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In a Biological World

Edited by
François Gros

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In a Biological World

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Edited by

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Foreword

It is clear that what follows should be regarded as some general reflections, hence by no means exhaustive, about the *spirit* of this meeting rather than a precise survey of the presentations themselves, however remarkable they have been. The reason being that, although having been partly responsible for the choice of the topics covered in this Symposium, I am far from having sufficient expertise to give each presentation the tribute it deserves. For the same reason I have deliberately avoided quoting the names of the speakers and adopted the attitude of an ignorant, albeit very involved and sincere observer...

Before starting, I have the great honour to express our deepest respect and admiration to the president of the French Republic, François Mitterand who has bestowed on us his eminent patronage, for his sustained recognition of the role of Science at the service of human "causes".

It is my agreeable duty to express my deepest appreciation to the members of the organizing committee (W. Arber, G. Bernardi, B. Hess, R. Petrov and P. Talwar) without whom this Symposium, of a rather unorthodox nature (since it is at the front line between exact and human sciences) would have never come of age !

The initiative clearly was that of the World Institute of Science. Frequent and exhaustive preparatory discussions among the members of the WIS "bureau" were exceedingly helpful. I want to acknowledge the continuous encouragement and very active support from Prof. A. Lichnérovicz, former President of WIS as well as that of the WIS Secretary General, L. Albou.

But (as it is often the case...) Giorgio Bernardi really was the true motor for this whole endeavour. He deserves our most sincere thanks for having taken a decisive part in shaping and organizing the Symposium itself in a way which has made it a lively and fruitful gathering.

Last but not least, let me thank warmly, on behalf of all the participants, as well as on my own behalf, UNESCO, whose Director General, Professor F. Mayor has been, as always, immediately open to our ideas and has provided considerable help. Likewise, we are quite grateful to Professors P. Fasella and B. Hansen for the generous and instrumental support which we have received from the European Union; and the CNRS particularly, the Director of the Life Science Department, Pierre Tambourin, as well as ICSU and its Executive Director, Mrs. Julia Marton-Lefèvre, all of whom have also given us their concrete and active sponsorship.

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The Message of Life

by Christian de Duve
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Does life have a message? Is there any meaning to the existence of life on Earth and to our own membership in the biospheric community? Many scientists have derived a negative answer to these questions from the achievements of modern biology. According to their views, the development of life is a highly improbable event, so improbable that it could be unique in the entire universe and even might well, but for a fantastic combination of fortuitous circumstances, never have occurred on Earth. The subsequent evolution of life, these scientists further maintain, is itself the product of innumerable chance events. That it should have given rise to humankind is a fluke, a cosmic joke. There is no message, no meaning. In the words of the late Jacques Monod, "The universe was not pregnant with life; nor the biosphere with man."

My reading of the facts is different. In my opinion, life and mind are written into the fabric of the universe. They are cosmic imperatives, manifestations of matter bound to arise wherever and whenever conditions are favorable, which probably means many places and many times in the history of the universe. From which I conclude that the universe is not meaningless (C. de Duve, *Vital Dust, Life as a Cosmic Imperative*, New York: Basic Books, 1995).

As far as the origin of life is concerned, the view that life is the product of a highly improbable chance event cannot be right. Life is a chemical process; therefore a deterministic one. So was its emergence, the product of a long succession of chemical events that, like all chemical events, were bound to take place under the prevailing conditions. That this process involved a very large number of successive steps reinforces this point. Something as complex as a living cell could not possibly arise in a single shot, nor even in a small number of steps. In the requisite long succession, almost every step must have had a high probability of taking place where and when it did. Otherwise the process would necessarily abort, in accordance with the laws of probability.

For these and other reasons, I conclude, in agreement with most of my fellow biochemists, that life is bound to arise, in a form not very different from its form on Earth, wherever and whenever the conditions that obtained on the Earth four billion years ago are reproduced. Most cosmologists believe that many planets with a history comparable to that of the Earth exist elsewhere in the universe. If they are right and if my views are correct, there must be many life-bearing planets in the universe. Perhaps one day technical advances will make it possible to test this statement.

What of the subsequent evolution of life, in particular the emergence of conscious, intelligent beings? Here, a dominant role of chance seems inescapable. According to Darwinian theory, overwhelmingly supported by molecular biology, every step in evolution starts with a chance mutation. The ability of the mutant to survive and reproduce under the conditions that happen to prevail -- again by chance -- is then

tested by natural selection. True. But this does not exclude inevitability. All depends on the constraints within which chance operates.

A first constraint is imposed by the sizes and structures of genomes, which limit the number of distinct mutations the genomes can undergo. This number is large in absolute value, but small relative to the number of mutations that take place, so that the odds of a given mutation occurring when and where useful are far from negligible. Many cases of drug resistance among both prokaryotes and eukaryotes attest to this fact. If such wide-ranging changes can take place in just a few decades, evolutionary times of millions of years or more surely must allow for almost every possible contingency. Contrary to an often entertained notion, evolution does not so much follow the vagaries of chance mutations -- although this may occasionally happen -- as do mutations wait, so to speak, for an opportunity to affect the course of evolution.

Existing body plans impose additional constraints on evolution. Effective mutations are restricted to the small number of genes that control the development of an organism, for example, homeotic genes; they must not disrupt the developmental process and they must be conducive to evolutionary success, or at least, compatible with it, under prevailing environmental pressures. In most instances, the changes that meet these conditions are trivial and do not alter the basic body plan. They characterize "horizontal" evolution and lead to biodiversity. It is in this kind of diversification that contingency plays its leading role, by providing some chance circumstance in which a given mutation happens to bring a selective advantage.

Much fewer, because much more constrained, are the changes that increase the complexity of body plans, that is, the changes responsible for "vertical" evolution. Life is channelled by the stringency of its internal constraints, and these are all the more stringent the more complex the developmental blueprint. The constraints no doubt leave room for developments that failed to happen on Earth but could happen elsewhere. But some directions may be compelling. In particular, the emergence of humans or, at least, of conscious, thinking beings could be much less improbable than is often intimated. The consistent increase in polyneuronal complexity in the animal line supports this view. Once neurones emerged and started creating networks (which occurred very early), there was a relentless drive toward the formation of increasingly complex networks, no doubt furthered by the strong associated selective advantages. Six million years ago, the mind of a chimpanzee was the apex of this evolutionary drive. Three million years ago, it was Lucy's. Today, it is the human mind. What it will be three or six million years hence, let alone one billion years hence -- or has already materialized elsewhere -- is anybody's guess.

From his understanding of life and mind as products of blind chance, Monod derived a view of the universe as absurd and meaningless. My own understanding of life and mind as cosmic imperatives leads me to a more optimistic and meaningful notion.

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Intron Phase Correlations and the Evolution of the Intron/Exon Structure of Genes

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Abstract

Two issues in the evolution of the intron/exon structure of genes are the role of exon shuffling and the origin of introns. Using a large database of eukaryotic intron-containing genes, we have found that there is a large excess of phase zero introns and non-random correlations between intron phases leading to an excess of symmetric exons and symmetric exon sets. These extremely significant excesses hold both for all genes and for plant or animal genes separately. We interpret these excesses as manifestations of exon shuffling and make a conservative estimate that at least 19% of the exons in the database were involved in exon shuffling, suggesting an important role for exon shuffling in evolution. Furthermore, these intron phase correlations and excesses of symmetric exons hold for those regions of eukaryotic genes that are homologous to prokaryotic genes: the ancient conserved regions. This last fact cannot be explained in terms of an insertional theory of introns but rather supports the concept that some of the introns were ancient, the exon theory of genes.

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Gene Products Implied in the Generation of Microbial Diversity

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Biological evolution ensures a steady development, long-term maintenance and diversification of life on earth. In this contribution I discuss evidence for the view that the evolutionary process does not only rely on illegitimate chance events, but is importantly influenced by genetically determined biological functions with evolutionary implications. A number of arguments taken from microbial genetics will give support to the following *thesis*:

Specific genes carried in the genome (and on accessory genetic elements) encode products that fulfil evolutionary functions (1) by generating genetic variation or (2) by limiting genetic plasticity to tolerable, but evolutionarily useful levels. In spite of this genetic determination, biological evolution is not directed.

Evolutionary processes are known to rely on mutation, selection and isolation. They can ideally be studied with haploid microorganisms which rapidly manifest phenotypic alterations due to mutation events. Bacteria and their viruses have extremely short generation times and are ideally suited for population genetic investigations, e.g. for studies on competition between mixtures of parental and mutant types submitted to various selection pressures. In addition, molecular genetic approaches can reveal the molecular nature of individual mutations, such as nucleotide substitution, small deletions and insertions, and larger DNA rearrangements. For simplicity, spontaneous mutation will here be defined as any alteration occurring to DNA sequences without an intended intervention by an investigator. More often such mutations will be detrimental, sometimes even lethal, than beneficial to the organisms. Therefore, tolerable mutation rates should be smaller than one mutation per genome and per generation. However, we should be aware that generation time is difficult to define for resting bacteria in their stationary phase. In cultures of exponentially growing *E. coli* bacteria the rate of mutagenesis is in the order of 10^{-2} new genetic alterations per cell and per generation. This results in a relatively high degree of genetic polymorphism in colonies grown from a single cell. Natural, spontaneous mutagenesis thus seriously limits the size of clones formed by genetically fully identical individuals upon propagation of bacteria. By mutations occurring in phases of rest, members of pure clones undergo further genetic diversification.

Many mechanistically different mechanisms contribute in parallel to the generation of mutations. For this discussion, we group these processes into four categories: (1) reproductive infidelity; (2) effects of environmental and internal mutagens; (3) DNA rearrangements; (4) DNA acquisition

The action of these processes on individuals in large populations generates new genetic diversity. However, overall genetic diversity is kept in balance by natural selection and, after all, the size of the biosphere, which can hold in the order of 10^{30} living cells. In addition, the efficiency of processes of the first two categories, reproductive infidelity and effects of environmental mutagens, is considerably attenuated by the activity of

various enzymatic repair processes, while DNA acquisition (category 4) encounters a number of natural limits to DNA transfer.

It is generally thought that a major source of nucleotide substitution is the occurrence of short-living tautomeric forms of the nucleotides, that are structural variants of the normal forms and present different specificity of base pairing. A mispairing which results when an incorporated tautomeric base reassumes its normal form should thus not be qualified as a mistake in the incorporation, it is rather the consequence of a statistically occurring structural variation of a biochemical compound. Many, but not all of such cases of primary infidelity are efficiently repaired before such mutations become fixed.

The following discussion on DNA rearrangements will be limited to bacterial systems. In these haploid organisms, homologous recombination cannot be attributed the same role in the generation of genomic diversity as is done for higher, sexually reproducing organisms with diploid genomes, although bacterial conjugation may sometimes substitute for the lacking recombinational reassociation of alleles from different sets of chromosomes. General recombination can also bring about major alterations in the genome structure and content by unequal crossing over at homologous sequences located at different sites in the genome.

In bacteria, enzymatic systems of site-specific recombination and of transposition are widespread. These and still other processes, often referred to as illegitimate recombination, widely contribute to genomic plasticity. Most of these processes are catalyzed by specific enzymes and thus result from the action of genetic determinants. Some of the DNA rearrangements mediated by these systems have been studied to great mechanistic details and are thus well understood. This is e.g. the case for site-specific DNA inversion and transposition of IS elements.

Site-specific DNA inversion is a source both of gene fusion and operon fusion. In the well-studied genetic flip-flop systems, one of two - or in some cases more - possible, alternative genomic arrangements is periodically assumed. This process can rapidly result in mixed populations of individuals with different phenotypic properties, if different genomic structures influence gene expression differently. Examples are the connection or disconnection of a promoter with an open reading frame or the fusion of a variable part with a constant part of a gene. The sites of crossing over in this enzymatically mediated process are consensus DNA sequences. Deviations from the consensus can still serve in DNA inversion, although with different efficiencies. Interestingly, the reaction can still take place with very low probability on DNA sequences widely diverting from the consensus. It is possible that short-term structural variations of the interacting partners, recombinase and its substrate DNA, thereby play a critical role. Many different DNA sequences can thus occasionally serve for DNA inversion. This rare use of secondary crossing over sites is thought to represent an important natural source of novel gene fusions and novel operon fusions with evolutionary relevance.

Another source of genomic rearrangements is the transpositional activity of mobile genetic elements. A number of different such "inserted sequence" elements, IS elements, reside each in a number of copies in bacterial genomes. Once in a while they undergo enzymatically mediated DNA rearrangements, which include simple

transposition, the formation of adjacent deletions and DNA inversion, as well as the cointegration of plasmids or of a plasmid with the chromosome.

Interestingly, transposition also occurs in resting bacteria. E.g., bacterial subclones kept alive for decades in a stab culture accumulate genetic polymorphism due to transposition, and the resulting diversity increases linearly with the time of storage of the bacteria. The degree of genetic diversity thus obtained depends on the target selection criteria of the participating IS elements. Depending on the IS element these criteria show different levels of sequence specificities.

Composite transposons are defined as two identical IS elements flanking one or several genes unrelated to the transposition process. Composite transposons can originate when two copies of the same IS element subsequently insert into different sites of a DNA segment. Although the two participating IS elements can still transpose alone, they sometimes transpose together as a unit with the DNA segment carried between them. This can happen intramolecularly as well as intermolecularly, e.g. to a natural gene vector such as a conjugative plasmid or a phage genome. This then opens the possibility of horizontal transfer not only of the IS element involved, but also of the gene(s) carried between the two IS elements. This has been widely documented, e.g. by the horizontal spreading of genetic determinants for antibiotic resistances. This latter example also nicely illustrates the important role played by selection for genetically altered forms, depending on changes in the environmental conditions.

The acquisition of genetic information from a donor by a receptor strain is at the basis of classical microbial genetics, i.e. (1) transformation of a receptor strain by the uptake of free DNA of a donor strain, (2) conjugation, in which donor and receptor bacteria enter in close contact and in which a conjugative plasmid serves as vector for the transfer of donor genes to recipient bacteria and (3) phage-mediated transduction, in which a viral genome serves as gene vector. In all of these processes gene transfer is followed by the establishment of the acquired genes in the receptor cell. This can be brought about by a recombination process or else by the establishment of the transferred vector together with its passenger DNA as an autonomous replicon.

As was already mentioned, various limitations reduce the efficiency of gene acquisition. These limits include the requirement of surface compatibilities for the DNA uptake, respectively the infection processes, the action of restriction-modification systems on penetrating DNA molecules, the requirements for the already described establishment step, and finally the responses given by the receptor cell to the expression of the acquired functions, which may risk to perturb the functional harmony of the host cell. This latter limitation is less severe for the acquisition of only small portions of genetic information, a condition strongly favored by the action of restriction endonucleases. These enzymes cleave larger DNA molecules into small fragments, the free ends of which are recombinogenic. DNA acquisition thus follows a strategy of acquisition in small steps.

In view of the multitude of processes contributing not only to vertical biological evolution by alterations occurring within the genome of an organism, but also to horizontal biological evolution by the occasional acquisition of small portions of foreign genetic information, the evolutionary tree should schematically be drawn with horizontal connections allowing for a gene flux between different branches.

It should be emphasised that the mechanistically different processes providing genetic diversity can only partially substitute for each other. Rather, they often fulfil different biological functions. This can be seen in a comparison of the processes of (1) nucleotide substitution resulting from infidelity upon DNA replication, (2) intragenomic DNA rearrangements and (3) DNA acquisition. The first of these processes serves in the strategy to stepwise develop new biological functions and to improve and adjust available biological functions. The second process, DNA rearrangement by any of the described enzyme-mediated recombinations, can lead to an improvement of available capacities, particularly by the fusion of different functional domains and by the fusion of expression control signals with coding sequences leading to different expression controls. Finally, the acquisition of sequence domains and motifs, of functional genes, and of clusters of genes offers the receptor cell a chance to profit of a successful development made by others.

The attribution of primarily evolutionary biological functions to DNA recombination systems acting as generators of genetic variations, to systems providing means for horizontal gene transfer, and also to natural limiters of genetic plasticity, such as mismatch repair systems or restriction-modification systems, is largely a matter of attitude of an investigator towards the object of his investigations, nature and life. Is it reasonable to assume that genetically encoded biological functions serve only to meet the needs of individual lives, by providing housekeeping and accessory functions required during the life span of the organisms? Alternatively, one can consider the process of a steady biological evolution as equally important for both the past and the future development of a multitude of life forms able to adapt to changing living conditions and to withstand contra-selective forces.

What has been described here for bacteria with a few selected examples might well have a more general validity. Analogous genetic variation generators act also in the development of antigenic variations in higher animals. I am aware that a very strict subdivision of biological functions into (1) those serving to maintain intact the cellular physiology, (2) those others serving for developmental purposes of multicellular organisms and (3) still others serving for the biological evolution of populations would not correspond to reality. Indeed, some genetically determined products serve for more than one of these purposes. But some specific gene products may very well primarily be used for biological evolution and they may also have been in the long term selected for this purpose. Since the selection of genetic variation generators is made mostly on variations concerning gene products which are not determined by the evolutionary genes in question, this kind of group selection - or selection between bacterial clones - must be exerted at the population level and must depend on the presence of enough appropriate variants to fulfil the selective needs. This can help to explain why in spite of the genetic determination of the evolutionary process, the direction of evolution remains undetermined and is a matter of interplay between the aleatoric occurrence of particular mutations and the action of natural selection exerted on the sustained mixed populations of organisms.

Additional readings

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Diversification of Allelic Specificities

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Individuality and Allelic Polymorphism.

For over half a century, geneticists have believed that the uniqueness of the individual could be explained by the generation (through recombination and reassortment) of a vast number of different genotypes. This can only happen in a population whose members differ among themselves in many genes. If we consider genes (A, B, C, D, E) that have at least one allelic alternative (a, b, c, d, e), then for each gene there are three alternative genotypes (AA, Aa, aa). For n genes, there are then 3^n possible genotypes. Early on, the number of identified human genes was large enough to generate a number of genotypes that exceeds the size of the total human population. Of course, this primitive calculation does not prove that each human being is genetically unique. Many genes have been identified through rare alleles; and genotypes where rare alleles are compounded will scarcely occur. Also, the population does not mate and mix at random; the degree of nonrandomness being still a topic of current debate.

Molecular genetics has clarified the question in one sense and perhaps muddied the waters in another. DNA sequencing showed a diversity far greater than was implied by known genetic variation. This established the genetic uniqueness of the individual beyond reasonable doubt. This fact is useful for lawyers in rape or paternity cases, but of less obvious interest to the biologist. This is because much of the observed DNA variation constitutes silent changes that have no effect on the phenotype of the individual. Biologists would like to know why individuals are unique in how they look, feel or act; and seek a genetic explanation for this uniqueness. Thus attention can be focused on allelic alternatives that (1) affect phenotype and (2) are reasonably frequent in the population. The latter condition is most easily satisfied where selection favors a stable polymorphism, with two or more alleles present at equilibrium. Thus special attention attaches to such stable polymorphisms.

Lambdoid phages. I call attention here to the relevance of prokaryotes (specifically the lambdoid bacteriophages) to the study of genetic polymorphisms. Numerous natural relatives of phage l have been identified. They share a common genetic map, can recombine in the laboratory, and seem to do so in nature. If we consider all of them as members of one recombining population, the population is functionally polymorphic in many genes; determining traits such as repression, antitermination, replication and integration. Each of these is represented by several different functional specificities, with corresponding divergence of DNA sequence. In the natural population, such specificities are mixed and matched to give the same kind of combinatorial array of genotypes as in the human population. (1, 2, 3). (Because replication is primarily asexual, the "individuals" in this case are individual lineages rather than individual phage particles.)

Lambda integrase. Available information (not presented in this abstract) allows some conclusions to be drawn about the evolutionary relationships among the integrases of

different lambdoid phages. Integrase is the protein that causes insertion of phage DNA into specific sites on the DNA of the bacterial host. The integration reaction can be separated into two steps: formation of a multiprotein-DNA complex, and the actual exchange of DNA strands within this complex. Comparative analysis indicates that the first step has shown substantial evolutionary divergence with respect to the location of binding sites on the DNA; in the second step, the DNA relations seem to be more conserved, but major changes in sequence recognition have taken place (4). How relevant the details of this case study are to the main issues of this essay remains to be decided. The overall goal of the work is to understand how proteins with different specificities arose from a common ancestor.

Uniqueness and Universality.

The individual lambdoid phage is unique for some of the same reasons that the individual human being is unique - because of stable polymorphisms within a recombining population. Although perhaps not universal, this basis for uniqueness is thus widespread in the biological world.

What maintains stable polymorphisms? The subject has been extensively analyzed by population geneticists. Some of the possible mechanisms (such as heterozygote superiority or heterogeneity among offspring of heterozygotes) are only applicable to diploid organisms, and as generally analyzed to those where (as in humans) recombination and replication and obligatorily coupled. Such mechanisms probably account for some polymorphisms, but the phage example underscores the fact that they are not the only important factors. Another mechanism (operable in diploids as well as haploids) is frequency dependent selection, where any rare type is selected because it is rare. A plausible argument can be made that this applies to phage specificities for characteristics such as insertion or repression. This is basically because individuals of the same specificity type can exclude one another, whereas different types coexist, in the same cell; so individuals of a rare type are seldom excluded, whereas those of a common type frequently are. The advantage of rareness causes rare types to become more common, thereby losing their advantage.

Stable Polymorphism and Evolution.

Stable polymorphisms contribute to variation among individuals within a population (or species) and, when reassorted by recombination, may suffice to give each individual a unique identity. The biological world is composed of many different species. How does the uniqueness of each species relate to the uniqueness of individuals within a species?

This is a fundamental evolutionary question, which (along with related questions) has been analyzed extensively (5). Clearly natural selection must act on existing variation. However, the new types or species may depend on rare pivotal events unrelated to the major sources of intrapopulational variation. Two types of such events were discussed in this meeting: exon shuffling (by Gilbert) and interspecific gene transfers (by Arber). To be sure the issue is clear, let us consider the possible fate of a new gene created by exon shuffling. Most such new genes will be disadvantageous, and selection will eliminate them; others will be neutral, and will increase only through the vagaries of genetic drift; a few will be advantageous and increase through selection.

The typical advantageous mutation will increase without limit; i.e., in a panmitic population, it will eventually displace its predecessors throughout the population. This is an evolutionary change for the species, and might constitute one step in the creation of a new species. The important point in the present context is that the process does not increase the variability of the population. Before the new mutation arose, all individuals in the populations were alike at the locus in question; at the end of the process, all individuals are again alike, because they all carry the mutation. Only transiently does this event increase the heterogeneity within the population. The only new mutations that permanently increase the heterogeneity (and therefore contribute to the uniqueness of individuals within the population) are those which, for some reason such as frequency-dependent selection, lead to stable polymorphisms.

Prokaryotic Research.

For several generations, research on prokaryotes has been useful in revealing basic mechanisms of processes that were hard to analyze in higher eukaryotes. They still serve that purpose, although the techniques that flowed out of prokaryotic research have rendered many questions directly accessible in macroorganisms. Mechanistic studies of growth and replication are not the only areas where prokaryotic research can contribute. In the future, prokaryotes may play an increasing role in testing the universality (or defining the limits) of populational and evolutionary principles.

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The Distribution of Genes in the Human Genome

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The term *genome* was coined three quarters of a century ago (Winkler, 1920) to designate the haploid chromosome set. While current textbooks of Molecular Biology do not yet go beyond the purely operational definition of the genome as the sum total of genes and of intergenic sequences, many molecular biologists have been thinking for some time that the genome is more than the sum of its parts. This concept is, however, still far from an acceptable definition of the genome, since this can only be based on specific properties. The properties of the genome that we have discovered and that will be briefly outlined here are the compositional patterns of DNA fragments (or molecules) and of coding sequences, the compositional correlations between coding and non-coding sequences and, above all, the gene distribution and its associated functional properties.

The mammalian genomes are mosaics of *isochores* (see Fig. 1), namely of long (>300 Kb) DNA segments that are homogeneous in base composition and range from 30 to 60% GC (Thiery *et al.*, 1976; Macaya *et al.*, 1976). This is an extremely wide range, almost as wide as that covered by all bacterial DNAs (25-72% GC). In the human genome, isochores can be assigned to two GC-poor families (L1 and L2) representing 2/3 of the genome, and to three GC-rich families (H1, H2 and H3) forming the remaining 1/3 (Fig. 2).

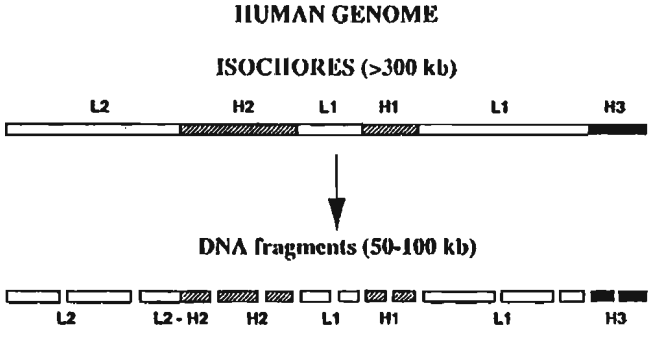


Fig. 1 - Scheme of the isochore organization of the human genome. This genome, which is a typical mammalian genome, is a mosaic of large (>300 Kb) DNA segments, the isochores. These are compositionally homogeneous (above a size of 3 Kb) and can be subdivided into a small number of families, GC-poor (L1 and L2), GC-rich (H1) and (H2), and very GC-rich (H3). The GC-range of the isochores from the human genome is 30-60% (from Bernardi, 1993).

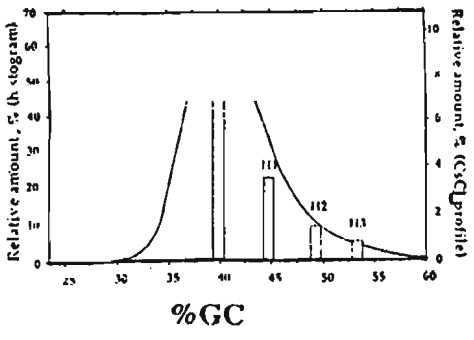


Fig. 2 - Histogram of the isochore families from the human genome. The relative amounts of major DNA components derived from isochore families L (*i.e.*, L1 + L2), H1, H2, H3 (see Saccone et al., 1993) are superimposed on the CsCl profile of human DNA (from Mouchiroud et al. 1991).

The *compositional distributions* of large (>100 Kb) genome fragments, such as those forming routine DNA preparations, of exons (and particularly of their third codon positions) and of introns represent *compositional patterns* (Bernardi et al., 1985; Mouchiroud *et al.*, 1987). These correspond to *genome phenotypes* (Bernardi and Bernardi, 1986), in that they differ characteristically not only between cold- and warm-blooded vertebrates, but also between mammals and birds and even between murids and most other mammals (see Figs. 3 and 4).

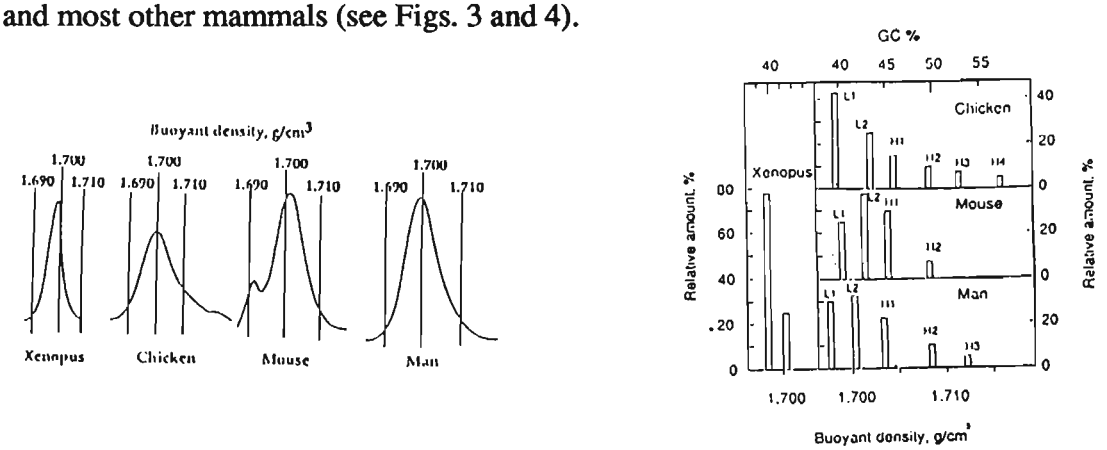


Fig. 3- Compositional patterns of vertebrate gênomes. Left : CsCl profiles of DNAs from *Xenopus*, chicken, mouse and man (from Thiery *et al.*, 1976). Right : Histograms showing the relative amounts, modal buoyant densities and GC levels of the major DNA components from *Xenopus*, chicken, mouse and man, as estimated after fractionation of DNA by preparative density gradient in the presence of a sequence-specific DNA ligand (Ag^+ or BAMD; BAMD is bis (acetato mercuri méthyl) dioxane). The major DNA components are the families of large DNA fragments (see Fig. 1) derived from different isochore families. Satellite and minor DNA components (such as rDNA) are not shown in these histograms (from Bernardi, 1993).

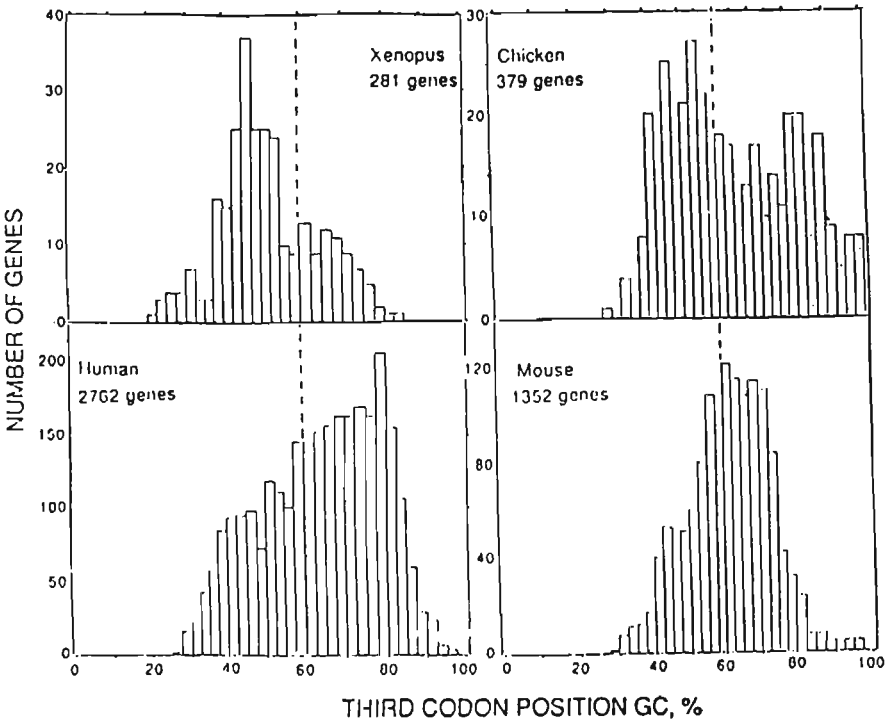


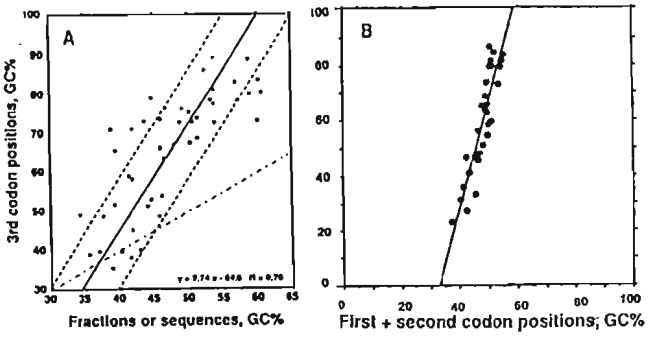
Fig. 4 - Compositional distribution of third codon positions from vertebrate genes. The number of genes taken into account is indicated. A 2.5% GC window was used. The broken line at 60% GC is shown to provide a reference (from Bernardi, 1993).

Compositional correlations (Bernardi et al., 1985) exist between exons (and their codon positions) and isochores (Fig. 5), as well as between exons and introns (Aïssani *et al.*, 1991). These correlations concern, therefore, coding and non-coding sequences and are not trivial since coding sequences only make up about 3% of the genome, whereas non-coding sequences correspond to 97% of the genome. The compositional correlations represent a *genomic code* (Bernardi, 1990; 1993). It should be noted that a *universal correlation* holds among GC levels of codon positions (third positions against first and/or second positions). This is apparently due to compositional constraints working in the same direction (towards GC or AT), although to different extents, on different codon positions, as well as on the isochores.

Fig. 5

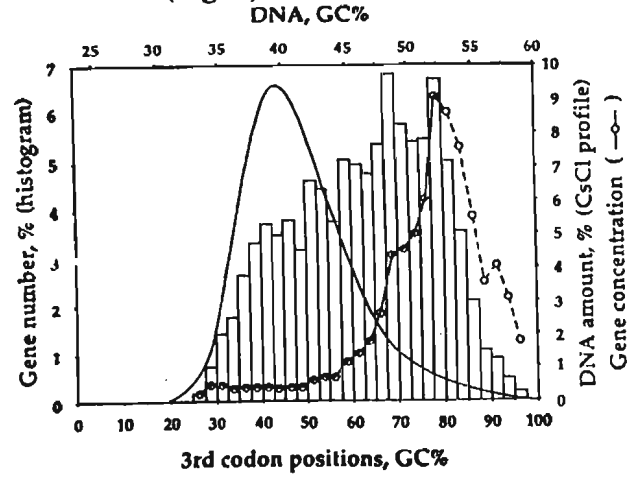
A. GC levels of third codon positions from human genes are plotted against the GC levels of DNA fractions (dots) or extended sequences (circles) in which the genes are located. The correlation coefficient and slope are indicated. The dash-and-point line is the diagonal line (slope = 1). GC levels of third codon positions would fall on this line if they were identical to GC levels of surrounding DNA. The broken lines indicate a ±5% GC range around the slope (from Mouchiroud *et al.*, 1991).

B. Plot of GC levels of third codon positions of genes from prokaryotic and eukaryotic genomes are plotted against GC levels of first + second positions. All values are averaged per genome (or per genome compartment, in the case of compositionally compartmentalized genomes) (from D'Onofrio and Bernardi, 1992).



The compositional correlations between GC3 (the GC level of third codon positions) and isochore GC have a practical interest in that they allowed us to position the coding sequence histogram of Fig. 4 relative to the CsCl profile of Fig. 3 and to assess the *gene distribution* in the human genome (Mouchiroud *et al.*, 1991; Bernardi, 1993). In fact, if one divides the relative number of genes per histogram bar by the corresponding relative amount of DNA, one can see that gene concentration is low and constant in GC-poor isochores, increases with increasing GC in isochore familes H1 and H2, and reaches a maximum in isochore family H3, which exhibits at least a 20-fold higher gene concentration compared to GC-poor isochores (Fig. 6).

Fig. 6 - Profile of gene concentration in the human genome as obtained by dividing the relative amounts of genes in each 2.5% GC interval of the histogram by the corresponding relative amounts of DNA deduced from the CsCl profile. The apparent decrease in gene concentration for very high GC values (broken line) is due to the presence of rDNA in that region. The last concentration values are uncertain because they correspond to very low amounts of DNA (from G. Bernardi, 1993).



The H3 isochore family has been called the *human genome core* (Bernardi, 1993), because if corresponds to the functionally most significant part of the human genome. Indeed, H3 isochore family is not only endowed with the highest gene (and CpG island) concentration, but also with an open chromatin structure (as witnessed by the accessibility to DNases, as well as by the scarcity of histone H1, the acetylation of histones H3 and H4 and wider nucleosome spacing; Tazi and Bird, 1991), with the highest transcription and recombination levels and with the earliest replication timing. The genes of the genome core have the highest GC_3 levels relative to their flanking sequences, have the shortest exons and introns (Duret *et al.*, 1995), exhibit an extreme codon usage and encode proteins characterized by amino acid frequencies differing from those of proteins encoded by GC-poor isochores (D'Onofrio *et al.*, 1991).

The human genome core is located in T(elomeric)-bands (Saccone *et al.*, 1992), which are essentially formed by GC-rich isochores (mainly of the H2 and H3 families). In contrast, R'-bands, namely the R(everse) bands exclusive of T-bands, comprise both GC-rich isochores (of the H1 family) and GC-poor isochores. Finally, G(iemsa) bands are formed almost exclusively by GC-poor isochores (Saccone *et al.*, 1993; see Fig. 7). The difference in GC level between G-bands and T-bands is about 15%. About 20% of genes are present in G-bands and about 80% in R-bands (60% of them in T-bands). The location of a majority of genes in T-bands is of interest in view of the association of telomeres with the nuclear matrix and envelope (de Lange, 1992).

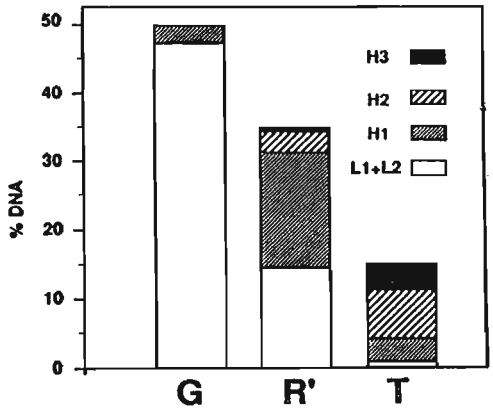


Fig. 7 - A scheme of the relative amounts of isochore families L1 + L2, H1, H2 and H3 in G-bands, R'-bands and T-bands; R'-bands are R-bands exclusive of T-bands (from Saccone et al., 1993).

To sum up, what has been outlined here is that the human genome is characterized by a specific compositional pattern of the isochores that form it, by compositional correlations between coding and non-coding sequences and by a specific gene distribution.

The compositional pattern of the human genome, which is typical of those of most mammals and similar to those of birds, is strikingly different from the compositional patterns of cold-blooded vertebrates which exhibit a much lower degree of heterogeneity and are not accompanied by R-banding in their metaphase chromosomes These different genome phenotypes of warm- *versus* cold-blooded vertebrates are due to compositional changes that are apparently due to adaptive regions (see below).

The compositional correlations indicate the existence of a genomic code implying similar constraints, although to different extents, on coding and non-coding sequences.

In turn, if the compositional patterns are largely due to adaptive reasons, non-coding sequences are not junk DNA, but must fulfill some functional role.

Finally, as far as gene distribution is concerned, two remarks should be made. First, the gene distribution reported for the human genome seems to have been conserved in evolution, genes showing their highest concentration in the GC-richest isochores of all vertebrates (Bernardi, 1993). Second, the gene-poor, GC-poor isochores have undergone little or no compositional change in vertebrates genomes. The gene-rich, GC-rich isochores are those which underwent compositional changes in evolution. More detailed discussions on the evolutionary implications of the results reported can be found in Bernardi (1993), Cacciò *et al.* (1995) and Zoubak *et al.* (1995).

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Transfer of a Genetic Reflex Epilepsy

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A reflex epileptic seizure is one which does not occur spontaneously, it is provoked by precise factors having a triggering or favouring role. It is considered as the consequence of a generalized hyperexcitability of the whole brain or of certain of its circuits. This hyperexcitability may be the result of one or several genetic abnormalities, but no specific data exist.

Reflex epilepsy occurs in Man and in numerous animal species. While in Man as in *Papio papio* the best known stimulation to induce reflex epilepsy is intermittent light stimulation (ILS) (for Man see reviews by Newmark and Penry, 1979, and Naquet and Poncet-Ramade, 1982; for *Papio papio* see Killam *et al.*, 1967 ; Menini and Naquet, 1986; Naquet and Meldrum, 1986; Silva-Barrat and Menini, 1990), in rodents, an audiogenic epilepsy is one of the models of generalized reflex epilepsy which is most studied (see Faingold, 1988; Jobe *et al*, 1993; Le Gal la Salle and Naquet, 1990; Marescaux et al., 1992).

Photogenic epilepsy was known from the end of the sixties (Crawford, 1970) in the chicken, but the audiogenic one was described only recently (Fadlallah *et al.*, 1995; Naquet *et al.*, 1994). In Fayoumi epileptic chicken (Fepi), the seizure symptoms differ according to the type of stimulation. An alerting reaction obtained with initiation of stimuli is followed by either myoclonia of the neck under ILS or a violent running fit with intense sound stimulation (ISS). These preconvulsive symptoms are followed by generalized convulsions in both cases.

All Fepis show an abnormal paroxysmal interictal EEG. Under ILS the seizure is characterized by a desynchronisation often followed by a depression of the rhythm. After ISS only the desynchronization is present. From the clinical and EEG points of view these seizures are completely different from those brought on by *Metrazol* in the Fepi (Guy *et al,* 1994).

Electrophysiological studies using microelectrodes have shown that at the beginning of a seizure under ILS certain neurones discharge in bursts of high frequency in the mesencephalon. On the wulst, as in the other prosencephalic structures, a cessation of the interictal neuronal bursting discharge which is correlated with the spike of the EEG is seen at the beginning of the seizure.

Knowing these characteristics of reflex epilepsy of the chicken and in view of the possibility of working on their eggs, this model was chosen to try to see if it was possible to transfer the reflex epilepsy from a Fepi embryo to a normal one.

To demonstrate this, the technique described by the group of N. Le Douarin based on the embryonic construction of chimera from Guinea fowl and chicken seemed to be the best (Balaban *et al.*, 1988). To study this reflex epilepsy the chimeras were constructed " *in ovo* " the second day of incubation by an exchange of brain primordia of a Fepi and a normal chicken embryo (Teillet *et al*, 1991). Different types of chimeras were constructed on the proposition that the neuronal circuits implicated in photogenic epilepsy can be dissociated from those involved in audiogenic epilepsy. The results were the following:

a. The chimeras with a Fepi prosencephalon had an interictal EEG analogous to that of the Fepi. They also showed ILS and ISS induced behaviour and EEG arousal reaction, but no preconvulsive symptoms.

b. The chimeras with a Fepi pro- and mesencephalon presented seizures typical of Fepis both for ILS and ISS. The interictal and ictal EEG were analogous to those of Fepis.

c. The chimeras with a Fepi mesencephalon had a normal interictal EEG. Under ILS they presented myoclonia of the neck at the frequency of the ILS which stopped with the ILS. Under ISS they presented complete seizures including convulsions which persisted after cessation of ISS.

d. The chimeras with a Fepi rhombencephalon had normal interictal EEG and did not react to ILS or ISS.

The differences of circuits utilized by the seizures induced by ILS or ISS were confirmed by examining *c*-fos expression .

Expression of the proto-oncogene *c*-fos differed on "western blots" carried out on the Fepi brain having had a seizure under ILS and those having had one under ISS. In both cases there was no expression in the hippocampus contrary to what is found following a seizure provoked by *Metrazol*. The differences were seen in the mesencephalic structures: after ILS, *c*-fos was expressed in the rostral parts of the mesencephalon while after ISS it was in the caudal parts where expression was the most prominent.

These results have demonstrated that it is possible to transfer a genetic reflex epilepsy from one embryo to another. In addition they have shown that there exist at least two types of reflex epilepsy that may be transferred from the Fepi chicken, one induced by ILS, the other by ISS. But if both start at the level of the brain stem, the circuits utilized by the two types of seizures are not superimposable and the structures responsible for triggering the seizure are specific and depend on the type of stimulus (for example, to obtain the complete pattern of the seizure in a chimera a Fepi prosencephalon is necessary under ILS and not under ISS). Finally the generator of the motor seizure is located at the level of the mesencephalon for both types

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Gene Therapy

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Gene therapy, that is to say the therapeutic use of genes as drugs, has two main fields of application: hereditary diseases, and any disease in which a therapeutic protein can, in principle, be replaced by its structural gene, or cDNA.

Genes can be transferred through transplantation, mainly auto-transplantation of *ex vivo* genetically modified cells, or by direct administration, *in vivo*, using viral or non viral vectors. Four viral vectors have hitherto been tested for their efficiency as gene therapy vectors : adenovirus, retrovirus, adeno-associated virus and *Herpes simplex* virus, adenoviral and retroviral vectors being the most intensively characterized. Cationic liposomes seem to be the most promising type of non-viral vector. The indications of these different types of vectors are very different. DNA transferred by adenoviral vectors of liposomes remains extrachromosomal; therefore, these vectors allow for a prolonged transfer and expression in dividing cells only. DNA transferred by retroviral vectors is integrated into chromosomes, but the vector is efficient on dividing cells, only. In these cells, a stable expression can be expected, provided that authentical stem cells have been infected.

Different types of cell can be used for gene therapy through autotransplantation of cells genetically modified *ex vivo*. Sometimes, the nature of the cells is imposed by the disease to be treated, for instance bone marrow stem cells for hematologic diseases or immunodeficiencies, or hepatocytes for liver metabolic diseases. Sometimes the therapeutic transgene product is active extra-cellularly and at a distance from the secretion site, which is the case for hormones, cytokines, clotting factors, etc... In these cases, the most convenient cells can be used : myoblasts, endothelial cells, fibroblasts, etc. ..Genetically modified fibroblasts can be retransplanted under the form of organoids, that are artificial neo-organs specialized in synthesizing and delivering the product of a therapeutic transgene. Organoids are composed of synthetic fibers coated with collagen and acidic FGF and then seeded with genetically modified cells; up to now, they have been implanted into the abdominal cavity, but could probably be used as bio-pumps in other sites.

The direct administration of a therapeutic transgene *in vivo* is indispensable when the cells that should be corrected are not identified, are disseminated throughout the organism or cannot be cultured *ex vivo,* then retransplanted, which is the case, for instance, for myotubes, nervous system cells and tracheobronchial cells. Adenoviral vectors and liposomes have been especially tested as *in vivo* gene therapy vectors.

Successful gene therapy has been used in animal models of human diseases : liver metabolic diseases, lysosomal diseases, muscular dystrophy, neurological diseases, cancer, etc...About 100 phase I-II clinical trials in human beings have been authorized around the world, but the successes remain limited, except in the case of children suffering a combined immunodeficiency due to ADA defect and , to a lesser degree, of young woman with familial hypercholesterolimia. The main difficulties encountered in

the development of gene therapy are related to the efficiency of gene delivery, the immune response against vector or product of the transgene, level and stability of the expression of the transgene.

In some cases, a transient transfer and expression of the transgene could be sufficient, for instance in cancer. The goals of gene therapy of cancer can be to increase the immune response against cancerous cells, or to transfer into these cells a gene encoding a drug-activating enzyme, thus sensitizing the cells to administration of the prodrug. The success of these strategies will depend on improving the immunotherpay strategies or the efficiency of the antioncogenic or cytotoxic genes transferred.

In conclusion, although the different tools used to day for gene therapy should be considerably improved before genes become a common form of drug, it is clear that a new versatile and potentially powerful type of treatment, and then a hope, are born.

Gene Therapy: the Artificial May Improve the Natural (As for Chromosomes)

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Since the advent of recombinant DNA and genetic engineering in the early '70s, it has been common to hear statements relative to rate and extent of the impact of those techniques on biomedical sciences and in general on the biological world. After nearly a quarter of a century it is time to attempt an evaluation.

As for the biomedical sciences, there is general consensus that the achievements in the biosynthesis of polypeptides of pharmacological importance are by and large satisfactory; on the other hand, even on this synthetic aspect one should not indulge in excessive complacency, since only a limited success has been obtained in the attempts either to induce the synthesis of valuable foreign proteins by entire and complex hosts, such as mammals and birds, or to manipulate living cells to produce secondary metabolites. Also in diagnosis successes have been relevant: the power of genetic diagnostics and modelling, strengthened by the development of transgenic animals and gene knock-out, has occasionally outsmarted actual therapy, to the point that some people, in those cases where disease symptoms appear late and therapy is unavailable as in Huntington chorea, discourage the use of presymptomatic diagnosis, especially on children (1). The substantial failure of the original but somehow naive concept that a monogenic dominant defect would be amenable to molecular correction by simply cutting the defective allele away from the genome of the affected cells and replacing it with a normal sequence (2), has convincingly demonstrated that, differently from in vitro reactions, for *in vivo* genetic recombination what is true for *Escherichia coli* or even for *Saccharomyces cerevisiae* is not necessarily true for *Homo sapiens*, and not even for *Mus musculus*. Experimental evidence seems to indicate that, compared to the microbial cells, in mammals there is a bias against homologous recombination and a preference for terminal, and generally random, insertion for introduced DNA sequences (3).

It is not the purpose of these introductory remarks to outline even a short historical account of gene therapy, but rather to underline the facets of its development and expose reflections of an outsider, leaving the task of a more informative revue of the field to the experts who will intervene later (4).

Unquestionably, even if still young, gene therapy of somatic cells is no longer considered an esoteric practice of futuristic medicine, suspected of invasive reverberations on generations to come. Indeed manipulations of germ line cells seems at present unfeasible, both on scientific and political grounds. Still the list of the mapped human clinical disorders had more than 500 entries by 1991 (1): by then the diseases practically amenable to gene therapy were already at least a dozen (5).

A novel and important concept, strengthened by a few but significant experimental results, is that gene therapy is better defined, rather than by the genetic nature of the

target diseases, by the instrumental use of genetic material and/or techniques. The promising field of vaccines, especially against tumors, is a good example (6). Along the line of a better definition, efforts should not be spared to emphasize the vital distinction between gene therapy on one side and, on the other, eugenics (with its racial implications) and procreatics (with the exploitation of the twenty or so variations on the theme of fertilization, mostly for the gratification of would-be parents, operators, lawyers etc. rather than of the resulting new human being). Abuses of these potentials should be considered with attention and probably a moratorium should be imposed on them as well as on germ cells interventions, similar to what was agreed upon by the practitioners themselves at Asilomar in 1975 for recombinant DNA. This until animal systems, especially anthropomorphic primates (due to their similarity to humans and also to the risk of their extinction) can prove the safety and validity of these interventions.

But with all the advantages deriving from a more precise definition, there remains a variety of practical problems to be solved: thus it is believed that in order to become effective forms of cure, DNA- and RNA-mediated interventions against genetic disorders necessitate to overcome several fundamental draw backs (5): (i) gene vectors and protocols are needed which allow genetic material to be injected directly into the patients, *in vivo*, rather than into their cells removed *ex vivo*, and persist intracellularly in the original form of introduction, be it episomal, as with adenovirus-based constructs, or integrative, as with retroviruses; (ii) the use of polynucleotide sequences themselves as therapeutic agents, rather than through their protein gene products, needs to be improved, as e. g. in the case of antisense RNA (8) and ribozymes (9); (iii) the necessity of precisely targeting the correct allele in the exact place of the "disease" one, or at least in a non critical region of the recipient genome or chromosome; (iv) the size of the DNA fragment to be transplanted should better contain exons and introns, plus the genetic signals necessary for its tissue- or stage-controlled expression. This latter requirement would seem even more stringent if the transplanted gene were to express metabolically complex products rather than a single and necessarily short (less than 30 Kd) polypeptide, as in with cDNA as templates. Also in view of the limited capacity and the questionable safety of the viral and retroviral vectors, innovations in gene delivery are urgent.

Among the various candidates increasing attention is being given to MAC, or Mammalian Artificial Chromosomes (7). Their development is contingent upon a more thorough characterization of both the centromere of high eucaryotes chromosomes and of the sequences deputed to the initiation and control of DNA replication. The availability of MAC would help unravel several basic but still rather obscure questions relative to the organization of the genetic endowment of higher eucaryotes, such as the mosaic structure of chromosomes (10), and the relevance of chromosomes to genes and genomes, as highlighted by the muntjak paradox (11): two phenotypically similar but distinct *cervidae* sub-families package their genomes, one in 46 diploid chromosomes (the M. reevesi), and the other in 8 (the M. muntjak). On a more health-related key a MAC may help clarifying some of the issues related to the lethal or at least devastating effects of errors in humans due to the number of chromosome as evidenced for all the except the Y (12), and possibly some of the complex transactions involving replication, expression, recombination and segregation of mammalian chromosomes.

Two considerations converge toward the usefulness of a MAC.

The first has experimental value: extra chromosomes are not necessarily detrimental to the organism, but rather it seems that the dosage of specific genes, and not that of chromosomes, may be the culprit of the associated pathologies; thus, a MAC should be assembled so that the number and quality of the genes can be precisely controlled.

The other is historical in flavour: artificial or synthetic genetic elements have so far provided outstanding contributions to the development of molecular biology, as shown by the synthetic repetitive mRNAs produced first by Ochoa and Nirenberg, and then by the full codon set produced by Khorana, and later again by Khorana with the assembly of the first totally synthetic genes. More recently the use of the yeast artificial chromosomes, or YACs, has impressed an unpredicted acceleration to the analysis of complex genomes. It is probable that the eventual availability of a MAC will catalyse scientific as well as practical effects of a similar magnitude, and possibly refine the therapeutic and ameliorative use of genetic intervention in cells and organisms (13).

In conclusion and in due respect of the general scope of the Symposium, it should be stressed that recombinant DNA, in contrast to what is often raised as a criticism can indeed contribute to the uniqueness of biological individuals, in view of its potential to extend beyond species boundaries the effects of one of the main sources of genetic variability, natural recombination. These effects are possible because of the universality of the genetic system and the emerging technologies, but may unnecessarily degenerate because of short-sighted and excessive commercialization of the cloning potentials.

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Role of Non-Coding Sequences in the Creation of Uniqueness and Biodiversity

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Coding sequences represent the most conservative part of the eukaryotic genome. At the same time they comprise only a few per cent of the total geonmic sequences. Other sequences located in spacers between genes in introns have no ovbious functions and sometimes designated as "junk DNA". The latter consists of mobile elements belonging to different classes and non-mobile sequences among which are mini- and micro-satellites. This fraction of genome is much less conservative than coding sequences. It was first shown by Ilyin et al. that repetitive sequences in Drosophila melanogasters have quite a variable location on chromosomes. Now it is clear that the individuals of the same species dramatically differ one from another by distribution and properties, size, etc., of many sequences belonging to "junk DNA". The question arises whether these sequences may influences the expression of coding ones and, in this way, to create phenotypic differences between such individuals. The report describes several examples for this obtained in the Institute of Gene Biology.

First are the experiments in the mtsl gene which was shown to be involved in the control of the tumor metastasis. Usually this gene is not re-arranged in tumor cells. However, in one mouse tumor cell line, two mRNAs encoding by mtsl were detected. Cloning of genomic and cDNA copies of mts1 gene from these cells showed that one mts1 allele is normal while another contains an insertion of IAP mobile elements resulting in the formation of chimeric mRNA containing IAP LTR and mts1 gene. The mutant mts 1 gene is controlled by IAP LTR and transcribed at a much higher level than its normal counterpart. Thus, insertion of mobile element switched the mts1 gene on, changing the phenotype of cell line. There are many examples in literature about strong influence of mobile elements on expression of many genes, in particular oncogenes.

Another less trivial example is the presence of microsatellite sequence in the first intron of the mts1 gene. This microsatellite was shown to interact with specific nuclear protein, as was shown by *in vitro* and *in vivo* footprinting. The presence of a micro-satellite creates a small but significant decrease in mts1 transcription. Thus, micro-satellite and mini-satellite can also modulate to some extent gene expression and create individual differences between representatives of the same species.

Sometimes mobile elements induce more complex effects, for example, DNA bridging. The group of control of genetic processes studied several mutations in D. melanogaster and found that the presence of mobile elements (gypsy, jockey and others) might create interactions between regions located at a large distance (from dozens of Kbase pairs to few Megabase pairs). Transcription of genes is strongly modulated by such interaction.

Finally, a series of mutations was obtained in D. melanogaster characterized by some unusual properties. With a high frequency, the mutant alleles convert one to another giving reversible pairs or groups of mutations. Also, irreversible changes take place,

and as a result, a large number of phenotypes appeared. For example, more than one hundred different phenotypes were recorded for the yellow incus. The mutations were induced by chimeric mobile elements consisting of two identical defective P element copies at the ends and genomic fragments from different regions in between. Mutation changes usually depend on the changes in the internal part of chimeric elements. Such mutations creating genotypic (phenotypic) diversity may be important for evolution.

Thus, mobile elements and other types of junk DNA create phenotypic uniqueness of individual and inter-species biodiversity. Therefore, they are important both for population survival and the evolutionary process.

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Genetic Diversity and Molecular Evolution of Pathogenic Viruses

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We have been working on the molecular evolution of pathogenic viruses, in order to elucidate the elementary processes of evolutionary mechanisms at the molecular level (1, 2). In particular, we collected nucleotide sequence data, as many as possible, of the pathogenic viruses such as HIV (human immunodeficiency virus) , HCV (hepatitis C virus), HBV (hepatitis B virus), HTLV (human T-cell leukaemia virus), Influenza A virus and others from carriers and patients over the world. Using these data, we have constructed phylogenetic trees for the viral isolates, and we successfully inferred from them the origin of the viruses and their geographical routes of infection. This kind of pathogenic viruses become a powerful tool for studying viral evolution.

With the aim of elucidating the evolutionary mechanism of pathogenic viruses at the molecular level, we now pay our attention to population dynamics of HIV and HCV within a human body as a host. Both HIV and HCV have an evolutionary rate approximately million times greater than human DNA genes. In fact, the rates of nucleotide substitution for the genes of HIV and HCV have been estimated to be of the order of 10^{-3}/nucleotide site/year. Thus, it is possible to trace the evolutionary divergence of these viruses within a single host during an observable period of time. It also enabled us to see the evolutionary response of the viruses to the immunological pressure from the host.

For HIV, we constructed a phylogenetic tree of the viral isolates which were obtained periodically since initial infection from each of four HIV carriers or AIDS patients. The tree was constructed by use of the nucleotide sequence data for the V3 regions and its surrounding regions of the *en* gene. The tree obtained clearly showed that the dominant type of the virus isolates always changed with the time. It suggests that HIV may be escaping from the immunological pressure of the host by changing continuously its dominant type within a human body.

For HCV, we performed the same approach as that of HIV. In particular, we constructed phylogenetic trees of HCV isolates which were obtained periodically from a human body, in order to examine the relationship between the genetic diversity of the HCV isolates and interferon treatment. For some patients, the genetic diversity of the HCV isolates was drastically reduced immediately after the treatment of the patients. After a few months, however, one of minor types of viruses which survived from the pressure by interferon became a source of resumption of genetic variability. These observations imply that within-a-host evolution of the pathogenic viruses can be actually useful for tracing the evolutionary pathways of the viruses within a single host.

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Some Physical Constraints of Molecular Evolution.

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A prerequisite for an understanding of the evolution of biology is the knowledge of the prebiotic availability of biological building-stones as well as their general interaction mechanisms and localisations. Based on the work of S. Miller, L. Orgel, A. Eschenmoser and others, we know that primary building stones such as aminoacids, purins and pyrimidines, carbohydrates, porphyrines and others are spontaneously generated and generally available. The work of Manfred Eigen and Peter Schuster [1] led to an understanding of the importance of the autocatalytic reaction cycle for amplification and molecular selection processes intrinsically linked to metabolic conversion. Indeed, evolution cannot proceed under equilibrium conditions, under which the directionality of turnover is lost, but needs a permanent flow of energy passing through the reaction systems. As shown by Schroedinger, the energy flow creates the conditions for strong deviation from thermodynamic equilibrium. This results in the phenomena of selforganization, the parameter of which are set by prebiotic constraints, and opens the possibility of autonomous pattern formation. Only the flow of energy converts molecular chaos into macroscopic order and coherence. The transition from equilibrium to nonequilibrium regimes passes a bifurcation point. Technically, a bifurcation generates solutions displaying broken symmetry. Under prebiotic conditions, energy input might have been realized by anaerobic substrate input, by irradiation and photosynthesis and later by respiration. In the nonequilibrium regime, the nonlinear character of the general dynamics - due to autocatalysis and a manifold of interacting combinations - yield a great number of complex dynamic states, such as oscillatory and aperiodic dynamics or deterministic chaos determined by two or more variables allowing coexistence of states, ready for proper selection. A puzzle is the problem of prebiotic spatial organization of reaction systems. How do we visualize the generation of spatial compartments which might be a precursor for later localization of prebiotic reaction passways within cellular compartments? We do not know, whether the earliest biological cells have been large with sizes in the range of length of meters or whether there have been, from the very beginning, specialized localisations in the order of microns, as we see it generally today. After all, it would be misleading to expect that processes of selforganization in living cells, as we experience today, are simply a reduced copy of the pattern formation phenomena in macroscopic reaction diffusion systems which presumably might have been prevailing in the prebiotic time range [2]. Thus, it might be appropiate to consider these constraints when discussing molecular aspects of the origin of genes, their evolution and mechanisms of diversification.

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Homeobox Genes in the Developing Brain and Gastrulation

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The study of vertebrate homologues of regulatory genes operating in the trunk of *Drosophila* has provided invaluable information about the genetic control of positional values in development. Many of these genes contain a homeobox and it is now well established that many homeobox genes control cell identity in specific regions or segments both in invertebrates and vertebrates. *Hox* genes (McGinnis and Krumlauf, 1992) stand out among the various homeobox gene families so far identified as the vertebrate cognates of *Drosophila* homeotic genes. They control the specification of body regions along the vertebrate axis and provide positional cues for the developing neural tube from the branchial area to the tail.

Despite this progress, the development of the anteriormost body domain, the head or better the anterior head, has remained relatively obscure in invertebrates and vertebrates alike (Finkelstein and Boncinelli, 1994). In the insect embryo, the nature of anterior head segmentation has been controversial, and the genes that govern it mostly unknown. In vertebrates, the very existence of compartments or segments in the forebrain and midbrain has been contested, and underlying molecular mechanisms of pattern formation undetermined.

A recent breakthrough has come with the identification of genes in *Drosophila* and their homologues in vertebrates that appear critical to anterior head and brain specification. We will focus here primarily on four vertebrate homologues of two of these genes, called *empty spiracles* (*ems*) and *orthodenticle* (*otd*) in the fruitfly (Finkelstein and Perrimon, 1991; Cohen and Jürgens, 1991 for reviews). These four genes are *Emx1* and *Emx2* (Simeone *et al.*, 1992a,b), related to *ems*, and *Otx1* and *Otx2* (Simeone *et al.*, 1992a, 1993; Finkelstein and Boncinelli, 1994), related to *otd*. The two *Otx* genes code for homeoproteins containing a homeodomain of the *bicoid* class. Homeodomains of this class contain a characteristic lysine residue at position 50, corresponding to position 9 of the recognition helix. The restricted family of homeobox genes encoding a homeoprotein of the *bicoid* class only include so far *bicoid* itself, *orthodenticle*, the two vertebrate *Otx* genes and *goosecoid* (*gsc*), a regulatory gene originally isolated in *Xenopus* where it has been suggested to play a role in executing Spemann's organizer phenomenon (Cho *et al.*, 1991).

The four *Emx* and *Otx* vertebrate genes are expressed in extended regions of the developing rostral brain of mouse midgestation embryos, including the presumptive cerebral cortex and olfactory bulbs. Here we summarize expression data and discuss a possible role of the four genes in establishing the boundaries of the various embryonic brain regions.

Expression of the four genes in E10 mouse embryos

At day 10 of development (E10) the developing neural tube of the mouse shows recognisable presumptive regions corresponding to the future anatomical subdivisions.

The entire neural tube consists of neuroepithelial cells in active proliferation and most of the specific differentiative events have not yet occurred. In E10 mouse embryos all four genes are expressed. Their expression domains (Simeone *et al.*, 1992a) are continuous regions of the developing brain contained within each other in the sequence *Emx1<Emx2<Otx1<Otx2* (Fig. 1). The *Emx1* expression domain includes the dorsal telencephalon with a posterior boundary slightly anterior to that between presumptive diencephalon and telencephalon. *Emx2* is expressed in dorsal and ventral neurectoderm with an anterior boundary slightly anterior to that of *Emx1* and a posterior boundary within the roof of presumptive diencephalon. This boundary most probably coincides with the boundary between first and second thalamic segment which will subsequently give rise to ventral thalamus and dorsal thalamus, respectively. The *Otx1* expression domain contains the *Emx2* domain. It covers a continuous region including part of the telencephalon, the diencephalon and the mesencephalon with an anterior boundary approximately coincident with that of *Emx2*. Laterally, the posterior boundary of *Otx1* domain coincides with that of the mesencephalon. In median sections a strong hybridisation signal extends only half way along the mesencephalon, dividing the mesencephalic dorsal midline in two domains. Finally, the *Otx2* expression domain contains the *Otx1* domain, both dorsally and ventrally, and practically covers the entire fore- and mid-brain, to the exclusion of the early optic area.

Expression of *Emx* and *Otx* genes identifies several regions in the forebrain (Fig. 1). Some of these regions seem to correspond to presumptive anatomical subdivisions, whereas the significance of others remains to be assessed. Dorsally, for example, it is clear that the two *Emx* genes identify a presumptive cortical region, part of which will be neocortex and archicortex. *Emx2* expression also appears to define the boundary between future dorsal and ventral thalamus. On the other hand, it is notable that expression of these genes does not offer an unambiguous cue for the boundary between presumptive ventral thalamus and posterior dorsal telencephalon.

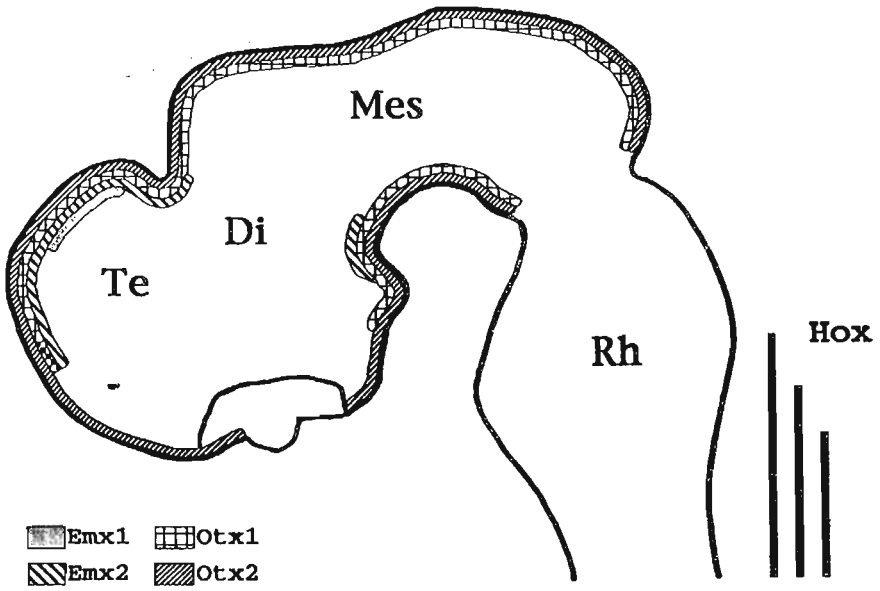

Figure 1. Summary of the expression domains of *Emx* and *Otx* genes in the developing central nervous system of E10 mouse embryos. Expression of members of the *Hox* gene family is also indicated. Di, diencephalon; Mes, mesencephalon; Rh, rhomboencephalon; Te, telencephalon.

In summary, analysis of E10 brain shows a pattern of nested expression domains of the four genes in brain regions defining an embryonic rostral, or pre-isthmic, brain as opposed to hindbrain and spinal cord. The first appearance of the four genes is also sequential (Simeone *et al.*, 1992a): *Otx2* is already expressed in E5.5, followed by *Otx1* and *Emx2* in E8-8.5 and finally by *Emx1* in E9.5. It seems reasonable to postulate a role of the four homeobox genes in establishing or maintaining the identity of the various embryonic brain regions. In this line, the specification of the regions of the early rostral brain seems to be a discrete process with its center in the dorsal telencephalon.

Expression of *Emx* and *Otx* genes in midgestation mouse embryos

Emx gene expression
Emx1 and *Emx2* are expressed in presumptive cerebral cortex in a developmental period, between day 9.5 and day 16 of development, corresponding to major events in cortical neurogenesis (Simeone *et al.*, 1992b). In its full extension, E12.5 to E13.5, the *Emx1* expression domain comprises cortical regions including primordia of neopallium, hippocampal and parahippocampal archipallium. *Emx1* expression seems characteristic of cortical regions, mainly but not exclusively hexalaminar in nature. In the same period, the *Emx2* expression domain comprises presumptive cortical regions including neopallium, hippocampal and parahippocampal archipallium and selected paleopallial localisations, but no basal internal grisea. In E12.5 embryos, the hybridization signal is uniformly distributed across the cortex without major differences but starting from E13.75 it appears to be confined to the germinal neuroepithelium of the ventricular zone, excluding the intermediate zone and cortical plate. From day 14.5 on, *Emx2* cortical expression progressively declines in anterior and ventrolateral regions and at day 17 of development is confined to specific cell layers in hippocampus.

Otx gene expression
In midgestation mouse embryos the two *Otx* genes are expressed in specific restricted regions of the developing brain (Simeone *et al.*, 1993). Both are expressed in basal telencephalon, in diencephalon and mesencephalon but not in spinal cord. Their expression domains in mesencephalon show a sharp posterior boundary, both dorsally and ventrally, approximately at the level of rhombic isthmus. From E9.25 onward, the expression of both genes clearly marks the posterior boundary of mesencephalon to the exclusion of presumptive anterior cerebellar domains. *Otx1* is also expressed in dorsal telencephalon, whereas *Otx2* expression has disappeared from this region at day 11.75.

Otx1 and *Otx2* are also expressed in restricted regions of diencephalon of midgestation embryos: epithalamus, dorsal thalamus and mammillary region of posterior hypothalamus. In these regions, the hybridization signal is almost exclusively confined to cells of the ventricular zone. Their expression domain does not include the ventral thalamus. A two-layered narrow stripe of expression is detectable at the level of the boundary between dorsal and ventral thalamus, that is the *zona limitans intrathalamica*, the precursor of *lamina medullaris externa* and mammillo-thalamic tract. Other localizations are: fasciculus retroflexus, the precursor of habenulo-interpenduncular tract, *stria medullaris*, including the region surrounding the posterior commissure, *primordium* of mammillotegmental tract, epiphysis, fornix and *sulcus lateralis hypothalami posterioris*. Posterior to diencephalon, *Otx1* and *Otx2* are expressed in mesencephalic regions of tectum and tegmentum, possibly at the level of presumptive periventricular bundles.

Both *Otx* genes are also expressed in the olfactory epithelium, as well as in the developing inner ear from early expression in the otic vesicle to epithelia in auricular ducts of sacculus and cochlea and in the devoloping eye, including the external sheaths of the optic nerve.

Areas and boundaries in diencephalon

Expression of *Otx* genes in diencephalon and mesencephalon of E12.5-14.5 embryos colocalizes with boundary regions and presumptive axon tracts, including anterior and posterior commissure (Fig. 2). This expression is confined to precursor cells surrounding these structures as if these cells could be used as borders of pathways for the pioneer axon tracts. This is particularly evident in posterior commissure and along the zona limitans intrathalamica. *Otx* gene expression in posterior commissure is limited to cells of ventricular epithelium, whereas primary fibers running on its surface are not labelled. Expression of *Otx* genes along the zona limitans intrathalamica might constitute a framework for the axon patterning of lamina medullaris and other structures physically separating dorsal thalamus from ventral thalamus. The existence of this barrier might account for the sharp dorsal boundary of the expression domain in ventral thalamus of *Dlx* genes (see Boncinelli, 1994 for a review; Simeone *et al.*, 1994) and for the sharp ventral boundary of the expression domain in dorsal thalamus of other genes like *Wnt3* (Salinas and Nusse, 1992). Both *Otx* genes are also expressed around the developing optic nerve. This localization is similar to that along the zona limitans intrathalamica in providing clues to axon pathfinding and patterning. In this light, expression of *Otx* genes might provide a global framework for the primary scaffold of specific axon pathways in the early neuroepithelium of the forebrain. We are currently testing this hypothesis with in vitro analysis of axon growth and propagation on *Otx*-transfected cells.

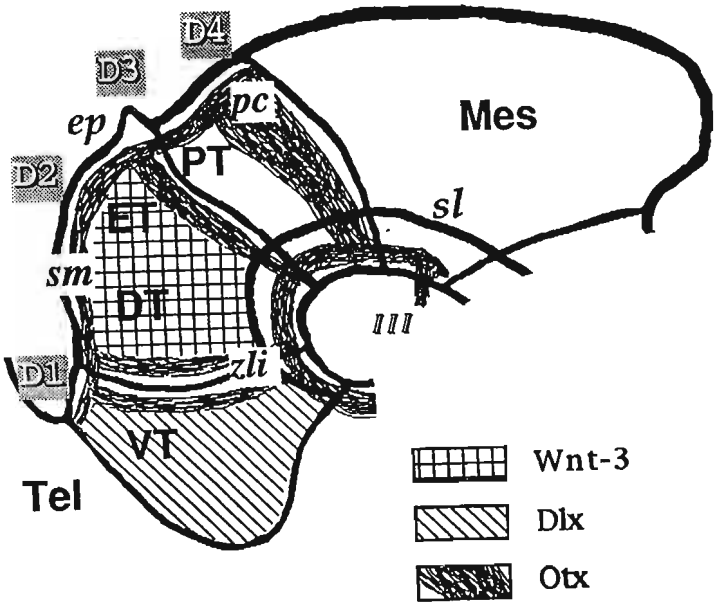

Figure 2. Schematic representation of the expression domains of *Otx1* and *Otx2*, *Wnt3*, and *Dlx* genes in diencephalon of E12.5 embryos. Within the diencephalic regions bold letters designate the columnar nomenclature: DT, dorsal thalamus; ET, epithalamus; PT, pretectum and VT, ventral thalamus. Outside the profile, the proposed new subdivision into four neuromeres, D1 to D4 (Figdor and Stern, 1993), is indicated. *ep*, epiphysis; Mes, mesencephalon; *pc*, posterior commissure; *sl*, *sulcus limitans*; *sm*, *stria medullaris*; Tel, telencephalon; *zli*, *zona limitans intrathalamica*; *III*, 3rd cranial nerve.

It is of interest to consider the possibility that *Otx* genes play a different role in the development of the head in at least two different stages. They first specify territories or areas in rostral brain of E8-E10 mouse embryos and provide later on a set of positional cues required for growing axons to follow specific pathways within the embryonic central nervous system. It is not clear whether the two functions are independent. It is also of interest to consider that in flies mutant for *orthodenticle*, pioneer axons of the posterior commissures fail to develop normally as if appropriate positional cues were missing (Tessier-Lavigne, 1992).

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Molecular Unity and Diversity in Glycoproteinic Hormones Regulating Fertility

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Put in perspective are a family of proteins with important hormonal properties. three of them are made in the pituitary and one in placenta. These proteins are crucial for achievement of reproduction in mammals. The ability to reproduce is one of the essential definition of living organisms and has assured the perpetuation of species.

These proteinic hormones are all composed of two subunits, α and β. The α subunit, a glyco-peptide of 92 amino-acid is common to *all* the 4 hormones and the β subunit in each case confers hormonal specificity.

The association of α with β subunits takes place in a defined way non-covalently to generate a confrontation which has biological activity. Neither the isolated α nor β exercise alone the hormonal properties.

The α subunit can, not only combine with the β subunit of FSH, TSH, LH OR hCG to generate the respective properties, namely that of FSH, TSH, LH or hCG within the same species, but also the α subunit from a hetero-species, such as ovine can combine with human β to give rise to a hetero-species dimer (HSD).

The hetero-species dimer created by joining of βhCG and α of ovine origin is more potent than the homospecies α + βhCG (HCG) in its steroidogenic properties. Moreover, it has better immunogenicity and generates antibodies of enhanced capacity for neutralization of the bioactivity of hCG (1). The sites at which association between α + β takes place are largely conserved amongst species.

Another feature of both the α and β subunits is the presence of cystines, ten half-cystines in α and 12 in β . These are linked within the molecule in disulphide bonds to create the right conformation of the subunit for association with each other. In case the S-S bonds are reduced and chemically modified, say by carboxy methylation preventing their reformation, the subunits loose the ability to join with each other.

Recent elucidation of the structure of hCG has provided insights on the specific position of S-S linkages in the α + β subunits of the hormone (2). A cardinal feature of these studies is the observation on the presence of a characteristic cystine knot in the hCG molecule. Another interesting feature revealed by the study is the spatial superimposition of the α and β subunits, in particular in the region where cystine knots are formed. Similar cystine knots are seen in the three other pituitary hormones, LH, FSH and TSH β reflecting a good deal of resemblance on the way that the molecules of this family are disposed. Inspite of these apparent similarities, the biological properties of 2 and 4 hormones, namely FSH and TSH divert completely from those possessed by LH and hCG. TSH acts on the thyroid, stimulating production of the

thyroid hormones influencing energy metabolism, growth and development. FSH acts on the gonads (ovaries and testes) just as LH does, but the receptors for the two are different. Their actions differ, but they complement each other in making of sperm in the males and egg in the females. They also stimulate production of sex steroid hormones in both males and females. The receptor for LH and hCG is conserved within the species and across the species, with the result that hCG can act not only in humans but also in mice and monkeys. Conversely the ovine LH can act on the human ovaries or testes besides in the proper species.

Immunological uniqueness and diversity

Amongst the 4 hormones sharing the common α, TSH and FSH have diverged sufficiently to have non cross-reactive antibodies so long as these are generated to the entire hormone. Antibodies raised to the isolated α subunit, however, can react with all the 4 hormones. This epitope common to these 4 hormones gets masked when the α subunit is joined to the β to give rise to the entire hormone.

LH and hCG have large homologies in amino-acid sequences. The disulphide bonds are exactly at the same position and both molecules fold in a manner to bind to the same receptor. There are also distinct differences in the two hormones, not only in terms of replacement of some amino-acids along the entire sequence but also βhCG has an extra tail of 30 amino-acids, non-existent in βhLH. This extra length has presumably materialized from an ancestral gene with one base deletion and read-through of the untranslated 3'- part of the gene. The tail is also heavily glycosylated with 4 serine linked carbohydrate residues rich in sialic acid. These contribute to a longer biological half life of the hCG as compared to hLH. The C-terminal tail is, however, not involved in binding with the receptor and has no participation in biological activity of the hormone. It is also a poor immunogen.

The immune system forms antibodies that are either specific to hCG or hLH and thus there is a recognition of the individuality of the two hormones by such antibodies (3). The immune system also makes antibodies which bind with determinants common to both LH and hCG.

hCG was the first choice of investigators for developing contraceptive vaccines. The rationale in this choice is the consideration that interception at the level of hCG would not interfere with the pituitary-ovarian axis and that the female would continue to make, LH and FSH as well as the ovarian sex hormones oestrogen and progesterone normally. Immunological interception would be only at the stage at which hCG is made, soon after conception but before the embryo can implant e.g. before pregnancy can be established. Thus inactivation of hCG by circulating antibodies imposes a pre-implantation block by preventing blastocyst to implant as well as by preventing endometrium to reach a receptive state for implantation. Two different strategies have been followed for developing the hCG vaccine, and both are safe and reversible (4,5). The vaccine which has demonstrated its ability to accord protection against pregnancy is the HSD-hCG vaccine, in which a hetero-species dimer is tagged to diphteria or tetanus toxoid. Women of proven fertility immunized with this vaccine do not become pregnant so long as the antibody titres stay about 50ng/ml (6). Immunization does not

disturb ovulation nor menstrual regularity. The effect is fully reversible and on decline of antibodies, in the absence of boosters, the women regain fertility.

The basis of the first contraceptive vaccine developed against a key hormone involved in the establishment of pregnancy is a strategy taking advantage of diversity within the molecular unity of hormones sharing a common backbone.

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Immune Response Diversity (IR-Genes) and Phenotype Correction

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Immunological diversity has at least two sides. One includes variations in tissue, cell and protein antigens of the body, blood groups, antigens of Maine Histocompatibility Complex, isoforms of proteins, enzymes etc... Antigenic structure of any individual is completely unique. To have well compatible donor for bone marrow transplantation we need to have a bank of not less than 20000 donors. Real identity exists only between two identical twins.

Another aspect of immunological diversity is the immune response diversity. Before the discovery of genes, controlling immune response (IR- genes), the diversity in strength of immune response was explained as a result of life experience. Differences in antigenic contacts during life span create antigen-priming mosaic in immunological memory. This is a good explanation of diversity of strength of immune reactivity among representatives of any human or animal population.

The IR-genes control of high or low response level is specific to each antigen. As a result, depending on the IR-genes composition, an individual may be a high responder to one group of antigens and a low responder to another group. So, if you immunize a large population with a vaccine or antigen, you will have many representatives with low immune response. It means that they will not be effectively immunized to resist infection against which you have immunized the population.

It is why the problem of avoiding the IR-gene control is a very important problem in both aspects; as an interesting scientific problem and as practically important task to create effective vaccines against non controlled infections.

Immune response after antigen application is a result of collaboration of 3 cells: macrophage-T-lymphocyte-B-lymphocyte. Majority of antigens are T-dependent, immune response can not be triggered without T-helping cells. So if we want to avoid IR-gene control we have to convert T-dependent antigens into T-independent. To check realisation of IR-gene control avoiding we have check our technology with known antigen, controlled by known IR-gene.

As a model to avoid IR-gene control we used TG (AL) antigen, synthesised by M. Sela. Immune response to TG (AL) in mice is controlled by IR-1 gene.

We found a group of linear polyelectrolytes both polyanions and polycations to be conjugated to antigens. Among them are polyacrilic acid, poly-4-vinyl pyridinium, polycondinium and their derivatives. The last one is biodegradable and non toxic. All of them are immunostimulants if their size is not less than 1000 monomeric units. We were working with polyelectrolytes, consisting of 10-40 thousands units.

Chemical linkage of the TG (AL) to the polyelectrolytes carriers transform it from T-dependent into T-independent, working even in athymic nune/nude mice. Linkage γBovine Serum Albumin or Human Gamma Globulin with the carriers convert them into T-independent antigens, TG (AL) antigen conjugated with polyelectrolyte carriers stimulates high immune response in animals (mice CBA genotype) bearing of IR-1 gene, controlling low response of the host. It was demonstration of avoiding T-dependence of the immune response, by conjugation of antigens to polyelectrolyte carriers.

On the basis of these achievements we have created three very effective conjugated vaccines: (a) against *Salmonella typhymurium* using O- polysaccharide as a linked antigen; (b) against influence virus using hemagglutinin as a linked antigen; (c) antihelmints vaccine using P-63- one of the common among helmints protein with molecular weight 63 k-K Da. The last one is really the first effective vaccine against helmints. We have named it H-polyvac.

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Regulation of Acetylcholine Receptor Genes Expression in Developing Muscle and Brain

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As the adult motor endplate, the AChR protein (a2bg/ed) as its subunits mRNAs are localized exclusively under the motor nerve ending. Denervation of the adult muscle causes a reappearance of unspliced and mature mRNA in extra-junctional areas. On the other hand, in the non-innervated embryonic myotube, the a, b, g, and d subunit mRNA are distributed all over the cell. A compartmentalization of gene expression at the level of subneural "fundamental" nuclei therefore takes place during development and is analyzed by the methods of cell biology and recombinant DNA technology (transfection, transgenic mic, DNA injection, adenoviral vectors) with both cultured and developing muscles in situ.

The data are interpreted in terms of a model which assumes that: 1) in the adult muscle fiber, nuclei may exist in different states of gene expression in subneural and extrajunctional areas; 2) different second messengers elicited by neural factors such as CGRP (cAMP) or ARIA (tyrosine kinase) (under the nerve endings) or electrical activity (Ca++, protein kinase C) (outside the endplate) regulate the state of transcription of these nuclei via trans-acting allosteric proteins binding to distinct cis-acting DNA regulatory elements; 3) in the chicken a-subunit enhancer, consensus E Boxes (CANNTG) play a differential role in the regulation by electrical activity while in mouse e-subunit promoter a different 83 nucleotides sequence confers preferential synaptic expression; 4) a regulation of the expression of myogenic protein genes takes place during endplate formation; 5) multiple post-transcriptional processes involving, in particular, the *Golgi apparatus*, proteins from the basal lamina and from the cytoskeleton (e.g., the 43 KD protein) contribute to the clustering, and stabilization of the AChR in the postsynaptic membrane.

A similar "promoter approach" is extended to the analysis of the expression of brain nicotinic receptor genes a2, a3, a4, b2, b4 by *in situ* hybridization, transfection of cultured nerve cells (b2-subunit promoter) and transgenesis in mice (chick a2-subunit gene and mouse b2-subunit promoter).

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Homeoproteins and Individuation

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Homeoproteins, a class of position-dependent transcription factors, are expressed at early develomental stages when they define the morphological fate of the embryonic territories and participate in decisions on cell lineages. In addition to this first period of expression, a second period that can last throughout adulthood has been described in several biological systems and, most importantly, in the nervous system.

The latter expression in the vertebrate nervous system starts during the period of neurite growth and synaptogenesis. It is thus possible that homeogenes are involved in the control of these processes. In the adult, the same genes would be responsible for the morphological remodeling that accompanies neuronal activity.

To gain access to the study of the putative role of homeogenes in the later stages of neuronal development, we have taken advantage of 2 distinct properties of the homeoproteins. A first property is the high structural conservation of the homeodomain, a 60 amino acid-long sequence responsible for homeoprotein binding to dsDNA. A second property is that the homeodomain target sites on the promoters are also very much conserved with a core ATTA sequence repeated several times.

From these two properties it can be foreseen that almost any homeodomain will compete very efficiently with full lenght homeoproteins for occupying their genomic target sites. This hypothesis was verified by synthesizing the homeodomain of Antennapedia and by demonstrating that it could compete in vitro with homeoproteins expressed in the cortex or the spinal cord.

We then decided to inject the homeodomain (abbreviated pAntp) into nerve cells and to verify if neurite elongation by post-mitotic neurons would be affected. In the course of these experiments we observed, to our surprise, that the homeodomain of Antennapedia was spontaneously internalized and conveyed to the nucleus of all cells in culture. Internalization was followed by an enhanced neurite elongation, both by spinal motorneurons and by cortical neurons. This neurotrophic effect of pAntp was not obtained when we used mutated versions of the peptide still capable of translocating across the membrane and to accumulate within nuclei but with weak DNA-binding activity. We produced cell lines with reporter genes under the control of promoters transactivated by homeoproteins and verified that pAntp-induced neurite elongation was specific and through transcriptional regulation.

From this series of experiments we conclude that homeoproteins can regulate neurite morphogenesis in post mitotic neurons. We propose that this might also be their role in vivo and, in particular, in the adult where they could modify neurite shape and, consequently, memorisation.

The finding that an homeodomain could be internalised led us to analyse the process of pAntp translocation and to demonstrate the implication of its third helix, also called recognition helix because of its role in the binding to dsDNA. This importance of the

third helix was intriguing because, due to the high conservation of this structure, it implied that translocation across membranes could be a property shared by several homeodomains. This might well be case since we have demonstrated that, in addition to the homeodomain of Antennapedia, those of Engrailed, Hoxa-5 and fushi tarazu are internalized by cells in culture and addressed to their nuclei.

More recently, we have demonstrated that a full length homeoprotein (Hoxa-5) was also internalized and could be retrieved intact from the nuclei. Internalization is rapid, occurs at 4°C and requires the presence of the homeodomain. The latter observation lends weight to the proposal that cells could exchange positional information by the local trading (secretion and translocation) of homeoproteins, an exchange followed by a specific modification in cell shape or activity.

In our model, homeoprotein exchange could occur in the adult, thus giving a physical basis for epigenetical modifications accompanying learning and memorization. Considering that homeoproteins are position dependent transcription factors and participate, in evolution, to the genetic memory of the animal species, it might come as a surprise that they might also participate in the construction of the individual memory during the life-long process of individuation.

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Neuroethological Prerequisites for the Evolution of Speech

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Molecular geneticists have estimated that *Homo sapiens* shares more than 98% of his DNA with his closest relative, the chimpanzee. This contribution deals with an essential part of what this little difference amounts to. It deals with the evolutionary aspects of the motor component of language, that is speech.

Talking to each other is the highest form of social communication and is possible only for humans. But animals throughout the animal kingdom have also developed various forms of social communication. Among the vertebrates audio-vocal communication has developed and expanded into an increasingly complex system, comprising the larynx, which produces the species-specific signal, the motor system, which generates the patterning of the vocal signals, the ear, which receives the species-specific signals, and the cerebral decoding device, which transforms the signal into a message. The way in which the cerebral motor patterning and the decoding systems are linked seems to be the key to understanding communication processes in general. All the subsystems have been subject to enormous changes during their co-evolution (Ploog 1992). This contribution deals only with the executive part of the system.

The most important change in the larynx of man concerns the muscular microregulation of the vocal folds, which can be controlled not only in length, tension, and degree of opening and closing but also in small segments, and, most importantly, at will.

The other equally important change in the peripheral apparatus is the considerable transformation of the supralaryngeal tract. The configuration of the mature human vocal tract enables a human to produce sounds that a chimpanzee cannot produce, for instance the vowels [i]; [a]; and [u], which are universal, occurring in all languages. Interestingly, a similar transformation of the vocal tract takes place in human ontogeny (Lieberman 1984).

A further very important difference between apes and man concerns the articulatory apparatus. While the nonhuman primates produce their sounds mainly with the larynx humans use their tongue and lips for articulatory movements. By a concerted action of these articulatory gestures with laryngeal movements the airstream produced by the lungs is transformed into a sound wave of high temporal and spectral complexity. In perception this continuous signal is resolved into a sequence of discrete entities, referred to as phonemes, each of which can be related to a particular configuration of the speech organs. How these synergistic motor configurations are produced by the central nervous system is still virtually unknown (Ploog 1988).

The central nervous organization of phonation has been investigated in the squirrel monkey, a small South American primate that is endowed with a rich vocal repertoire. The great variety of calls regulate the social life; certain different calls warn of aerial and terrestrial predators (Winter *et al.* 1966).

How is vocal behavior represented and organized in the brain? Answers to this question contribute in two ways to basic issues in communication research. First, we gain direct access to the brain system that is responsible for the vocal expression of emotions and thereby indirect information about the system that generates and controls emotions. Second, we gain access to a non-verbal communication system that we largely share with the nonhuman primates. This system operates not only in our non-verbal communication system with conspecifics but also in prosodic features in our language--in conversation, in songs without words, in jubilating, complaining and cursing, to name just a few.

The results of electrical brain stimulation in monkeys provide enough information for an outline of the system involved in vocal behavior, and hence in the vocal expression of emotions (fig. 1). Only natural calls can be elicited by electrical stimulation of the brain sites above a certain level in the pons. The responsive structures reach from subcortical orbitofrontal and temporal structures, through thalamic and hypothalamic structures, into the midbrain and pons and their intimate connections with the limbic system. Not a single vocally responsive site was detected in the neocortex (Jürgens & Ploog 1970/76).

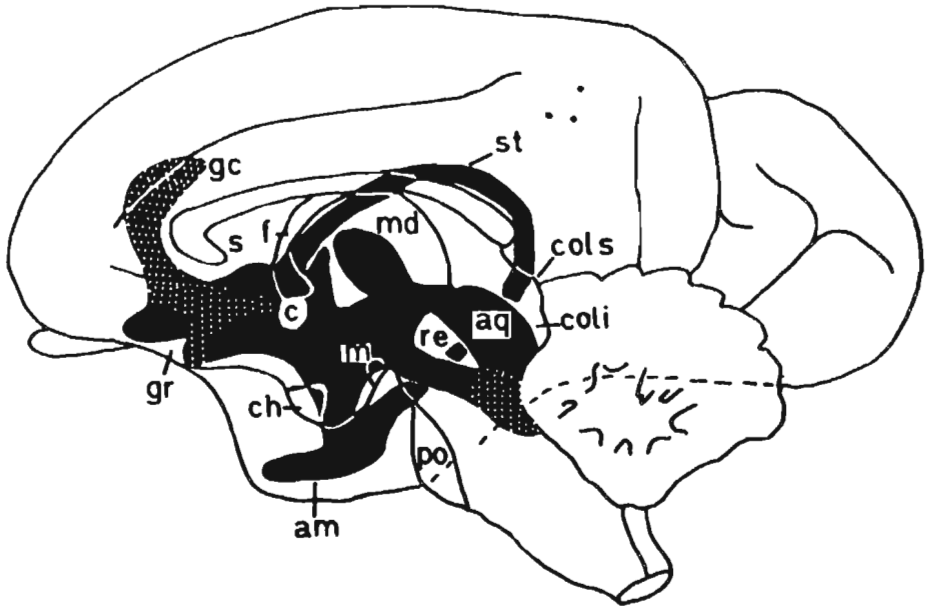

Figure 1: Subcortical brain areas (black) yielding species-specific vocalizations when electrically stimulated. Stipulated areas of particular functional significance.

There are two regions which are of particular functional significance in both monkey and man: the (stippled) periaqueductal gray in the midbrain on the one hand and the (stippled) anterior cingulate gyrus on the other. The central gray in the caudal midbrain and laterally adjacent tegmentum is the phylogenetically oldest structure for the generation of species-specific calls. Its electrical stimulation yields vocalizations in

Summarizing the results on the vocal cerebral system in primates including man we see from figure 2 that the system is hierarchically organized. Level IV on the right shows a lateral view of a primate brain with the cortical representation of the larynx, pharynx, tongue, and lips (LR). The dotted area above the anterior cingulate gyrus (GC) indicates the supplementary motor cortex (SMA). The connecting lines represent anatomically verified direct projections in rostrocaudal direction. All areas marked with a dot yield vocalization when electrically stimulated (except for LR in the monkey). This is in level IV the cingulate gyrus, in level III the amygdala, midline thalamus, and different hypothalamic areas, in level II the periaqueductal gray and laterally bordering tegmentum, and in level I the reticular formation of pons and medulla, feeding into the nucleus ambiguus (NA) which innervates the vocal cords. With one exception, all structures shown in this figure are homologous in man and monkey. The one exception is the direct motor pathway from the laryngeal representation (LR) in the primary motor cortex to the laryngeal motoneurons in the medulla (RF/NA) which does not exist in the monkey. This direct connection serves as the neuronal basis for the voluntary control of the vocal folds in man which is not possible in the monkey. This control is a necessary, if not sufficient prerequisite for the evolution of speech. The high degree of precision, and extremely fine motor control of the vocal cords as well as the articulators is indispensable for the development of learned speech. There is no anatomical and functional basis for this faculty in monkeys and apes. One must remember that they can neither speak nor sing. The hypothesis is advanced that the last little step in the evolution of the phonatory system in the brain is the outgrowing of the fine fiber portion of the pyramidal system that serves the *direct* and *fast* innervation of the larynx muscles via the nucleus ambiguus. In the neocortical larynx representation more neurons of the pyramidal cell type may have been recruited to solve this task (Ploog 1990). It is a challenging question in what direction the biological communication systems, starting with non-verbal social signalling and, up to now, ending with human language, will further evolve.

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Universal and Language-Specific in the Development of Speech

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Languages vary widely in phonological structure. As simple a measure as segment inventory size exemplifies this: inventories range at least from 11 phonemes in the case of some Polynesian languages to 141 in the case of some African languages (the range for consonants is at least 6 to 95, for vowels at least 3 to 46). Furthermore, no single segment is found in all languages; no language of average inventory size (20-40) contains all the most common segments; and there is no strict hierarchy of segments such that if a language contains segment X it will always contain segment Y (all data from Maddieson, 1984).

Thus there is huge variation in the type of speech input that a newborn child is presented with. But we all learn the phonological repertoire of our native language in the first year of life. New evidence suggests that this complex task is facilitated by abilities which are present at birth.

Experiments on infant speech perception have most often used a *habituation* paradigm (e.g. Eimas, Siqueland, Jusczyk & Vigorito, 1971): repeated instances of an auditory stimulus are presented until the infant's response (amplitude of non-nutritive sucking, or fixation duration to a visual stimulus accompanying the sounds) decreases, then the sound is changed; if the response recovers, the infant is assumed to have noticed the change, i.e. discriminated the relevant contrast. Phonetic discrimination can also be measured via event-related brain potentials (Dehaene-Lambertz & Dehaene, 1994). With older infants, *preferences* can be measured by determining which of two types of stimulus an infant will turn its head towards and attend to longer (e.g. Jusczyk, Cutler & Redanz, 1993).

Research with all these methods shows that infants can discriminate speech sound contrasts both from their own and from other languages. Thus they can discriminate contrasts which they have never heard; and they can discriminate contrasts which adults fail to discriminate, because mature language users identify speech sounds only in terms of the categories of their native language.

However, the ability to distinguish non-native contrasts diminishes rapidly during the child's first year. By about 11 months of age it resembles adult ability: contrasts found in the native language can be distinguished, but contrasts not found in the native language are not discriminated. That is to say, by the end of a child's first year of life, the native phonological system is in place.

Recent research has shown, however, that not all phonetic contrasts are learned (or, respectively, lost) at the same rate. Specifically, the structure of the native-language vowel repertoire appears to be acquired earlier than the consonant repertoire. At six months, infants seem to have a concept of prototypical vowels of their native language (Kuhl, Williams, Lacerda, Stevens, & Lindblom, 1992). Prototypes are "ideal"

instances of a vowel category; adult listeners judge vowels to be more similar to the category prototype than to each other, even when acoustic differences are equal (Kuhl, 1991). Kuhl *et al.* studied six-month-old infants acquiring American English and Swedish, and found that each group showed a prototype effect in the native language (they did not detect a difference between the prototype and another vowel, although they could detect the same size difference between two non-prototype vowels), but neither group showed a prototype effect in the non-native language.

Likewise, in standard habituation tasks, the loss of discrimination for non-native vowels seems to occur between four and six months (Polka & Werker, 1994), whereas the loss of discrimination for non-native consonants does not occur till around ten months of age (Werker & Tees, 1984; Werker & Lalonde, 1988).

Why should vowel perception develop earlier than consonant perception? The answer may lie in acoustic differences between vowels and consonants (at least, the types of consonants that have been the subject of most infant speech perception studies). Vowels are generally longer in duration than consonants; perhaps more importantly, they exhibit a more marked periodic structure. Cutler and Mehler (1993) have argued that infants display from the earliest days of life what they termed a "periodicity bias", i.e. a preferential attention to periodically regular signals. Such a bias has evolutionary value in that it implies preferential attention to structured (potentially meaningful) signals over random noise. There is supporting evidence for this claim in other perceptual abilities that infants display. Thus infants are sensitive to prosodic boundaries: clause boundaries first, phrase boundaries later (Hirsch-Pasek, Kemler Nelson, Jusczyk, Cassidy, Druss, & Kennedy 1987; Jusczyk, Kemler Nelson, Hirsch-Pasek, Kennedy, Woodward, & Piwoz, 1992). Jusczyk and his colleagues propose a gradual refinement of ability to perceive patterns, from more obvious to less obvious instances.

The benefit of a "periodicity bias" for the infant language-learner is presumably connected with the ultimate aim of language acquisition: achieving communication. An inventory of phonemes is, after all, not an end in itself. Language is for communicating with other members of one's group. To achieve this, the child needs the units of communication - words. However, words are not clearly demarcated in the speech stream (for prelinguistic infants or for adults), because speech signals are continuous. In principle, an adult language user (thus, in possession of a stock of words) could recognise speech by recognising known units in the speech stream; but this option is obviously not open to infants who do not yet know any words. An infant's task is to build up a stock of known words (a lexicon), and for this the words must be extracted from the continuous speech stream.

It is with this task that a "periodicity bias" can assist. Attention to periodicity in the prosodic structure of speech offers a way of *segmenting* the speech stream, in that the speech rhythm of a given language reflects the characteristic prosodic shape of words of the language. (In fact, adult language users exploit this correspondence; that is, they too use language rhythm, in language-specific ways, to help with speech segmentation.) Experiments with neonates (two to four days in age) suggest that the relevant sensitivity is in place at birth. For instance, neonates can attend to syllables in preference to non-syllabic sequences (Moon, Bever & Fifer, 1992): they will suck more strongly given a signal which activates a recording of their mother's voice than given a

signal which precedes silence, and the three-phoneme syllables *pat, tap* work as signals in this paradigm, whereas the three-phoneme non-syllables *pst, tsp* do not. Likewise, neonates discriminate number of syllables but not number of phonemes (Bijeljac-Babic, Bertoncini & Mehler, 1993); they can perceive a change from a list of four-phoneme nonwords (with two syllables, e.g. *rifo, kepa*) to a list of six-phoneme nonwords only when the second list differs from the first in number of syllables (thus, *mazopu, rekivu* is perceived as different, but *suldri, treklu* is not). Finally, neonates discriminate bisyllables which contain a word boundary (the first syllable is somewhat lengthened) from phonetically matched bisyllables which do not (Christophe, Dupoux, Bertoncini & Mehler, 1994). These new studies with neonates suggest very early sensitivity to the components of speech rhythm, viz. syllables, and to the prosodic relations between them.

During the second half-year of life, infants' babbling gradually converges on the phonology of the native language; the sensitivity to prosodic structure is expressed in this development also. In particular, language-specific speech rhythm appears: thus after the age of six months the non-final syllables in babbled utterances of French infants become more regular in duration, those of English infants less regular (Levitt & Wang, 1991; Levitt & Utman, 1992). By nine months of age - but still before they can produce any words - infants display a significant preference for the characteristic prosodic shape of words of their native language (Jusczyk, Cutler & Redanz, 1993); that is, they have successfully acquired a concept of what words of their language are prosodically like. Laying the foundation for a lexicon is the principal achievement in the first year of language acquisition. The sensitivity to periodicity and prosody provides a basis for this.

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Biology International Special Issue N° 33 (1995)

The Hierarchical Uniqueness Of Biodiversity

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Biodiversity is now a very fashionable word. It became very largely used since some ten years ago, but with very different meanings. For the public at large, it evokes mostly the emotion of preserving some very charismatic species of animals and plants, like panda, elephant, Sequoia, etc. For a politician or an entrepreneur, the main concern is for its utilisation, including the problem of the property rights, and the transfer of know-how for applications in biotechnology. The overall geopolitical aspect of an equitable use of biodiversity at a world-wide level - and mostly between countries of the North and of the South - is now a very hot and debatable issue linked to the recent Convention on Biological Diversity. In addition, a multitude of myths on biodiversity pervades the media in their search for sensational or catastrophic news.

Even the scientific community uses very loosely the term "biodiversity", sometimes referring to given taxa (e.g., bird biodiversity, plant biodiversity, microbial biodiversity, etc.), sometimes to different levels of integration (e.g., molecular biodiversity, species biodiversity, or biodiversity of ecosystems).

Admittedly, biodiversity is therefore an almost intractable word to be defined. However, a better understanding on the implications of biodiversity has become a real necessity, not only to eliminate matters of contention during political negotiations leading to treaties and conventions, but also -much more important - to provide a kind of leitmotif to the biological world and to increase interactions among disciplines.
There are already two *official* definitions of biodiversity, that is to say, approved by all countries. Nevertheless, the participation of the scientific community to elaborate such definitions is far for having been satisfactory.

The longest definition is that of the United Nations in 1992, conceived while preparing the Convention on Biological Diversity. According to this definition, biodiversity means "The variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems". The shortest one is that of the Global Biodiversity Strategy in 1992. It reads as follows: " The totality of genes, species, and ecosystems in a region".

It is interesting and even surprising to note that, in spite of their relative shortcomings when analysed from a scientific viewpoint, both definitions specifically mention the three main components of biodiversity as an unitary principle: diversity within species is the *genetic* diversity; between species is the *species or taxonomic* diversity, and of ecosystems is the *ecological* diversity. In other words, biodiversity can be defined as the ensemble and the interaction of the genetic, the species, and the ecological diversity, in a given place and at a given time (see Fig. 1).

But these interactions are of a hierarchical nature, so that emerging properties appear when passing from the genetic to the species and to the ecological diversities. By

interlocking the three diversities, as shown in Fig. 2, the classical zooming effect of the hierarchical theory is reached, whereby new properties of biodiversity emerge according to the positions of the three blocks and of the level and intensity of the interactions. Interaction between and among different hierarchical levels is the central phenomenon of biodiversity, and the basis for the explanation of the different characteristics and functioning of biodiversity.

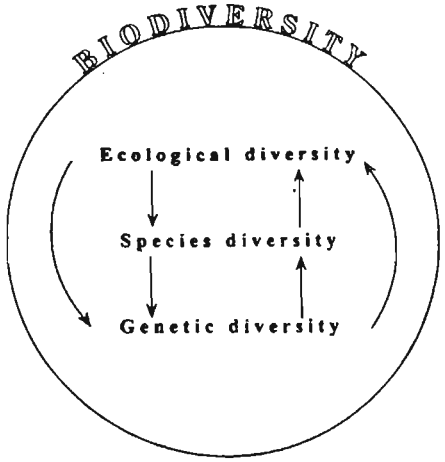

Figure 1: A definition of biodiversity based on its components and their interactions.

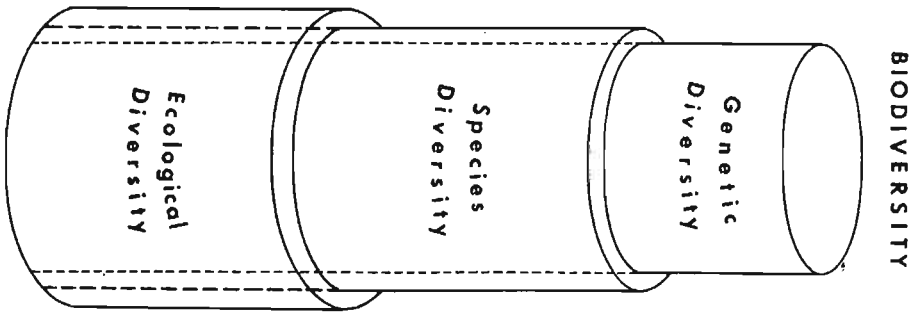

Figure 2: Biodiversity presented as a hierarchical zoom.

Nevertheless, the hierarchy shown above is not - admittedly - a "clean" hierarchy, since genes, species, and ecosystems do not belong all together to the same hierarchical category. The concept is expanded and made more accurately in Fig. 3, where the hierarchical patterns of biodiversity are shown as the interactions of three different scales of levels of organisation: the genetic, the taxonomic, and the ecological ones. In

this way, the universality of the biological world is represented, while the unifying principle and the uniqueness are provided by the hierarchical interaction of the various diversities.

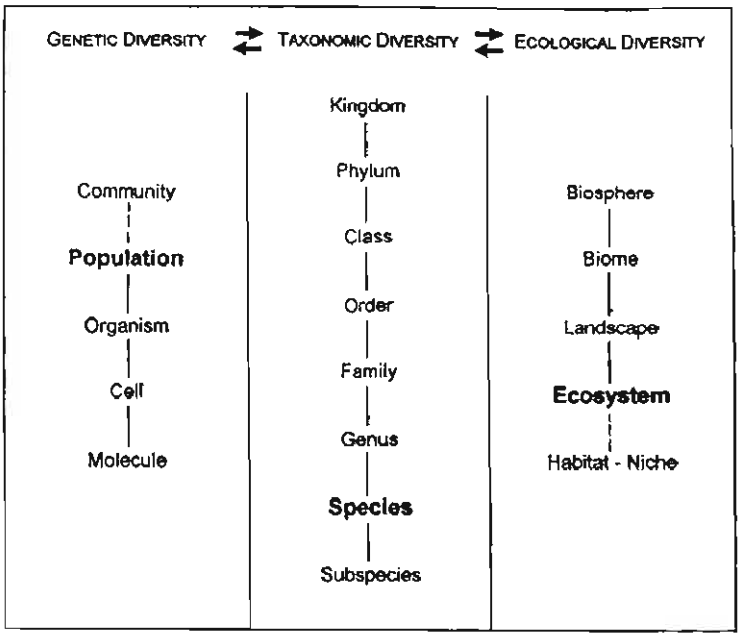

Figure 3: Hierarchical patterns of the three scales shaping the concept of biodiversity.

Populations, Species and Ecosystems are usually at the cornerstone of the intersection of the three scales. They are also the three main elements considered in Conservation Biology. But this is a restrictive misuse of the hierarchical approach.

As regards the genetic scale, for instance, molecular and population genetic should be very closely interlinked. Concerning the taxonomic scale, the overemphasis put on species diversity could lead to biased conclusions. For example, the marine environment is considered to be poorer of species as compared with the terrestrial one; however there are as many as 28 phyla in the marine environment (13 of them being endemic), against only 11 phyla in the terrestrial environment with just one endemic phylum. And in the ecological scale, the main factor of species extinction is the fragmentation of landscapes due to human impact, and not merely the lack of resilience at the ecosystem level or the decreasing fitness of populations.

This hierarchical organisation of biodiversity cannot be considered as a simple theoretical artefact. From a practical viewpoint, structural and functional attributes of system stability, productivity and sustainability, as well as possible patterns of ecosystem functioning, can only be clarified if most elements of the three genetic, taxonomic and ecological scales are considered in terms of their hierarchical interactions.

This is also true from an evolutionary viewpoint. Only these interactions can bring some highlights to understand whether a given level of biodiversity depends on the evolutionary time elapsed without great disturbance, or rather reflects the frequency of repeated disturbances in the evolutionary history (most likely both processes, under different ecological and evolutionary circumstances). The current debate on the origin of *hot spots* of megadiversity is very linked to the above considerations.
In addition, structural and functional attributes of biodiversity can only be determined by a proper consideration and definition of appropriate scales of space and of time. It is only with these principles in mind that the process of speciation and that of species extinction can be approached in scientific terms, being exempt of the emotional catastrophism that is prevailing today in the public opinion and in the media. Unfortunately, attitudes and actions of decision-makers at the national and international level are strongly influenced by the "catastrophe" approach, mostly in the countries of the North.

As concluding remarks, there are insurmountable obstacles to tackle in isolation the scientific problem of biodiversity, as well as some managerial implications for species preservation and conservation of natural areas. No single discipline, certainly not ecology nor biosystematic, can claim to be able to approach comprehensively the various aspects of biodiversity at a local or global level. If a general theory of biodiversity is based, as a central phenomenon of life, on the hierarchical theory of successive levels of organisations and of the subsequent emerging properties, it concerns the universality of all the biological world . To such universality, biodiversity can contribute by playing a unifying role in view of the pervasive and not exclusive nature of its approach.

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Stigmatization by Phenotype and Genotype

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In 1927 a book appeared in France which carried the provocative title "La trahison des clercs" (1). The word "clerc" is untranslatable. One could translate it as scholar, intellectual or - scientist. The author claimed that philosophers, sociologists and political scientists of his time had committed treason by abandoning either truth and reason or justice or both to serve the nation, race or class. In a new edition which appeared shortly after WW II he added one scientist to his long list of non-scientists, Alexis Carrel, a French Nobel Price winner and eugenicist. He could have added most German Non-Jewish professors of sociology, law or philosophy (2) and particularly almost all German Non-Jewish professors of human genetics (anthropology, psychiatry etc.) (3).

It is a curious fact that in 1927, the year of the appearance of this book, an International Congress of Genetics was held in Berlin. Hermann J. Muller lectured there about his discovery of the mutagenic action of X rays and so gave the first rationale of the existence of alleles of genes. The president of the congress was the German human geneticist Eugen Fischer. He had just become director of the Kaiser Wilhelm-Institute for anthropology, human genetics and eugenics in Berlin-Dahlem (the Kaiser-Wilhelm Society now calls itself Max-Planck-Society). He was an excellent scientist and most influential. He may listed among those who committed treason of justice, but not (or only rarely) treason of truth and reason. I would like to list here some of his treacheries:

- As elected rector of Berlin University he dismissed his Jewish colleagues in 1933 and 1934.

- He was instrumental in the phrasing and executing of the law which allowed the involuntary sterilisation of the feeble minded, schizophrenics, manic depressives, and carriers of Huntington's chorea. In between 1934 and 1939 about 350,000 German citizens were sterilized under this law.

- He was involved in the illegal sterilisation of all (about 700) coloured German children.

- He asked in 1933 for a law outlawing marriage and penalizing intercourse between (German) Jews and Non-Jewish Germans. This became real in 1935 as one of the Nuremberg laws.

- He asked for a law which would allow the involuntary sterilisation and incarceration in concentration camps of petty criminals ("Asoziale"). About one million people (including most Gypsies) were singled out. But the law never materialized. Many Gypsies were sterilized and about 20.000 were transferred to and murdered in Auschwitz.

- I could go on, but just refer to my book (3).

It is important to point out that Fischer and the experts involved in these and other such actions tried their best to be rational when defining the people that did belong or did not belong to a particular group. It was not the treason of science but the injustice which was offensive. It should be pointed out that neither Fischer nor any one of his colleagues suffered any serious inconveniences after 1945. The international scientists did not care and the German ones were loyal to each other.

It is also worthwhile to point out that the nature of genes (DNA) was not known at the time. This has since changed radically. Today the genotype can be determined in some of the cases mentioned above. It should also be said that the feeble minded are now treated by the authoritarian regime on mainland China as they were treated half a century ago by the German authorities. The most developed countries such as the USA, France or Germany may end in a different but equally dangerous abyss should they decide to rely essentially on the market economy with regards to differences of genotype. Let me explain:

- The genotype of Huntington's chorea can now be accurately determined. In the USA, where no public medical insurance exists, it does not make sense for an insurance company to insure a carrier of Huntington's chorea at a normal low rate. The economic rate may be so high that the person can not get insurance. He then belongs to a genetically defined underclass, or better, under-race.

- Similar tests do not exist for schizophrenia or manic depression. So far all attempts at isolating relevant genes have failed. This may be due to wrong or misleading definitions of the various phenotypes. But it is conceivable that sooner or later relevant genes and alleles will be discovered and that DNA tests will come into being. This would then hit one or more percent of the population. It is far from clear whether medication is the cheap effective solution of their social problems. Again stigmatization may become common if it is not outlawed in time.

- No gene has been isolated that is responsible for intelligence, however one may define intelligence. This does not imply that such a gene and its alleles can not be found. Today most of those who claim that IQ is inherited draw the conclusion that less money should be spent on the education of people with lower intelligence. I think just the opposite is necessary. Those who do not believe that the inheritance of IQ is already demonstrated rigorously, and I am one of them, should consider what to say when it will be demonstrated, particularly when ethnic, racial differences may be shown.

- Finally, a lot of effort is directed today to demonstrate the inheritance of what is called euphemistically "violent aggression" and what is identical with crime. In one case the successful isolation of a gene predisposing for violent aggression, rape, arson, etc. has been claimed (4). A more benevolent interpretation would say that the carriers of the inactive allele are acting spontaneously too often. I think it is totally wrong to connect the mutant genes with the phenotype of violent aggression i.e. crime. To claim that crime is a direct consequence of a mutant gene denies the free will of the afflicted person. It degrades him to a machine. It should not be done.

The geneticists of today will play a decisive role in the use of their science. Will it be used to create an underclass (under-race) to suit the market? Will it be used to help the afflicted? The next generation of geneticists will be judged eventually how they will act before this dilemma: Will they help or abandon the helpless?

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Scientists and Eugenics : a Recurrent Danger

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The social implications of human genetics are of such an importance that the subject *must* be treated. There is no other organism for which a detailed and extended information on anatomy, development, physiology, biochemistry, evolution and population statistics is available. These advantages have led to important discoveries , to cite only the nature of blood groups or the anomalies of haemoglobin.

The systematic study of human genetics has started before Mendel with the work of Francis Galton(1822-1911), a cousin of Darwin. Following 1900, we see an accumulation of information concerning the Mendelian inheritance of aberrant conditions in man, starting with Farabee's account of brachydactyly (short fingers) in 1905. As early as 1902, Garrod and Bateson had suggested that alcaptonuria (a benign metabolic abnormality bearing on the catabolism of aromatic amino acids, first described in 1649, and conferring a black color to urine) is due to a single recessive gene, but the evidence remained inconclusive until Garrod reported an adequate number of families in 1908 .

The more familiar and obvious differences, such as stature, hair, eye and skin color, fingerprint patterns , right- *versus* left-handedness, are difficult to analyze. In other mammals, eye and hair color are among the best understood of the inherited characteristics, but in man, there are so many intermediates that analysis is difficult. Red hair and blue eyes are often cited as due to recessive genes, but their classification is often uncertain, and one notes contradictory observations in the usual popular descriptions.

The mental characteristics , of the greatest interest and importance to society, .are even more difficult to analyze. At the sensory level, there are well-established Mendelian differences, such as the sensitivity to certain tastes, hemeralopia (night blindness), or daltonism (color blindness). At the other extreme, there are Mendelian characters which implicate serious mental disorders (e.g. phenylketonuria or Huntington's disease). The range between those extremes is the most difficult and interesting to analyze. One of the first attempts has been made by Galton. He is responsible for the expression " nature *versus* nurture". In 1869, he collected a series of pedigrees showing the concentration of particular kinds of exceptional achievements in certain families, such as musicians in the Bach family. He minimized the effect of family tradition and concluded that the results were primarily due to biological inheritance, despite one case that he pointed out but did not emphasize: in the Roman family of the Scipios, there was an extraordinary number of generals and orators, but one of them, Scipio Aemilianus, "was not of Scipio blood" but was adopted, suggesting (though not to Galton) the importance of family tradition rather than genetic composition.

The same approach was followed by Davenport in 1911. He describes the Tuttle-Edwards family of New Haven, where we find two presidents and one vice-president of the United States of America, six college presidents, and numerous notables. In the Lees from Virginia, one finds many generals and politicians. On the other hands, he

cites the Jukes and Kallikak families, with a dreary list of prostitutes, drunkards, thieves and paupers. Davenport also ignores the overwhelming importance of family environment and of the resulting lack of opportunities. He should have realized that a potential college president, or of a potential member of the Virginia legislature, would have had no chance of realizing his potentialities, had he been born into a Jukes family! Since 1900, other biologists, some of which definitely more sophisticated than Davenport, such as Bateson in his Herbert Spencer lecture of 1912, where he marvels "*that the more divergent castes of civilized humanity are capable of interbreeding and of producing fertile offsprings from their crosses. Nothing but this paradoxical fact prevents us from regarding many classes* ***even of Englishmen*** (my emphasis) *as distinct species in the full sense of the term*". In the same vein, Darlington writes in 1953 (The Facts of life): "*In England, for example, it is not lack of research which limits food production but the genetic unfitness of a large part of the tenant farmers, the legally secured occupiers who are organized to keep better men off the land*".

The discussions on this subject have been very emotional, because unambiguous objective evidence is difficult to get. The extreme proponents of genetic determination are in general conservatives, whose views are ultimately rooted in the caste system of feudalism, while the advocates of environmental control represent a political philosophy derived from the egalitarianism of the French Revolution.

The most effective approach was initiated by Galton in 1883 when he studied pairs of twins and recognized that they could be classified in two classes, "similar" and "dissimilar", and concluded that they arose respectively from a single fertilized egg and from two independently fertilized eggs, a conclusion since confirmed by embryological and genetical evidence. The two classes are referred to presently as monozygotic and dizygotic. He saw the opportunity offered to test the relative contribution of heredity ("nature")and environment ("nurture"), since the monozygotic twins should be alike in genetic makeup, while the dizygotic twins should be no more alike than ordinary brothers and sisters. He carried out a few tests on mental properties and concluded that the monozygotics were more alike in their behavior. In 1925, Mueller found a pair of monozygotic twins who had been separated in early life and brought up in different families. The psychological tests he conducted showed that the twins reacted in a similar manner. This type of studies was extended in 1937 to twenty separated monozygotics, with controls on a series of reared together monozygotics, and also of dizygotics. The authors were disappointed by the inconclusiveness of the results. Later series of such studies have also been quite disappointing, notwithstanding their importance. The difficulties encountered originated from 1) the different ages at which the separation had taken place, 2) the inaccuracy of the tacit assumption that twins reared together are exposed to identical environmental effects , 3) the fact that the separated twins were usually reared in rather socially similar families (none was brought up as a Lee and his twin as a Jukes!) 4) the uncertainty as to just the psychological tests were measuring. However, these studies have established that there is an appreciable inherited component in the determination of human mental differences.

The difficulties of an objective study of mental differences are even more striking in the case of racial differences. If we admit that there are inherited individual differences, we may conclude on general grounds that there are statistical differences between races. If one has a tendency to consider that individual mental differences have a predominantly

genetic origin, one is likely inclined to consider that the observed or imaginary cultural differences between races is being genetically determined and to conclude that some races(and in particular, the one to which one belongs) are inherently superior. The persons with this attitude have not generally received a scientific education; the terrible example is Adolf Hitler, but he was preceded by many pseudo-scientific writers (Gobineau, Houston Chamberlain, Madison Grant, etc...), many of which would probably have been horrified by Hitler's atrocities.

There are unfortunately *bona fide* biologists and geneticists who have professed such racist theories(see the book by Benno Müller-Hill, *Science Nazie, science de mort.L'extermination des juifs, des tziganes et des malades mentaux de 1933 à 1945*, Editions Odile Jacob, 1989 and that edited by Josiane Olff-Nathan, *La science sous le Troisième Reich. Victime ou alliée du nazisme?* Editions du Seuil,1993).

Galton has been the first to suggest the possibility of the genetic improvement of human populations, for which he introduced the word *eugenics*. Two types of eugenics have been described as " negative" and "positive". Negative eugenics proposes to decrease or eliminate the more extreme inherited defects- physical or mental- and positive eugenics proposes to increase the number of better individuals, increasing the possibilities to obtain even better ones.

Both approaches are valid for agricuture: a glance at a fruit market is sufficient to discover the splendid results obtained by the peasants of yesterday and the farmers of to-day. The existence of our beautiful cherries, apples, pears or grapes obtained after generations of selection does not need a justification. The same goes for animal breeding.

But what about man himself? Human genetics has influenced a great number of human sciences such as anthropology, psychology and psychiatry, to cite only a few . If one studies the effects of genetics on anthropology and psychiatry, recent history shows an immense field of ruins and of crimes. Many geneticists, anthropologists and psychiatrists have emerged from this nightmare only to plunge into the deep sleep of amnesy.(see the book by Müller-Hill, cited above).

Eugenics is not an ideology imposed to science by politicians. It finds its roots and developments among talented scientists and medical doctors. It has then been exported to the political domain with their blessing. Galton and Fisher are among the founders of human genetics, even if other eugenists like Davenport and Lenz have not left the same traces in the history of science.

Eugenics is based on the notion of the qualitative importance of the genes and that a means to change their distribution in the population lies in the change of reproductive habits; thus, one could encourage an increased reproduction among the carriers of "good" genes or a decreased reproduction among the carriers of "bad" genes. The first option (positive eugenics) being uneconomical has been rarely applied. A policy of limitation of the number of undesirable individuals, implying less direct costs, has appeared more attractive : there have been forced sterilizations among the mentally retarded and the individuals with an aberrant behavior, in the states of California and Virginia, and later in Nazi Germany. The linkage between eugenics and "racial hygiene" lead to the promulgation of laws on immigration and " cross-breeding". The

logical conclusion was finally the elimination of the carriers of undesirable genes by the genocide and the holocaust.

One remains dumb with amazement in reading the following lines, written by Emile Guyénot, a first class geneticist, corresponding member of the French Academy of Sciences, professor at the University of Geneva, in the fourth edition of his otherwise excellent textbook " L'Hérédité", published in 1948, at a time where the horrors of the Nazi politics were known by everybody :

Devant les progrès de la science de l'hérédité, beaucoup de savants, ayant voué leur activité à l'amélioration de la race humaine, ont jeté les bases d'une science nouvelle, l'Eugénique, qui se propose , entre autres choses , d'éliminer par un contrôle judicieux des unions, certaines de ces maladies héréditaires qui sont un fléau de l'humanité. Quelques-uns ont proposé une législation prohibitive; d'autres croient suffisant d'éclairer le public sur le danger de certaines unions. Il semble que l'on puisse de façon générale s'en tenir aux indications suivantes. Les individus, porteurs de maladies dominantes ,étant assurés de transmettre leur maladie à au moins une partie de leurs descendants, l 'abstention serait pour eux la seule règle de conduite. Les individus, ayant eu des ancêtres porteurs de maladies récessives, doivent savoir qu'une union consanguine a des chances d'être néfaste pour quelques-uns de leurs descendants.
Les efforts de l'eugénique ont été raillés par ceux des pseudo-savants qui prennent leurs illusions pour des réalités. La vérité est qu'il est scandaleux, alors que les lois de l'hérédité permettent d'améliorer les races de boeufs, de chevaux ou de cochons, que seule l'humanité continue à se reproduire au hasard comme elle le faisait à l'âge des cavernes. Situation d'autant plus grave que les progrès de la médecine, de la chirurgie, des conditions sociales tendent à conserver et à mettre en état de procréer les déchets humains que la sélection naturelle aurait autrefois éliminés. Les dégénérés sont légion; les hospices d'aliénés manquent de place; la létalité stérilise les familles. Les états qui n'auront pas su comprendre à temps paieront cher leur imprévoyance.

As François Jacob mentioned in *Le jeu des possibles* , there is a constant proportion of malevolent people and imbeciles in all the samples of a given population, among scientists and insurance agents, among writers and peasants, among priests and politicians.

Scientists should remain aware of the constant resurgence of eugenics, under a form or another.

Concluding Remarks

by François Gros

Biology often addresses questions or analyzes situations which situate at the hinge between exact, natural sciences, and activities which rather belong to social and human sciences. This has probably always been so but it is even more pronounced nowadays following the rapid break throughs in genetics, neurobiology and immunology. As a consequence, the end of this millennium is accompanied by an explosive flourishing of questions (and relevant committee's...) in the field of bioethics but, perhaps even more significant is the fact that almost everyone, from laymen to experts, is tempted to re-explore or re-visit classical concepts and interrogations concerning man and his place in our biosphere ! One such interrogation has to deal with the notion of individuality : what distinguishes each individual from each others ? what makes him unique, irreplacable and possessor of both actual and potential qualities ? Can molecular biology, often indicted of being a reductionist science, shed light on this problem ? What part does the biological background play if anything, in the most sophisticated expression of our personality, as far as we regard ourselves (and I would like to think this is justified) as responsible living beings ? These questions are conducive of an objective analysis of the implications of such situations as "uniqueness" and "diversity" in a living world. This has been the topics addressed in this meeting. This is not, indeed an easy paradigm to embrace, and it would be an impossible task for me, to provide, in what follows, a fair, exhaustive survey of the various facts and points of view which have been presented so far ! But, from a superficial standpoint, and for pure convenience, the kind of "bird's eye view" I can offer, leads me to subdivide the contributions and accompanying discussions that have taken place, into five levels of increasing complexity.

I will then discuss :

i) *the primary DNA level,* where focus was placed on genetic variation, in relation to evolutionary (phylogenetic) mechanisms.

ii) *the ontogenetic level,* comprising, among a variety of aspects, the embryological and developmental strategies involved in the morphological, physiological and even behavioral "shaping" of individual patterns. This is a level at which epigenetic events are postulated to play a major role.

iii) *the higher order organisational level,* the level "par excellence" at which, or by which, what we regard as our "personality" begins to emerge. Here neurological and psychological references to our individuality will predominate. This level is dealing with problems such as training, education, cultural imprinting, and elicits the most delicate interrogation of this meeting : is there a biological background to these characteristics often regarded as marking the difference between *Homo sapiens* and the other most evolved primates ? If so, what do we know ?

iv) *the ecosystemic level,* whose role in "speciation" has often been underlined by the specialists of evolution (for example due to the phenomenon of biogeographic isolation), but whose effect on the expression of individuality is less conspicuous.

v) *the ethical level...*

The primary DNA level

When we speak of genes, do we mean that all genes perform the same function, particularly as far as determining and maintaining individual features ? The concept of regulatory genes, introduced in the early 60's has clearly shown that our genome can be equated with an ensemble of networks related to integrated functions, each of these networks being able to respond to environmental modulations. As biologists turned their attention towards higher organisms, they soon discovered something that first presented itself as an embarrassing paradigm, namely the fact that very few genes are found as unique determinants of a given product within the cell. The most common situation is that which is referred to as genetic *redundancy* : in many instances genes can be recognized as being members of a multigene family, each member encoding products of very similar, albeit slightly different characteristics. This situation is often viewed as the net result of successive and cumulative recombinational events. The role of these "isogenes" and of their products, amazingly, is still a matter of debate. Do the existence and maintenance of multigene families correspond to (a) defined function(s) or does it constitute a purely gratuitous phenomenon, only reflecting some kind of genuine properties of DNA ? The question has remained unanswered, and there are arguments for both interpretations. But generally speaking, relatively little attention has been payed to the relevance of genetic redundancy at an *individual* level. This remark should be somewhat "tempered" in the case of mitochondrial DNA which, as was reported at this meeting and as is well known, is the site of very frequent and hence very recent mutations, and can be the target for the accumulation of age-related DNA damages, the physiological consequences of which begin to be relatively well documented. Their existence not only gives precious information about the age-related alterations of individual genotypes (at the cytoplasmic DNA level) but also provides to a certain extent some sort of a genetic signature of "individuality".

Some speakers at this meeting, drawing conclusions from an analysis of the microbial world have proposed to distinguish the role of two categories of genes : i) genes encoding functions which can be regarded as matching the biological needs of individuals in terms of survival, development, but also homeostatic adequation to "daily life", and, ii) genes which, although not obviously, or evidently advantageous to individuals under ordinary living conditions, might turn out to secure potentially better eco-adaption in special or extreme environments ; the genes of this latter category might be regarded as *conditionally useful*, depending largely upon the environmental context. This concept can perhaps apply also to explain the paradox of multigene families, once we realize that different members of the "family" might obey different regulatory patterns. Should we go as far as suggesting that this confers individual advantages as well as being a way for a given species to be "buffered" against extreme conditions...? This remains an opened possibility.

From the session devoted to the state-of-the-art knowledge about the genome programs (man, yeast...) it is clear that we are gaining considerable knowledge about the existence of *new* genes in various living forms (this is particularly striking in the case of yeast for example), but also about the fine-detailed genomic organization of the hereditary material in different species's. This is based, in particular, upon sophisticated, statistical and comparative analyses of the distribution of genetic sequences in the various gene banks, but also upon a better understanding of the overall organization of the genomes among different species's (cf. Bernardi's isochores) by looking, for example, at differences between cold-blooded and warm-blooded vertebrates. One of the most significant consequences of the efforts involved in the genome programmes analysis is, unquestionably, to "revolutionarize" our approach to public health (mapping of "candidate genes", genotypic diagnosis, gene therapy... etc). As to the consequences for human beings as *individuals,* they are more difficult to assess ; these might in a sense be contradictory since, on the one side, each person could, at a long range benefit from a more precise knowledge about the origin, nature and fate of genetic diseases (this is what is meant by predictive medicine) but, on the other side, as a result of the extensive categorization of these diseases, medicine at a long range might adopt an essentially "collective" and hence, slightly less "human" approach to patients. Systematization involves both good and bad aspects !

Several lectures have addressed the fascinating problem concerning *the origin of genes* themselves. This field is gaining support and enjoying progress from a variety of approaches, even though one must regret that the colloquium, due to the absence of some speakers, could not touch very much upon the rapidly evolving "RNA world"...

Genetic diversification, namely the formation of new genetic entities, or the modifications of pre-existing ones, is often contemplated by biologists as being both, the main driving force, and the mere reflection of the evolutionary pathway of the living species's from this earth. This "neodarwinistic" view is based upon the observation that point mutations, recombinational events, transpositions... together with exon reshuffling and loss of introns...etc., have played a major role, not only in the forming of new multigene families, but also in the constitution of new genetic entities by the joining of pre-existing motifs. But, to look at this problem from a different angle, if one takes into account the number of somatic mutations, the changes in RFLP patterns, or in satellite distributions... etc, it is clear that each individual from this planet, *differs from any other one by thousands and, most probably, even by millions of tiny DNA characteristics which represent like a "signature" of his uniqueness* (in some cases, these characteristics are utilized as markers to finger-print DNA samples and hence as means of identification...) But, with the (important) exception of genetic diseases or alterations in the HLA haplotypes, some of which are paralleled by the presence of these polymorphic traits on the DNA (ex : degree of gene amplification, satellite distribution, RFLP's...) one usually does not know whether, and to what extent, these "traits" are associated with defined physiological or behavioral consequences.

Ontogenesis and the biological basis of individuation

Many other facets of the mechanisms underlying the phenomenon of "individuation" as it manifests itself during ontogenesis have also been addressed during this meeting, some of which involve somatic DNA rearrangements (acquisition of the antibody

repertoire) while others comprise more complex epigenetic phenomena. The number of combinatorials which are involved in the formation of immunological and neurobiological networks is enormous ; at least, is it sufficient to explain why each individual on earth harbors a unique spectrum of defence mechanisms against environmental agressions, or can adopt typical behavorial or emotional attitudes while facing a specific situation, *due to training, experience and also at a neurobiological level to the stabilization of very complex arrays of functional synaptic connections, in response to specific stimuli*. The various combinatorials which are at the basis of, or reflect the biological individuality, often involve proper macromolecular interactions. But phenomena, like clonal selection, cell migration, positional information, programmed death... etc., also contribute largely in "shaping" this individuality at the tissular or organ level, a situation which gives rise to different, and in most cases, unique morphological, metabolic or physiological patterns. Biological uniqueness during ontogenesis is also achieved through the differential synthesis and positioning of a complex array of tissue specific antigens present *at the surface* of the cells or by the existence of various spectra of surface receptors, adhesins, ion channels... Thus, our tissues, or the whole of our body, displays a genuine, unique susceptibility to exogenous stimuli (neurotransmitters, hormones, growth factors...etc).

To what extent is this mosaïc of antigens and receptors any different from one individual to another ? Although this problem is, thus far, very poorly documented, recent studies carried out on the olfactory receptors shed some light on their extraordinary diversity, some of these receptors being highly specific in responding to defined chemical signals (pheromones) while others display a broader spectrum of recognition but their "decoding" by the central nervous system reflecting largely an individual behavior acquired by training and experience.

Neurobiological aspects of individuality

In highly evolved animals and in *Homo sapiens*, individuality is largely "neurobiological", or at least neurobiologically-based, given the considerable development of the Telencephalon with respect to other more "primitive" areas of the brain.

Biologists, over the last decade, have achieved great progress in elucidating the mechanisms underlying the development of the central nervous system (neurogenesis). This is largely due to combined approaches to the problem, such as : embryology, ultrastructure studies, electrophysiology, as well as molecular genetics. The utilization of simple models, particularly drosophila, has paved the way to the fine-detailed analysis of the genetic control operating during the segmental morphogenesis of the nervous system. As is presently well known, homeotic genes - a superfamily of genes coding for a special category of transcription factors - operate like master regulatory devices which direct the topological ordering of the segmental parts of our body and, more particularly, the dorso-ventral positioning of the various anatomical elements of the central nervous system (CNS). Moreover, these genes are often located themselves on chromosomes in a tandemly arranged fashion and expressed according to a vectorial and temporal cascade reflecting their functional involvement in the control of morphogenesis. If one realizes that these genes are expressed in a strictly integrated and interactive fashion, and that they display a high degree of conservation in their

sequences and relative order on chromosomes throughout evolution, it is clear that modern biology has unravelled what appears as a major clue in the ontogenetic control of the nervous system. In addition, studies of mutations which alter the organization of the nervous system in drosophila have revealed that other classes of regulatory genes (with an HLH motif) do operate as integrated networks, or so called "syntagma's (G. Bellido), which are involved in the developmental positioning and anatomical location of certain neurons or groups of neurons, such that precise correlations can presently be drawn (however preliminary they might be) between the functioning of genetic "syntagma's" and the three dimensional assembling of certain CNS components. Lineage studies in nematodes have also provided considerable information. Last, but not least, the embryonic neural chimera technique (quail-chicken transplantation experiments) extensively developped by the Le Douarin school, enables one to analyze the relation existing between well defined portions of the brain, and certain specific behaviors (the nature of the song in birds for example, or the sensibility to manifest seizures following an epileptic shock). One central problem in neurobiology, and also the most relevant to analyze the very complex mechanisms of "individuation", is to understand the neurobiological determinism of behavior, and also symmetrically the way training, learning, education... etc (more generally speaking, individual experience) might exert some imprinting effect by modifying our nervous system. The first question can receive certain answers from the comparative analysis of anatomical and behavioral disorders in certain neuropathological situations. As to the second question - the role of experience in the patterning of our neuronal ensembles - it is presently tackled on simplified models and takes largely into account the synaptic consolidation model (Changeux, Danchin, Courrège) largely derived from studies related to the control of acetylcholine receptor genes, at the neuromuscular junction, following moto-neuronal stimuli. It is nonetheless evident that the "mapping" of the major brain functions, sometime announced by the community of biologists like one of the most significant achievement from ongoing neurosciences in the forthcoming decades, will continue to gain considerable support from the convergent efforts from ethologists, neurophysiologists, neuroanatomists and psychologists... not to forget biophysicists who are applying positon-camera techniques to determine the physiological activation of specific brain areas during the expression of precise cognitive functions. *It is likely that multidisciplinary neurosciences should, at a long range, inform us about certain basic aspects of our individuality at a "cognitive" level.*

Ability to speak, which is correlative in evolution of a high degree of differentiation of the phonatory system and of the "Broca" and "Wernicke" areas of the encephalon, probably first appeared in *Homo habilis*, 1.5 million years ago... As was illustrated at this meeting, it implies, as far as its ontogenic manifestation, very precise neuro-ethological prerequisites in infants, such as learning categorization, namely loosing the multiple unordered impregnation of the brain, in favour of a preferred communication system. Ability to speak represents a major "jump" (together with the process of visual self-recognition) in the development of individuality, which begins with the self-consciousness of one's own identity !

Ecosystemic and ethical levels

It would take too much time to digress about the place of man in the universe and the problem of anthropocentrism. As was sayed in the opening session, that an individual being unique does not mean that he is alone and therefore "prisoner" of his uniqueness,

a uniqueness which he would contemplate with a feeling of superiority over other individuals or members from other species's.

Firstly, there is no need to emphasize the commonality of traits among living beings at molecular and cellular levels, for it is precisely the "credo" of molecular biology itself ! We have heard for example that human diseases, in terms of their underlying genetic alterations, can also have their genetic counterparts in... yeast.! Paradoxically so, it is the molecular biology, often designated as responsible for having spoiled the concept of biodiversity (due to its frequent utilization of simplified models...) which probably tells us best, how much universal is the biosphere, and how much respect human beings should pay to the so-called lower organisms... But, I would like to end up these concluding remarks by few ethical considerations. Biologists nowadays, cannot avoid or by-pass certain basic questions regarding the society as a whole. Time is over where science was contemplated as the universal receipt to reach human wisdom, peace and welfare... Eugenistic attitudes, derived from the wrong interpretation of scientific observations, but also, and quite sadly from the theories that have been put forward by certain scientists themselves, have often prevailed, in the past, and continue to prevail with their most inhuman connotation (as was recalled during this meeting). The risk is always very great to extrapolate to human beings, or human societies (with the argument that it is scientifically-established) certain concepts or theories based upon observations made on animals. Genetics, needless to say, when over or mis-interpreted can give rise to sociologically unreasonable and sometime murderous situations (*Cf.* B. Müller Hill), some of which have been amply illustrated during the national-socialism period of the last world war... Let us not forget that too often are scientific results "utilized" (and I should even say "captured") to propagate false messages, or even worse, to reject or stigmatize minorities on the basis of their phenotype. Scientists must be very attentive to it.

But, I think that very few of the biologists, nowadays, are of the belief that their only concern is to do science, *irrespective* of its social and ethical consequences. The majority of them is strongly committed to defencing the value of biodiversity, not only to protect species's which are about to disappear, like this is judiciously advocated by ecologists, but also because it feels it is his duty to describe and maintain the fantastic and wonderful diversity of living beings, *as individuals*. Each of these individuals is unique, each one is the representative of billion years evolution. Each one has a great symbolic value in himself ,"here and now", that is to say regardless of the species he belongs to and of its evolution.

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