test DNA to the cellular DNA on the slide is detected autoradiographically.

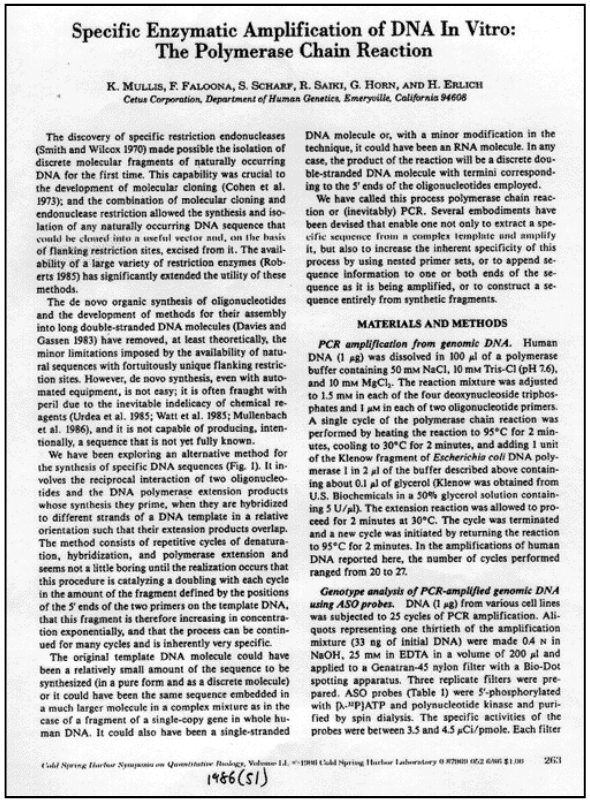
Materials and Methods.—(A) *Preparation of radioactive test DNA:* The radioactive DNA used in our hybridizations was extracted from tissue cultures[9] of the mouse and the toad *Xenopus* grown for several days in a medium containing 5 μc/ml of thymidine-H^3 (specific activity 11.3 C/mM). The cells were lysed in a sodium dodecyl sarcosinate-pronase solution (0.5% Sarkosyl Geigy, 50 μg/ml self-digested pronase, 0.1 *M* EDTA,



Mary Lou Parduel



Joseph Gall



Specific Enzymatic Amplification of DNA In Vitro: The Polymerase Chain Reaction

K. MULLIS, F. FALOONA, S. SCHARF, R. SAIKI, G. HORN, AND H. ERLICH
Cetus Corporation, Department of Human Genetics, Emeryville, California 94608

The discovery of specific restriction endonucleases (Smith and Wilcox 1970) made possible the isolation of discrete molecular fragments of naturally occurring DNA for the first time. This capability was crucial to the development of molecular cloning (Cohen et al. 1973); and the combination of molecular cloning and endonuclease restriction allowed the synthesis and isolation of any naturally occurring DNA sequence that could be cloned into a useful vector and, on the basis of flanking restriction sites, excised from it. The availability of a large variety of restriction enzymes (Roberts 1985) has significantly extended the utility of these methods.

The de novo organic synthesis of oligonucleotides and the development of methods for their assembly into long double-stranded DNA molecules (Davies and Gassen 1983) have removed, at least theoretically, the minor limitations imposed by the availability of natural sequences with fortuitously unique flanking restriction sites. However, de novo synthesis, even with automated equipment, is not easy; it is often fraught with peril due to the inevitable indelicacy of chemical reagents (Urdea et al. 1985; Watt et al. 1985; Mullenbach et al. 1986), and it is not capable of producing, intentionally, a sequence that is not yet fully known.

We have been exploring an alternative method for the synthesis of specific DNA sequences (Fig. 1). It involves the reciprocal interaction of two oligonucleotides and the DNA polymerase extension products whose synthesis they prime, when they are hybridized to different strands of a DNA template in a relative orientation such that their extension products overlap. The method consists of repetitive cycles of denaturation, hybridization, and polymerase extension and seems not a little boring until the realization occurs that this procedure is catalyzing a doubling with each cycle in the amount of the fragment defined by the positions of the 5′ ends of the two primers on the template DNA, that this fragment is therefore increasing in concentration exponentially, and that the process can be continued for many cycles and is inherently very specific.

The original template DNA molecule could have been a relatively small amount of the sequence to be synthesized (in a pure form and as a discrete molecule) or it could have been the same sequence embedded in a much larger molecule in a complex mixture as in the case of a fragment of a single-copy gene in whole human DNA. It could also have been a single-stranded DNA molecule or, with a minor modification in the technique, it could have been an RNA molecule. In any case, the product of the reaction will be a discrete double-stranded DNA molecule with termini corresponding to the 5′ ends of the oligonucleotides employed.

We have called this process polymerase chain reaction or (inevitably) PCR. Several embodiments have been devised that enable one not only to extract a specific sequence from a complex template and amplify it, but also to increase the inherent specificity of this process by using nested primer sets, or to append sequence information to one or both ends of the sequence as it is being amplified, or to construct a sequence entirely from synthetic fragments.

MATERIALS AND METHODS

PCR amplification from genomic DNA. Human DNA (1 μg) was dissolved in 100 μl of a polymerase buffer containing 50 mM NaCl, 10 mM Tris-Cl (pH 7.6), and 10 mM $MgCl_2$. The reaction mixture was adjusted to 1.5 mM in each of the four deoxynucleoside triphosphates and 1 μM in each of two oligonucleotide primers. A single cycle of the polymerase chain reaction was performed by heating the reaction to 95°C for 2 minutes, cooling to 30°C for 2 minutes, and adding 1 unit of the Klenow fragment of *Escherichia coli* DNA polymerase 1 in 2 μl of the buffer described above containing about 0.1 μl of glycerol (Klenow was obtained from U.S. Biochemicals in a 50% glycerol solution containing 5 U/μl). The extension reaction was allowed to proceed for 2 minutes at 30°C. The cycle was terminated and a new cycle was initiated by returning the reaction to 95°C for 2 minutes. In the amplifications of human DNA reported here, the number of cycles performed ranged from 20 to 27.

Genotype analysis of PCR-amplified genomic DNA using ASO probes. DNA (1 μg) from various cell lines was subjected to 25 cycles of PCR amplification. Aliquots representing one thirtieth of the amplification mixture (33 ng of initial DNA) were made 0.4 N in NaOH, 25 mM in EDTA in a volume of 200 μl and applied to a Genatran-45 nylon filter with a Bio-Dot spotting apparatus. Three replicate filters were prepared. ASO probes (Table 1) were 5′-phosphorylated with [γ-^{32}P]ATP and polynucleotide kinase and purified by spin dialysis. The specific activities of the probes were between 3.5 and 4.5 μCi/pmole. Each filter

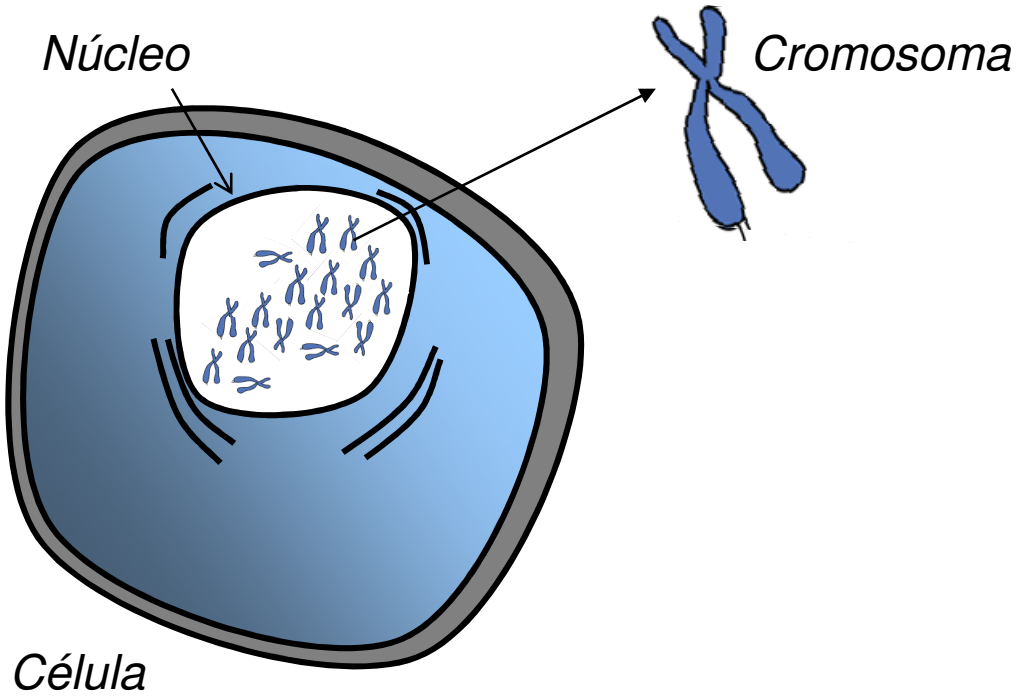
Cold Spring Harbor Symposia on Quantitative Biology, Volume LI. © 1986 Cold Spring Harbor Laboratory 0-87969-052-6/86 $1.00

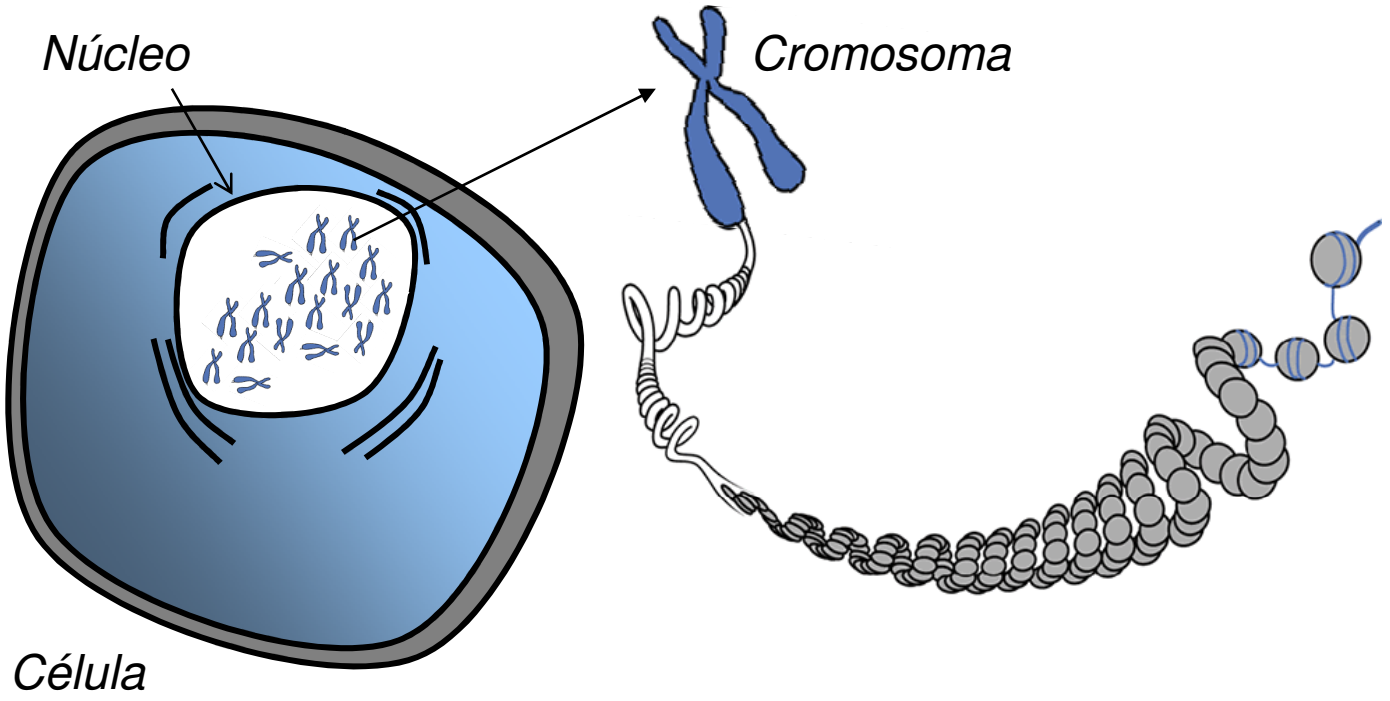
1986(51)

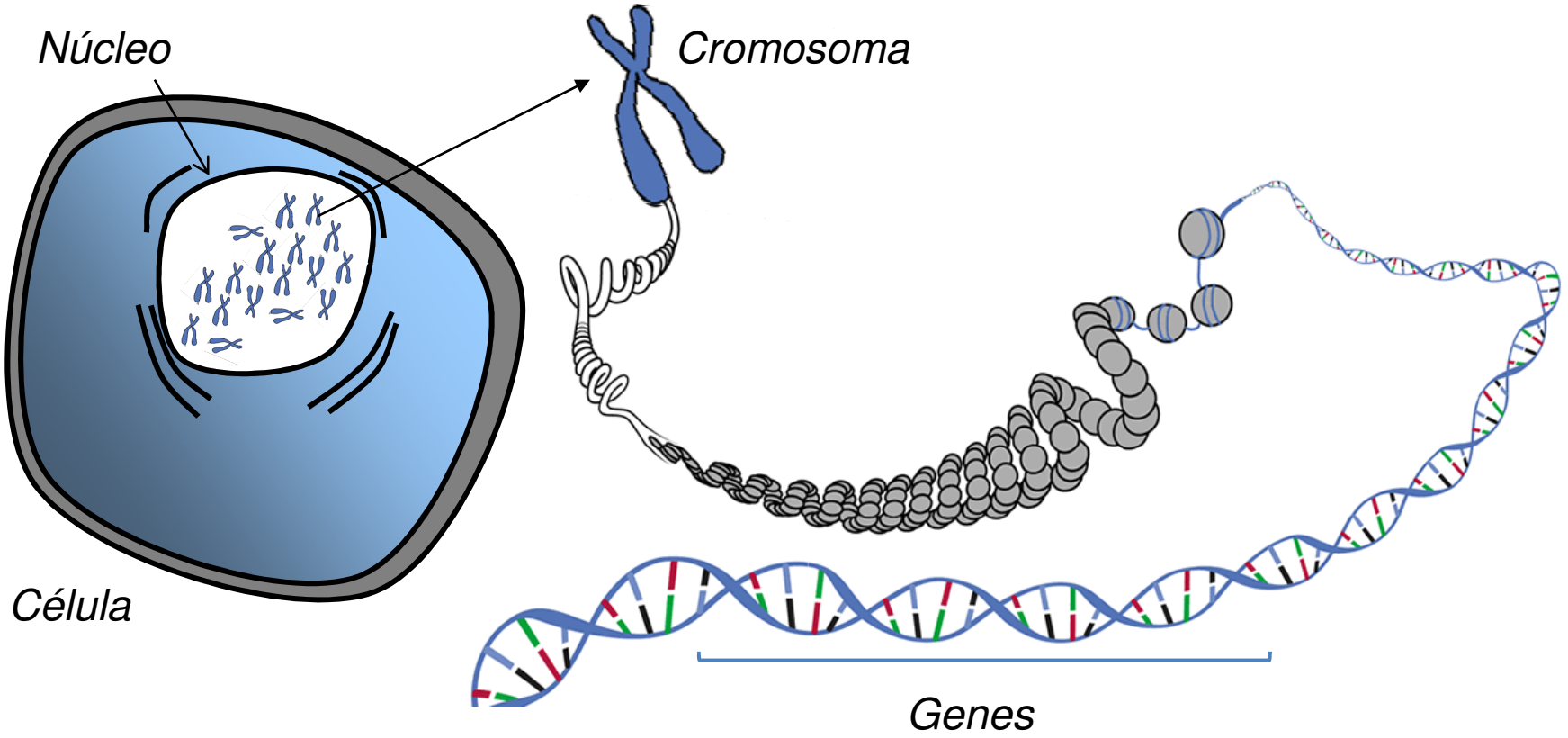
Invención de la PCR (1986)

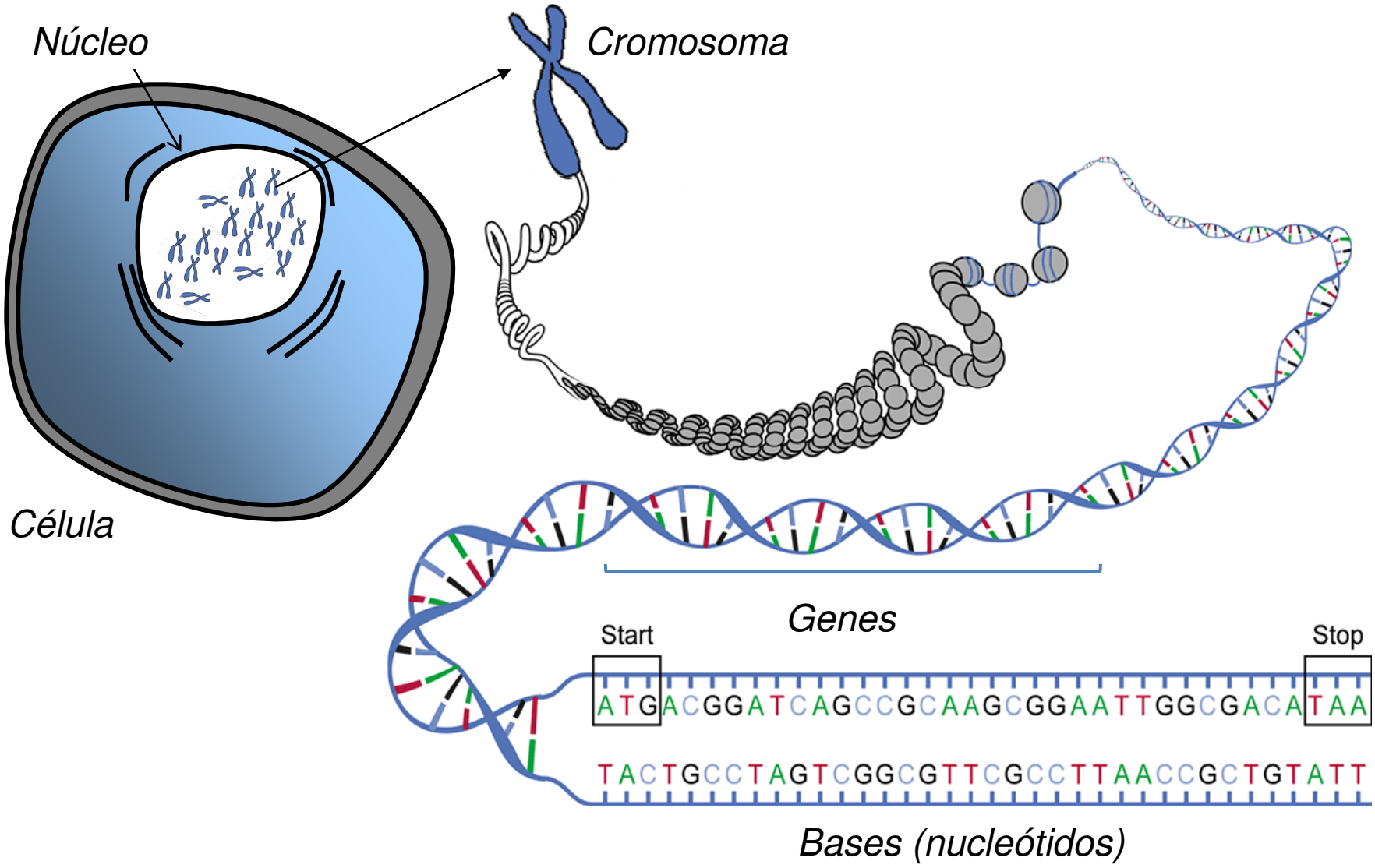
Kary Mullis

En resumen ...


Núcleo
Cromosoma
Célula


Núcleo
Cromosoma
Célula


Núcleo
Cromosoma
Célula
Genes

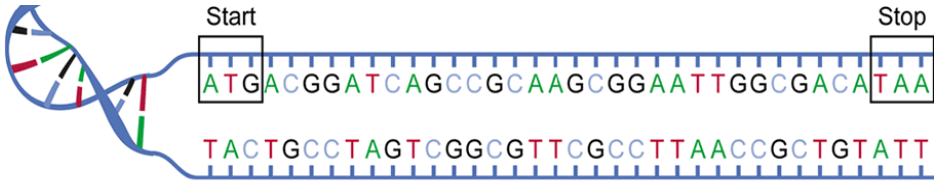

Núcleo
Cromosoma
Célula
Genes
Start
Stop
ATGACGGATCAGCCGCAAGCGGAATTGGCGACATAA
TACTGCCTAGTCGGCGTTCGCCTTAACCGCTGTATT
Bases (nucleótidos)

Conceptos básicos:

- Gen
- Cromatina y cromosomas
- Genotipo y fenotipo
- Mutación
- Enfermedades “genéticas” y herencia
- Diagnóstico genético

Gen

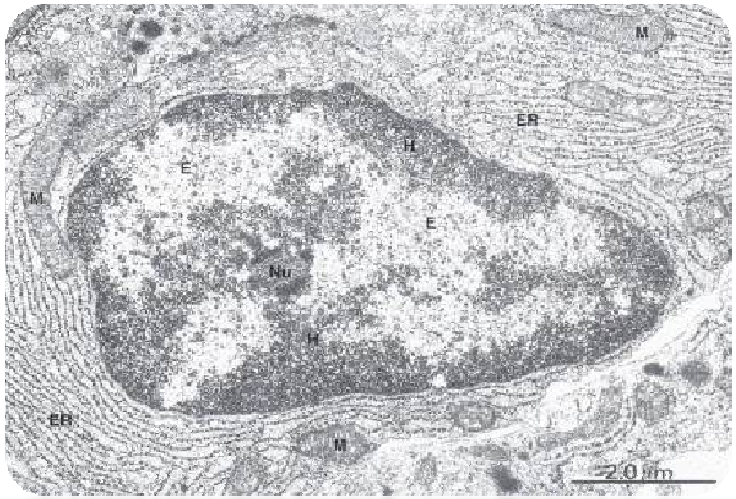
- Gen: secuencia de ADN compuesta por nucleótidos (A,T,G,C) donde está la información para la síntesis de las proteínas.
- El orden o secuencia de las 4 bases determina la proteína producida.
- Todas las células tienen todos los genes.
- Tenemos dos copias de casi todos los genes.
- Los genes están formados por una doble hélice de DNA, dos cadenas complementarias.

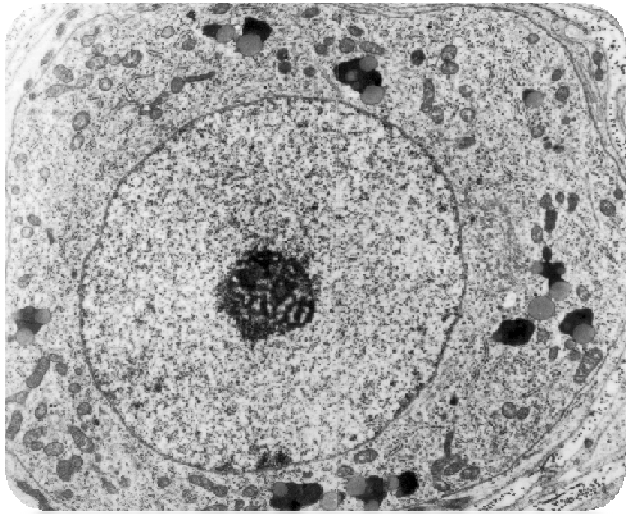


Conceptos básicos:

- [x] Gen
- [ ] Cromatina y cromosomas
- [ ] Genotipo y fenotipo
- [ ] Mutación
- [ ] Enfermedades “genéticas” y herencia
- [ ] Diagnóstico genético

¿Qué es el núcleo celular, la cromatina y los cromosomas?



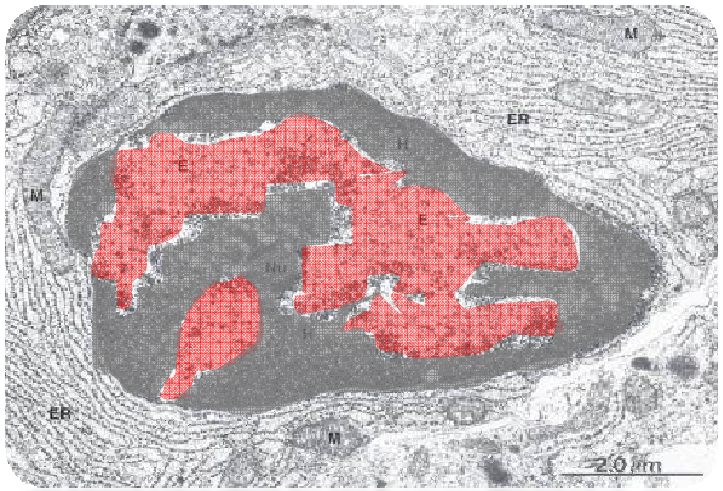


- El núcleo celular es un orgánulo complejo que contiene la mayor parte del material genético celular. Su función es mantener la integridad de los genes y controlar las actividades celulares regulando la expresión génica.

- La cromatina es la unión del ADN con proteínas para mantener la integridad del material genético.

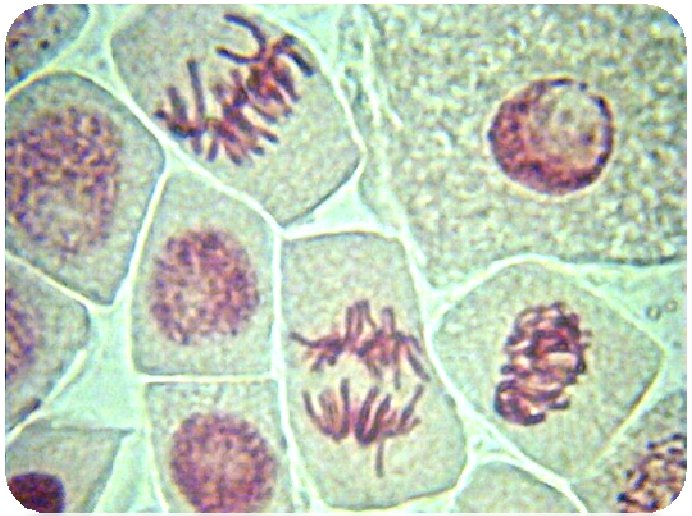
  - Eucromatina: cromatina activa, genes que están siendo transcritos. Está más descondensada (rojo).

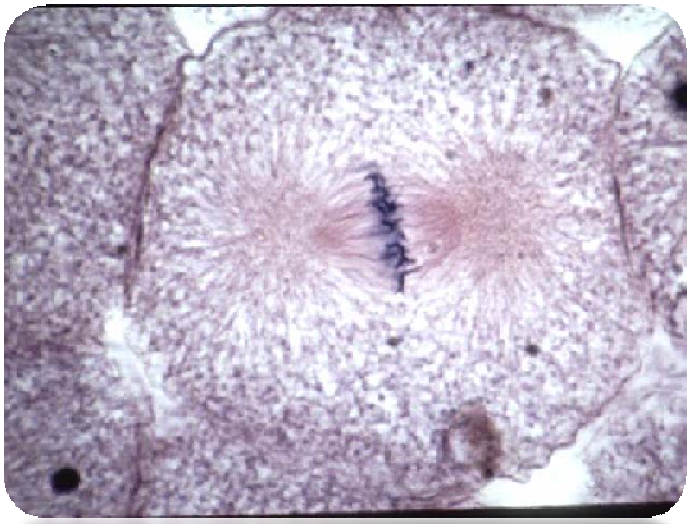
  - Heterocromatina: empaquetada más densamente, genes inactivos (gris).



- Los cromosomas:el máximo nivel de compactación que alcanza la cromatina durante la división celular.

¿Para qué “sirven” los cromosomas?





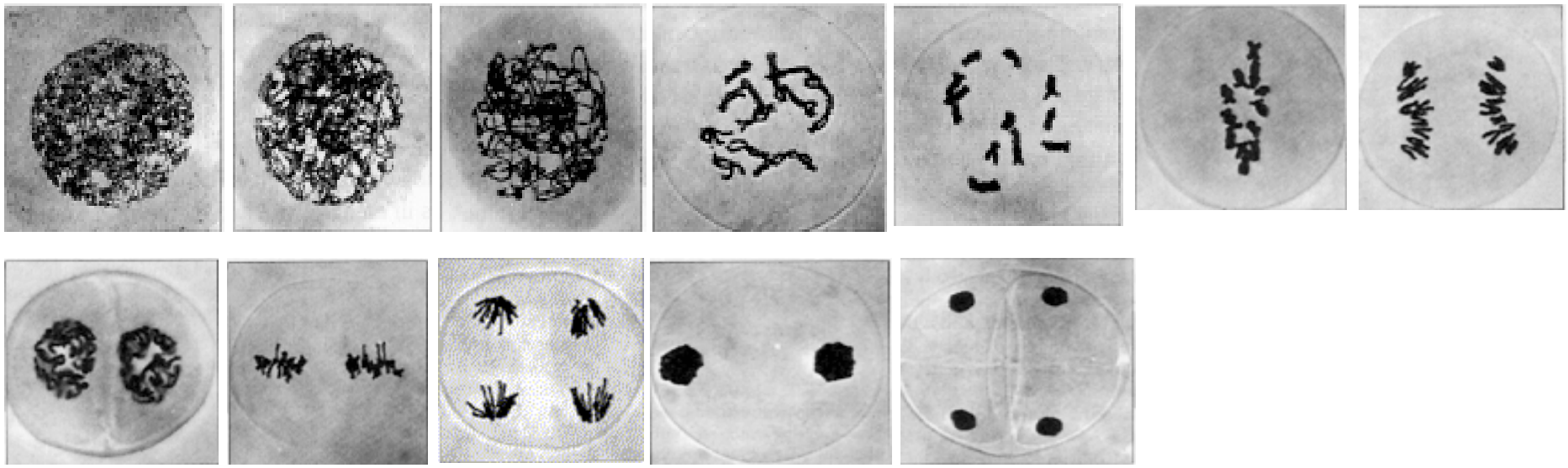
Los cromosomas permiten la transmisión de la información genética a las diferentes células en los procesos de división celular.

División celular:

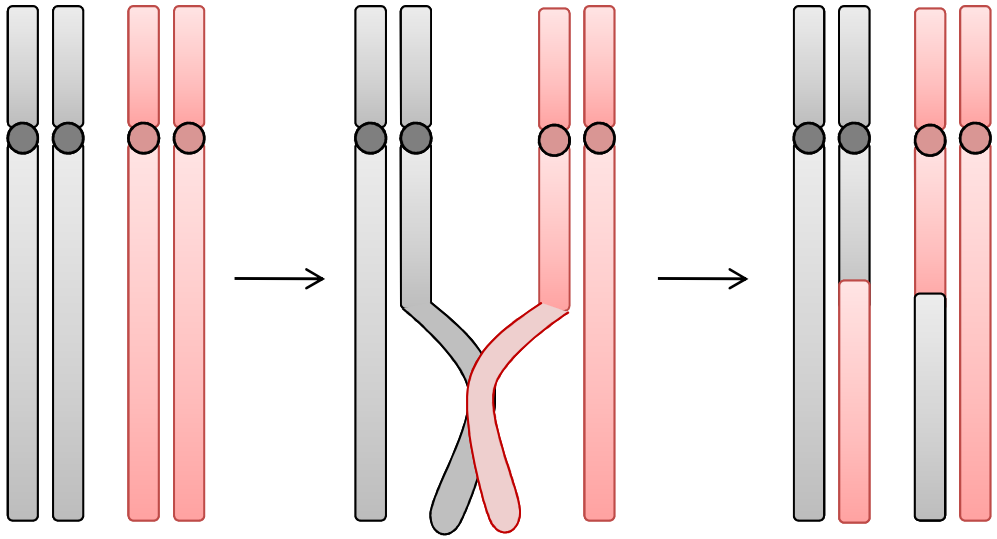
-

+

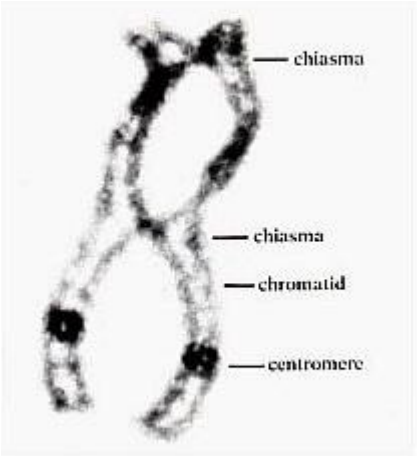
Compactación de la cromatina



Los cromosomas recombinan entre si durante la división celular de las células germinales (óvulos y espermatozoides). Esto permite la formación de cromosomas nuevos. Da lugar a individuos únicos, genéticamente diferentes a los padres.



Entrecruzamiento cromosómico



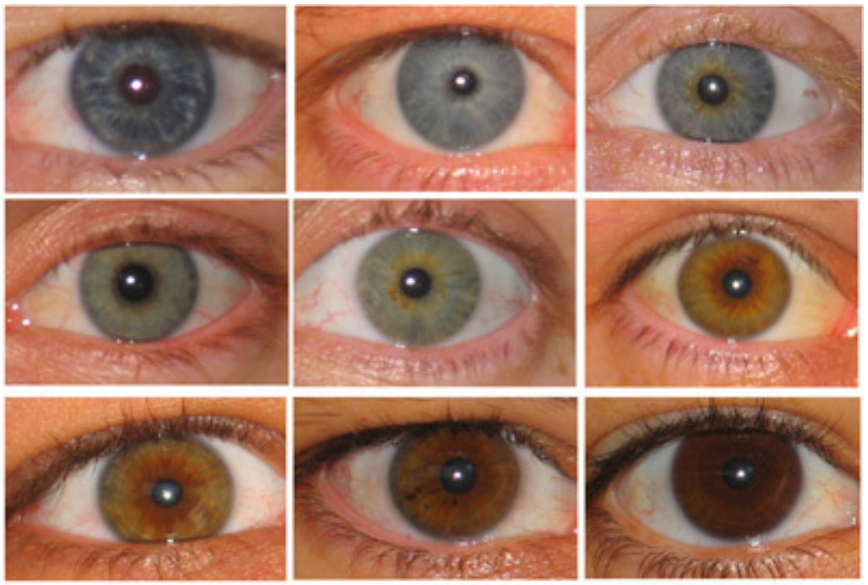
Conceptos básicos:

- [x] Gen
- [x] Cromatina y cromosomas
- [ ] Genotipo y fenotipo
- [ ] Mutación
- [ ] Enfermedades “genéticas” y herencia
- [ ] Diagnóstico genético

Genotipo y fenotipo

Genotipo: toda la información genética de un individuo.

Fenotipo: la expresión del genotipo en un individuo concreto y en un ambiente concreto (rasgos morfológicos, bioquímicos, de desarrollo, comportamiento, ...).



Conceptos básicos:

- [x] Gen
- [x] Cromatina y cromosomas
- [x] Genotipo y fenotipo
- [ ] Mutación
- [ ] Enfermedades “genéticas” y herencia
- [ ] Diagnóstico genético

"Mutación"

Es un cambio en la secuencia del ADN.

Normalmente se habla de mutación *cuando el cambio da lugar a una alteración patológica de la proteína y como consecuencia una disfunción.*

No todos los cambios son patológicos.