Paths of Discovery
Pontifical Academy of Sciences, Acta 18, Vatican City 2006
www.pas.va/content/dam/accademia/pdf/acta18/acta18-arber.pdf

THE IMPACT OF MICROBIAL GENETICS ON THE DEVELOPMENT OF GENOMICS AND BIOTECHNOLOGY

WERNER ARBER

Introduction

In the last 60 years, research in the life sciences has uncovered a wealth of information on biological functions. This has mainly become possible by introducing new research strategies including the experimental exploration of biologically active molecules and their interactions, in using among other means quantitative measurements of relevant parameters, improved imaging techniques, biophysical, biochemical and molecular biological approaches as well as bioinformatic tools. In this article, special attention will be paid to developments that have their origin in microbial genetics. Within this context we shall highlight a few particular discoveries in which the author had been personally involved.

Roots and Early Development of Genetics, Evolutionary Biology and Nucleic Acids Biochemistry

Classical genetics goes back to Gregor Mendel who described in 1866 that phenotypic traits of peas become transferred into the progeny and that plants with different traits could give rise to recombinants upon sexual reproduction. This scientific approach started to flourish in the first half of the 20th century.

Contemporarily with Mendel's findings, Charles Darwin published in 1859 the theory of natural selection representing the major root of evolutionary biology which can explain the wide natural biodiversity that forms a pillar of ecology.

It is around 1940 that scientists built a bridge between the hitherto independently developed fields of genetics and evolutionary biology. This so-

called modern evolutionary synthesis explained that genetic variants are at the basis of altered phenotypic traits and that they also form together with their parents the substrate for natural selection and thus the evolutionary development. At that time, histological evidence suggested that genes reside in chromosomes, but their chemical nature remained unknown.

A publication made in 1874 by Friedrich Miescher is at the root of nucleic acids biochemistry, which in 1953 culminated in the description by Watson and Crick (1953) of the double helical structure of DNA insuring a high degree of fidelity upon the replication of the filamentous DNA molecules.

The Early Days of Microbial Genetics

Bacteria are haploid, unicellular microorganisms that reproduce by cell division. For a long time they were thought to have no sexuality. It is probably for this reason that geneticists did not pay attention to bacteria (Zuckerman and Lederberg, 1986). This changed only in the early 1940s when microbial mutations were isolated and when particular mixtures of cells with different identified traits yielded recombinants (Lederberg and Tatum, 1946; Lederberg, 1947). At the same time Avery, MacLeod and McCarty (1944) could show that DNA, rather than protein, is the carrier of genetic information. In the crucial experiment they mixed highly purified, hence protein free, pneumococcal DNA with living cells of another strain of Pneumococcus bacteria. Under these conditions some of the latter cells acquired traits characteristic for the strain that had been the source of the DNA. It took about ten years until the biologists recognized the importance of this transformation experiment and the conclusion regarding the carrier of genetic information. The likely reason for this delay is seen in the fact that chromosomes are composed of DNA and of proteins. The higher degree of complexity of proteins, as compared to that of nucleic acids, had stimulated the scientists of that time to assume that the postulated complex nature of a gene could best be explained by proteins. The breakthrough to the acceptance of the conclusions of Avery *et al*. (1944) was greatly helped by the knowledge of the linear structural features of DNA molecules. This knowledge opened new avenues to explore the genetic code and the embedding of genes into the long DNA filaments.

Different Natural Means Provide Opportunities for Horizontal Transfer of Genetic Information Between Different Strains of Bacteria

As we have already seen, free DNA molecules liberated by a donor bacterium may sometimes be taken up in transformation by a genetically distinct acceptor strain (Avery *et al.*, 1944). In contrast, the process first explored by Lederberg (1947) is based on a kind of sexual mating between two bacterial cells. In this process of conjugation, donor DNA becomes linearly transferred into the acceptor cell through a bridge built between the mating partners. A third natural possibility of horizontal gene transfer depends on a viral particle as a vector for a segment of the donor genome. This process is called transduction and it was first described by Zinder and Lederberg (1952). It will catch our specific attention below.

In any process of transfer of genetic information from a donor to an acceptor cell, the transferred DNA must become firmly associated with the acceptor genome, should it get inherited into the progeny of the acceptor cell. Microbial genetics has described different natural ways to accomplish this requirement by recombinational processes or by maintaining the acquired DNA molecule as an autonomously replicating unit, a so-called plasmid.

Cointegration of a Viral Genome with the Bacterial Host Genome

It had been seen in the early days of microbial genetics that some bacterial viruses could strongly associate with their host bacteria for a more or less extended time. In this situation the host survives the infection and it propagates. Once in a while the genetic information of the virus can become active in one of the progeny cells and produce viral progeny particles. The host cell will thereby die by lysis. The underlying phenomenon is known as lysogeny. Its explanation goes back to studies by Lwoff and Gutmann (1950). Further explorations later revealed that some viruses integrate their genome temporarily into the host genome, while other viral genomes are rather maintained as plasmids.

When a cointegrated, endogenous virus, also called provirus, becomes activated again, its genome becomes excised from its chromosomal location. This is brought about by the same site-specific recombination enzyme that is also responsible for the cointegration in the establishment of lysogeny.

Study of Proviral Mutants

Most of the viral genes remain silent, unexpressed in the proviral state. A provirus may thus accumulate spontaneous mutations in the course of time. Some of these mutations will inactivate genes that are essential for viral reproduction. The study of such mutations was the topic of my PhD thesis. Some of the mutants analyzed were unable to produce intact viral particles upon reactivation of virus reproduction. The deficient structural elements (such as empty heads, filled heads, tails, association of empty heads with tails of the bacterial virus) could be quantitatively analyzed in the electron microscope (Arber and Kellenberger, 1958). This allowed us at least in some cases to identify the specific gene function that had been hit by the mutation.

In this electron microscopical study we also included a viral derivative which transduced the genetic information of bacterial origin encoding the fermentation of the sugar galactose (Morse, Lederberg and Lederberg, 1956). In its proviral form this derivative was unable to produce any viral structures visible in the electron microscope. The only way out of this surprising situation was to undertake genetic studies. These investigations revealed that a relatively important segment of the viral genome was fully absent. Obviously, it must have been substituted by a segment of the host genome carrying the genes responsible for galactose fermentation together with a few other genes (Arber, Kellenberger and Weigle, 1957, 1960; Campbell, 1962). As we will see below, this hybrid structure associating host genes with a part of the viral genome became in the early 1970s a model for gene vectors serving in recombinant DNA technology.

Horizontal Gene Transfer Encounters Several Natural Barriers

In the early years of microbial genetics, it became rapidly obvious that the efficiency of horizontal gene transfer varied widely in function of the genetic and evolutionary relatedness of the bacterial strains involved. Barriers act against DNA acquisition at various steps of horizontal gene transfer. First, the surface of the acceptor cells must be compatible with the needs for a successful uptake of the donor DNA. Second, enzymes of restriction-modification systems can distinguish between foreign DNA and the cell's own DNA, as we will more clearly explain below. Third, the transferred DNA has to become firmly associated with the acceptor genome in

order to become effective in the progeny of the hybrid. And, finally, the newly acquired genetic functions must be compatible with the functional harmony of the entire genome in order to withstand the pressure of natural selection.

The Discovery of Restriction Enzymes

In their work with bacterial viruses involving more than one bacterial host strain, several investigators observed in the 1950s that the involved virus grew very inefficiently upon a change of the host bacteria (see Arber, 1965b). In general, the few progeny viruses obtained could, however, efficiently re-infect the new host. But often, once adapted to the new host, the viruses did not any longer infect efficiently their previous host bacteria. This phenomenon was called host-controlled modification. The adaptation to a new host was called modification, and the inefficiency of infection upon the change of the host was called restriction. Since restriction was observed also upon periodic back and forth changes between a pair of two distinct hosts, the scientists correctly argued that modification could not be explained by a genetic mutation. Therefore, many scientists thought that modification was brought about by some host protein which became associated with the viral particles.

In 1960 I became unexpectedly confronted with the molecular basis of host-controlled modification. As a postdoctoral investigator at the University of Geneva I got engaged in a project to study the effects of different types of radiation, such as ultraviolet light, X-rays and radioactive decay, on living organisms. This research on biohazards of radiations was carried out in the context of a Swiss national program of research in view of the peaceful use of atomic energy. Our intention was to carry out our studies with different strains of *Escherichia coli* bacteria and with bacteriophage λ. In the course of preparing several *E. coli* strains which should also serve as hosts for growing the bacterial virus λ we encountered the phenomenon of host-controlled restriction. Driven by the curiosity to understand the molecular basis of this phenomenon we undertook a series of one cycle growth experiments. These revealed that against the general assumption it was the phage DNA rather than a host protein which was the subject of modification (Arber and Dussoix, 1962) and which was also the target for restriction. Indeed, restricted phage DNA became rapidly degraded upon infection of a restricting host and this explained the high inefficiency of the

infection (Dussoix and Arber, 1962). Interestingly, in other experiments it had been shown that phage DNA which had suffered radiation damage became also degraded upon infection, even in non-restricting hosts (Kellenberger, Arber and Kellenberger, 1959). For a while we wondered if the DNA degradation observed in the different types of experiments had the same roots. This stimulated us to follow the different situations in parallel. In addition, this argument served to justify our experimental investigation of host-controlled modification in the framework of the research project on biohazards of radiations, although this sideline had not originally been foreseen in the project.

Modification is an Epigenetic Phenomenon and Consists in Sequence-Specific Methylation of Nucleotides

Since DNA restriction and modification appeared to become of high prospective interest (Arber, 1965b) we decided to concentrate our upcoming research to the further exploration of this phenomenon. These studies resulted in the insight that the molecular basis of modification, the adaptation of a virus to grow efficiently on a given host strain, resided in the methylation of a nucleotide that is imbedded into a specific sequence of nucleotides of a length of four to about ten base pairs (Arber, 1965a; Arber and Linn, 1969; Smith, Arber and Kuhnlein, 1972). The attached methyl group affects neither the normal base pairing nor the correct expression of the concerned genes. Modification is thus an epigenetic phenomenon.

The Search for Restriction and Modification Enzymes

On the basis of the described findings it was postulated that bacteria usually possess one or more restriction and modification systems serving as a kind of immune defense against foreign DNA entering into the cell. Restriction enzymes were postulated to act as nucleases destructing foreign DNA. The cell's own DNA was postulated to be insensitive to this restriction cleavage because of its proper modification, the methylation in the strain-specific DNA recognition sequences. Within a relatively short time, this interpretation was confirmed by the isolation of restriction endonucleases and modification methylases and by the study of their *in vitro* activities (Meselson and Yuan, 1968; Linn and Arber, 1968; Smith and Wilcox, 1970; Kuhnlein and Arber, 1972).

In comparative studies of the activities of purified restriction enzymes it was confirmed that restriction cleavage becomes indeed activated on specific recognition sequences on the DNA as long as these sites carry no strain-specific methylation. We now know that some restriction enzymes (type II enzymes) cleave their substrate DNA molecules precisely at the recognition site (Roberts *et al.*, 2003), while some other restriction enzymes (type I) translocate the DNA after recognition and eventually cleave it at a more or less random location (Murray, 2000). Since the 1970s the type II enzymes widely serve as tools in genetic analysis and engineering.

In Vitro Recombinant DNA Techniques

A major difficulty in the attempts to study genetic functions at the molecular level remained still around 1970 the tremendous size of the filamentous DNA molecules carried in the chromosomes. In the search for means to sort out DNA fragments of handsome size, appropriate for sequence analysis and functional analysis, the scientists became aware of the naturally observed possibility of a covalent association of a given DNA segment with an autonomously replicating vector DNA molecule. We have already encountered this phenomenon with some bacterial viruses and it had also been shown to occur with conjugative plasmids (Jacob and Adelberg, 1959; Adelberg and Pittard, 1965). Experiments carried out to produce such hybrid DNA molecules *in vitro* in using a bacteriophage λ derivative as a vector were successful (Jackson, Symons and Berg, 1972; Lobban and Kaiser, 1973). This not only allowed the investigators to sort out a specific DNA segment from its genomic location, it also enabled them to amplify the sorted-out segment in order to obtain enough well purified material to carry out structural and functional analyses.

As soon as type II restriction enzymes became available genetic research benefited from their reproducible DNA cleavage function producing manageable DNA fragments. This enabled the researchers to establish physical genome maps (restriction cleavage maps). Specific DNA segments could be sorted out and used to produce *in vitro* recombinant DNA molecules (Cohen *et al.*, 1973).

By these developments, based on scientific knowledge on natural processes of specific interactions of bacterial enzymes with DNA molecules, molecular genetic studies became possible for every kind of living organism. Just a few years later, still another microbial enzyme, a ther-

mo-resistant DNA polymerase was at the basis of the introduction of the polymerase chain reaction. This PCR reaction enables the researchers to highly amplify specific DNA segments at their natural location under the only condition that short flanking sequences are already known (Saiki *et al.*, 1988).

Search for Nucleotide Sequences and Functions of DNA

Still in the 1970s, chemically based strategies were developed to determine the nucleotide sequences of selected and amplified DNA segments (Sanger, Nicklen and Coulson, 1977; Maxam and Gilbert, 1977). Once the DNA sequences became known, one could envisage to undertake functional studies on selected open reading frames as well as on elements controlling gene expression or maintenance functions of the DNA molecules. For this purpose strategies of local site-directed mutagenesis were developed (Shortle, Di Maio and Nathans, 1981; Smith, 1985). This enables the researchers to compare the phenotype of the wild type form of a gene with those of designed mutants. Alternatively, the deletion of a DNA segment or other kinds of DNA rearrangements by methods of genetic engineering can also serve for site-specific mutagenesis. These approaches unravel quite often, although not always, the biological function encoded by the gene or other genetic element in question.

Comparison of Research Strategies Used in Classical Genetics and in Molecular, Reverse Genetics

Investigations in classical genetics depend on the availability of mutants. These can have a spontaneous origin or they can be induced by a treatment with a mutagen. The mutant is recognized by an altered phenotype which becomes transmitted into the progeny. The phenotypic changes can give a reliable hint to the specific function affected by the mutation. Genetic crosses between independently isolated mutants serve to establish genetic maps, and specific genetic information can be localized on these maps. Note, however, that this approach of classical genetics does not depend on any knowledge on the chemical nature of the carrier of genetic information. In classical genetics the concept of the gene remains an abstract notion, without physical entity.

In contrast, most investigations of the new molecular genetics start with isolated DNA molecules with the aim to identify their biological functions. This research goes from the carrier of genetic information to the functions, while research in classical genetics, as we have just seen, goes from functions to a genetic map. In view of this strategic difference of the research approaches, molecular genetics is sometimes also called reverse genetics. In this strategy a manageable, well purified DNA fragment is sequenced, open reading frames and potential control signals are identified, site-directed mutations are then placed on strategic spots of the DNA under study, the mutated DNA segment is introduced instead of its wild type form into the cell under study, alterations in the phenotypes as compared to the wild type condition are looked for and this can, at least sometimes, allow one to conclude on specific biological functions of the DNA segment in question. This strategy is generally applicable to the genomes of most living organisms, at least with some appropriate modifications. It represents the essential basis for genomics and to some degree also for proteomics.

Note that the definition of a mutation differs in molecular genetics (changed nucleotide sequence) from that used in classical genetics (phenotypic change). Not all changes in a given nucleotide sequence will become manifested by a changed phenotype, while an inheritable change in a phenotype is always caused by a change in the nucleotide sequence.

Impact of Functional Genomics on the Development of Biotechnology

Both the molecular genetic research strategies and the thereby acquired knowledge offer wide novel possibilities for biotechnological applications. Generally speaking, biotechnology takes advantage of biological functions for the benefit of mankind and increasingly also of its environment. Such applications may, for example, specifically relate to an improvement of human, animal or plant health, to nutritional security, to agricultural production or to environmental remediation.

Specific knowledge on particular biological functions as a result of investigations in functional genomics can offer ample possibilities to make use of these functions in biotechnology. Thereby, methods of molecular genetics such as site-directed mutagenesis can serve for improvements of the functions in question, both with regard to their quality and quantity. Most importantly, the strategies of molecular genetics render it possible to transfer a given specific genetic information into other organisms that may

sometimes be unrelated to the original source of the biological function in question. This can, for example, be of high relevance for the biotechnological production of a gene product to serve as a medical drug. Recall that in classical biotechnology, that has been practiced for many centuries, one has to use the organisms as they are found in nature. At most, one can try to improve a function or the yield of a product by breeding techniques and by random mutagenesis. Still today this does often not include a thorough molecular genetic analysis of the resulting hybrids and mutants, respectively. In contrast, genetic modifications carried out with modern molecular genetic strategies usually include a careful analysis of the modified organisms both at the genetic and functional levels.

Conjectural Risks of Genetic Engineering

At a very early time in the application of *in vitro* recombinant DNA techniques, the involved scientists themselves raised the question of possible biohazards related to some of their experiments. In order to debate these questions an International Conference was held in February 1975 in Asilomar (Berg *et al.*, 1975). In brief, possible risks of genetic engineering may become manifested in a short-term or in a long-term. Pathogenicity, toxicity, allergenic effects and other harmful or undesirable effects can be counted among the short term risks. These can be carefully studied experimentally within a reasonable time before any of the genetically modified organisms are approved for biotechnological applications. In order to protect preventively the health of researchers and more generally that of the human population, appropriate guidelines were drawn up and these require that according to a scientifically based classification of a given risk, the research has to be carried out under precautions that are worldwide in use in medical diagnosis of pathogenic microorganisms.

The prediction and identification of long-term risks of genetic engineering is a more difficult task than that of the evaluation of short-term risks. Long-term risks may sometimes have an impact on the course of biological evolution, particularly with genetically modified organisms that are deliberately released into the environment, as it is for example the case for agricultural crops. As a matter of fact, the production and release of genetically modified organisms represents a human contribution to biological evolution. An important requirement to responsibly evaluate any long-term evolutionary risks associated with intentional genetic alterations is a good

knowledge of the natural process of biological evolution. In view of this consideration, I decided at the Asilomar Conference held in 1975 to concentrate my own future research on studies of the process of biological evolution at the molecular level.

Three Qualitatively Distinct Molecular Strategies Contribute to the Spontaneous Formation of Genetic Variants

Fortunately, a considerable amount of data on molecular mechanisms of spontaneous genetic variation was already available in the 1970s, mainly from microbial genetics. Many more data were subsequently obtained from specifically designed research projects. For these reasons, it is now possible to draw reliable conclusions regarding the mechanisms and strategies that contribute under natural conditions to genetic variation. At previous occasions I have reported to our Academy on this progress (Arber, 1997, 2002, 2003a). I will therefore just briefly summarize here the main facts and conclusions.

Several different specific molecular mechanisms, rather than a single mechanism, contribute to the formation of genetic variants. These mechanisms can be classified into three general strategies that possess different qualities with regard to their contribution to genetic evolution.

One strategy brings about small local changes in the sequences of the genome, such as a nucleotide substitution, the deletion or the insertion of one or a few nucleotides, or a scrambling of a few nucleotides. Some of these changes, in particular the substitution of a single nucleotide, can valuably contribute to the evolutionary improvement of existing biological functions. To make this point clear, it should be kept in mind that by far not each nucleotide substitution will result in a functional improvement. Rather, it is natural selection that favors rare spontaneous beneficial variants according to the rules of Neodarwinism. Local sequence changes can be brought about by replication infidelities involving often natural structural flexibilities (tautomerism) or chemical instabilities of the nucleotides, as well as by the action of chemical and some physical mutagens. In many of these cases nascent mutations are rapidly repaired by appropriate enzyme systems. For larger genomes the absence of efficient repair is detrimental for the organism.

A second strategy for the generation of genetic variants is a rearrangement of DNA segments within the genome. This DNA reshuffling depends

in general on activities of recombination enzymes such as for homologous recombination, for so-called site-specific recombination and for transposition of mobile genetic elements. These processes can yield a duplication and higher amplification of a DNA segment, the deletion of a DNA segment, the inversion of a DNA segment, the translocation of a DNA segment and, as is widely known for diploid organisms, hybrid chromosomes with genes from the two parents. Some of these reshuffling processes can bring about novel gene fusions as well as the fusion of a given open reading frame with an alternative expression control signal. Again, rare favorable rearrangement products providing functional benefits will be favored by natural selection. More often, however, a DNA rearrangement will reduce the functional harmony of the genome and thus cause a selective disadvantage.

The third strategy of generating genetic variants is DNA acquisition by horizontal transfer of genetic information from a donor organism into an acceptor organism. This phenomenon is best studied with bacteria since it is at the basis of bacterial genetics. This involves bacterial conjugation, virus-mediated transduction and transformation by free DNA molecules as transfer processes. Depending on the evolutionary relatedness of the donor and acceptor strains, horizontal gene transfer can give rise either to conversion (the substitution of a segment of genetic information by a different, but still homologous DNA sequence) or to the acquisition of genetic information that was hitherto not present in the acceptor genome. Again, it will depend on natural selection if the resulting hybrid will be favored or not in the long-term. For the concerned acceptor organism, the successful acquisition of foreign genetic information can represent a rapid and efficient functional innovation. DNA acquisition can be seen as a sharing in successful developments made by others.

Comparison of Genetic Alterations Obtained by Genetic Engineering with Those Occurring Spontaneously

Genetic engineering uses the same three strategies of genetic variation that serve in the natural world for the purpose of biological evolution. Genetic engineering may indeed involve a local change of nucleotide sequences, it may bring about a rearrangement of genomic sequences or it may consist in the acquisition of a segment of foreign genetic information. Similarities between the natural and the intended genetic changes are also seen with regard to the size of DNA sequences involved in these processes

(Arber, 2002). In this view, similar conjectural biohazards may be expected from genetic engineering and from natural genetic variation and, as a matter of fact, from classical breeding strategies.

However, these similar processes will of course generally not yield identical products in view of the tremendous number of possible unique genomic sequences. Therefore, absolutely precise predictions cannot be made. From these considerations one may deduce that a careful, responsible handling and long-term control of any organisms that had deliberately been genetically modified by human intervention is justified. This relates both to products of genetic engineering and to those obtained by classical breeding strategies. Particular attention should be paid to organisms into which genetic information from a genetically unrelated donor organism had been inserted, because of a lack of solid knowledge on the range and the probability of successful horizontal gene transfer under natural conditions. In this context, it is relevant to recall that deliberate mass production, as it applies to many plants of agricultural use independently of their origin, favors just by statistical means their occasional involvement in evolutionary processes.

The Theory of Molecular Evolution

Besides its practical relevance for the evaluation of conjectural risks of genetic engineering, a profound knowledge of molecular mechanisms that serve in the natural world for the generation of genetic variations represents a basic contribution to a deeper understanding of biological evolution. What has in fact become possible in the last few decades is a second evolutionary synthesis, integrating molecular genetics and Neodarwinism to become a theory of molecular evolution (Arber, 2003b; 2004). This represents an expansion of the Darwinian theory of biological evolution to the level of molecular processes, particularly those involved in genetic variation, in reproductive isolation and eventually also in natural selection.

From the short description that we have given for the three strategies for genetic variation it is obvious that in most of these molecular mechanisms specific enzymes are involved. Genetic studies with microorganisms have shown that many of these enzymes are not essential for the normal clonal propagation of bacteria from generation to generation. This is, for example, the case for transposition of mobile genetic elements or for site-specific DNA inversion. However, these processes are of obvious relevance

in the occasional production of genetic variants. The involved enzymes are the products of genes. In view of their functional relevance for biological evolution we call these genetic determinants evolution genes. The products of some of the evolution genes are actively involved in the production of genetic variants, as we have seen, these are in fact variation generators. The products of other evolution genes have the task to keep the frequencies of genetic variation low and tolerable for a long-term maintenance of the given kinds of organisms. These enzymes can serve for example in the repair of nascent mutations, or for the restriction of foreign DNA upon horizontal gene transfer.

The theory of molecular evolution postulates that the generation of genetic variations not only depends on activities of evolution genes. Rather, it assumes that a series of non-genetic elements play also their specific roles. This represents a making use of intrinsic properties of matter such as the tautomerism and chemical instability of nucleotides and various conformational flexibilities of biologically active molecules for the purpose of genetic variation. Other non-genetic elements influencing spontaneous mutagenesis are environmental mutagens and random encounter.

The Intrinsic Duality of the Genome

An interesting implication of the presence of the postulated evolution genes on the genome is a duality of the genomic information. We have to realize that not all the genes present on the genome exert their activities for the benefit of the individual organism in question. Other genes work for the benefit of the evolutionary development of the population. The evolution genes serve for a steady expansion of life, for the production and renewal of biodiversity. In contrast, the more classical housekeeping genes, accessory genes of use by all individuals under particular life conditions and developmental genes serve each individual for the fulfillment of its life. Note that the products of some genes can serve for both of these objectives and act for purposes of the individuals as well as of biological evolution.

Philosophical, World View Aspects of the Theory of Molecular Evolution

What has been outlined here on the basis of recently acquired scientific knowledge may have wide relevance for our worldview. We can general-

ly conclude that natural reality takes active care of biological evolution, as it also takes care of individual physical lives. Mutations should not be considered as errors or as caused by accidents. Rather, intrinsic properties of matter together with activities of evolution genes are at their origin. Different specific molecular mechanisms, different natural strategies, contribute in specific ways to the process of biological evolution.

The genomic duality can offer an unexpected, at least partial, solution to the theodicean question. Variation generating evolution genes exert occasionally their mutagenic activity in a particular individual of a population. We have learned that new genetic variants are generally only rarely favorable, beneficial under the living conditions encountered by the concerned individual. More frequent are either neutral or unfavorable mutations. These might perhaps be favorable under other living conditions. In this regard, the evolutionary progress resembles a trial and error process with relatively few winners. Therefore, individuals having suffered an unfavorable mutation can be considered to have become victims of the natural process of biological evolution. Under the assumption that biological evolution is brought about by a divine intention and as a consequence of the genomic duality, both with regard to the presence of evolution genes and with regard to their variation generator activities, one can see a possible answer to the question of theodicy in the juxtaposition of physically good and physically evil in the overall genetic activities, deserving both the requirements of individuals and of evolving populations.

Conformity Between Traditional Wisdom and Scientific Knowledge on Biological Evolution

Consider the narration of creation as it is given in the Genesis as a testimony of traditional wisdom. Scientific theories and firmly established scientific knowledge are sometimes considered to antagonize traditional knowledge. This was also the case for the Darwinian theory of evolution. It is thus indicated to re-inspect the situation in a search for conformities between traditional wisdom and scientific views. According to the Genesis, God created our world stepwise. This can well correspond to the step-by-step progress of biological evolution. In addition, genetic variations must be at the basis of the well-distinguished personal characteristics of prophets and other descendants from the first human beings on our planet. These characteristics are quite specifically described in the Genesis, indicating that

human beings were not considered as clones, they were rather seen as unique individuals. From a scientific point of view, this represents genetic diversity as a consequence of genetic variation. During creation God evaluated several times the quality of His work and He concluded that it was good. In today's scientific terms this includes the process of biological evolution as such, the generation of genetic variations and the genomic duality with its consequences that we have already described. Both, the health of the individual human beings and the prospective progress of biological evolution must correspond to God's will. From my laic point of view, I can see one of the missions of the son of God, Jesus Christ, to consist in teaching to the human society that it is a human duty to provide help for the suffering, and thus underprivileged people, by love and medical care. In the Christian faith, this can represent a responsible reaction to the theodicean problem that is linked to the process of continued creation anchored in biological evolution.

Concluding Remarks

After having been largely neglected by classical genetic research, microbial genetics, once initiated, has rapidly opened novel research strategies to identify DNA as the carrier of genetic information and then to investigate genetic information at the molecular level. This has given rise to molecular genetics that is applicable to all kinds of living organisms and that is now known as functional genomics. Some of these steps are here described in more detail, such as the identification of natural gene vectors and of restriction enzymes serving as valuable tools in molecular genetic research. It is also outlined how newly acquired knowledge on genetic functions can lead to fruitful biotechnological applications. In turn, such applications, particularly if they involve *in vitro* recombinant DNA techniques, raise questions of conjectural risks. Some of these risks relate to long-term evolutionary developments. Again, it is mainly on the basis of experimental data from microbial genetics and knowledge resulting from these experimental investigations that a theory of molecular evolution could be formulated. This theory postulates that spontaneous genetic variations are jointly caused by intrinsic properties of matter and by activities of evolution genes. This represents an expansion of the Neodarwinian theory to the level of molecular events involved in biological evolution. On the practical side, this forms a reliable basis for a responsible evaluation of long-term conjectural risks of genetic engineering. Besides this, the improved understanding of molecular processes involved in biological evolution has a strong impact on our world

view, the fundament of the orientational knowledge that can serve the civil society to assume co-responsibility for practical applications of scientific knowledge for the benefit of mankind and its natural environment. Consistencies between religiously founded traditional wisdom and recently acquired scientific knowledge are discussed, as well as questions with regard to the simultaneous presence on the genome of genes acting for the benefit of the individual organism, and of evolution genes that insure the evolutionary progress of populations of organisms and thus a rich biodiversity.

REFERENCES

Adelberg, E.A. and Pittard, J. (1965), 'Chromosome transfer in bacterial conjugation', *Bacteriol. Rev*. 29, 161-172.

Arber, W. (1965a), 'Host specificity of DNA produced by *Escherichia coli*. V. The role of methionine in the production of host specificity', J. *Mol. Biol*. 11, 247-256.

Arber, W. (1965b), 'Host-controlled modification of bacteriophage', *Annu. Rev. Microbiology* 19, 365-378.

Arber, W. (1997), 'The influence of genetic and environmental factors on biological evolution', *Plenary Session on the Origin and Early Evolution of Life* (part I), The Pontifical Academy of Sciences, Commentarii, vol. IV, n. 3, pp. 81-100.

Arber, W. (2002), 'Molecular evolution: comparison of natural and engineered genetic variations', *The Challenges of Science*, The Pontifical Academy of Sciences, Scripta Varia 103, pp. 90-101.

Arber, W. (2003a), 'Cultural aspects of the theory of molecular evolution', *The Cultural Values of Science*, The Pontifical Academy of Sciences, Scripta Varia 105, pp. 45-58.

Arber, W. (2003b), 'Elements for a theory of molecular evolution', *Gene* 317, 3-11.

Arber, W. (2004), 'Genetic variation and molecular evolution', *Encyclopedia of Molecular Cell Biology and Molecular Medicine*, R.A. Meyers (ed.), Wiley-VCH, Weinheim, vol. 5, pp. 331-352.

Arber, W. and Dussoix, D. (1962), 'Host specificity of DNA produced by *Escherichia coli*. I. Host controlled modification of bacteriophage λ', *J. Mol. Biol*. 5, 18-36.

Arber, W. and Kellenberger, G. (1958), 'Study of the properties of seven defective-lysogenic strains derived from *Escherichia coli* K12(λ)', *Virology* 5, 458-475.

Arber, W., Kellenberger, G. and Weigle, J. (1957), 'La défectuosité du phage Lambda transducteur', *Schweiz. Z. allg. Pathol. u. Bakteriol*. 20, 659-665.

Arber, W., Kellenberger, G. and Weigle, J. (1960), 'The defectiveness of Lambda transducing phage', *Papers on Bacterial Genetics*, E.A. Adelberg (ed.), Little, Brown & Co., Boston-Toronto, pp. 224-229.

Arber, W. and Linn, S. (1969), 'DNA modification and restriction', *Annu. Rev. Biochem*. 38, 467-500.

Avery, O.T., MacLeod, C.M. and McCarty, M. (1944), 'Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III', *J. Exp. Med*. 79, 137-158.

Berg, P., Baltimore, D., Brenner, S., Roblin, R.O. and Singer, M.F. (1975), 'Asilomar Conference on recombinant DNA molecules', *Science* 188, 991-994 and *Nature* 255, 442-444.

Campbell, A.M. (1962), 'Episomes', *Advanc. in Genetics* 11, 101-145.

Cohen, S.N., Chang, A.C.Y., Boyer, H.W. and Helling, R.B. (1973), 'Construction of biologically functional bacterial plasmids *in vitro*', *Proc. Natl. Acad. Sci. USA* 70, 3240-3244.

Dussoix, D. and Arber, W. (1962), 'Host specificity of DNA produced by *Escherichia coli*. II. Control over acceptance of DNA from infecting phage λ', *J. Mol. Biol*. 5, 37-49.

Jackson, D.A., Symons, R.H. and Berg, P. (1972), 'Biochemical method for inserting new genetic information into DNA of Simian virus 40: Circular SV40 DNA molecules containing Lambda phage genes and the galactose operon of *Escherichia coli*', *Proc. Natl. Acad. Sci. USA* 69, 2904-2909.

Jacob, F. and Adelberg, E.A. (1959), 'Transfert de caractëres gènètiques par incorporation au facteur sexuel d'*Escherichia coli*', *Comptes Rendus des Séances de l'Académie des Sciences* 249, 189-191.

Kellenberger, G., Arber, W. and Kellenberger, E. (1959), 'Eigenschaften UV-bestrahlter λ-Phagen', *Z. Naturforsch.* 14b, 615-629.

Kuhnlein, U. and Arber, W. (1972), 'Host specificity of DNA produced by *Escherichia coli*. XV. The role of nucleotide methylation in *in vitro* B-specific modification', *J. Mol. Biol.* 63, 9-19.

Lederberg, J. (1947), 'Gene recombination and linked segregation in *E. coli*', *Genetics* 32, 505-525.

Lederberg, J. and Tatum, E.L. (1946), 'Novel genotypes in mixed cultures of biochemical mutants of bacteria', *Cold Spring Harb. Symp. Quant. Biol.* 11, 113-114.

Linn, S. and Arber, W. (1968), 'Host specificity of DNA produced by *Escherichia coli*. X. *In vitro* restriction of phage fd replicative form', *Proc. Natl. Acad. Sci. USA* 59, 1300-1306.

Lobban, P.E. and Kaiser, A.D. (1973), 'Enzymatic end-to-end joining of DNA molecules', *J. Mol. Biol*. 78, 453-471.

Lwoff, A. and Gutmann, A. (1950), 'Recherches sur un Bacillus megathérium lysogëne', *Ann. Inst. Pasteur* 78, 711-739.

Maxam, A.M. and Gilbert, W. (1977), 'A new method for sequencing DNA', *Proc. Natl. Acad. Sci. US*A 74, 560-564.

Meselson, M. and Yuan, R. (1968), 'DNA restriction enzyme from *E. coli*', *Nature* 217, 1110-1114.

Morse, M.L., Lederberg, E.M. and Lederberg, J. (1956), 'Transduction in *Escherichia coli* K-12', *Genetics* 41, 142-156.

Murray, N.E. (2000), 'Type I restriction systems: sophisticated molecular machines', *Microbiol. Mol. Biol. Rev*. 64, 412-434.

Roberts, R.J., Vincze, T., Posfai, J. and Macelis, D. (2003), 'REBASE: restriction enzymes and methyltransferases', *Nucleic Acids Res*. 31, 418-420.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988), 'Primer-directed enzymatic amplifications of DNA with a thermostable DNA polymerase', *Science* 239, 487-491.

Sanger, F., Nicklen, S. and Coulson, A.R. (1977), 'DNA sequencing with chain-terminating inhibitors', *Proc. Natl. Acad. Sci. USA* 74, 5463-5467.

Shortle, D., DiMaio, D. and Nathans, D. (1981), 'Directed mutagenesis', *Annu. Rev. Genet*. 15, 265-294.

Smith, H.O. and Wilcox, K.W. (1970), 'A restriction enzyme from *Hemophilus influenzae*. I. Purification and general properties', *J. Mol. Biol.* 51, 379-391.

Smith, J.D., Arber, W. and Kuhnlein, U. (1972), 'Host specificity of DNA produced by *Escherichia coli*. XIV. The role of nucleotide methylation in *in vivo* B-specific modification', *J. Mol. Biol*. 63, 1-8.

Smith, M. (1985), '*In vitro* mutagenesis', *Annu. Rev. Genet*. 19, 423-462.

Watson, J.D. and Crick, F.H.C. (1953), 'Genetic implications of the structure of deoxyribonucleic acid', *Nature* 171, 964-969.

Zinder, N. and Lederberg, J. (1952), 'Genetic exchange in Salmonella', *J. Bacteriol*. 64, 679-699.

Zuckerman, H. and Lederberg, J. (1986), 'Postmature scientific discovery?', *Nature* 324, 629-631.