THE EXCITING POTENTIALS OF BIOLOGY

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It is a very great honour to receive one of this year's Kyoto Prizes. I feel extremely grateful to the Inamori Foundation and to the selection committee for their choice. I wish to take this opportunity to express to them my deepest thanks.

Auguste Comte, nineteenth-century French philosopher and father of positivism, gave a classification of sciences according to their generality and complexity in which mathematics came first, followed by physics, while the study of living organisms, that is, biology, given its enormous complexity, could hardly ever hope to merit the term of science at all, but would be better called "natural history." Such a notion, which has perhaps dissuaded some bright people from embracing the career of biologist, was based on the exclusively descriptive character that biological sciences have displayed for so long. This notion is no longer valid.

Since the 1950s, biology has undergone dramatic changes. This veritable revolution is the result of discoveries that have radically changed our way of looking at the living world. They have shown that biological specificity, which makes each individual unique, is derived from the information stored in the DNA molecules constituting its chromosomes. The way in which this information is encoded and translated is now largely understood and the possibility of deciphering the fine structure of the genome is now real.

Initiated on bacteria and viruses, research in molecular biology has, by virtue of recent technological progress, gained access to the genetic material of the more complex eukaryotic organisms. One knows nowadays that the mammalian genome, that of a human being, for example, contains a thousand times more DNA than a bacterium, although it contains only 20 to 30 times more genes. Technological advances have been so gigantic during the last decade that current debate is centred, not on the feasibility of sequencing the entire human genome, but on the opportuneness of doing so.

Physical sciences have brought revolutionary changes in the daily lives of men, moulding social and industrial organization through their applications to the domains of energy, transportation, communications and, more recently, of electronics and computer

science. In the same way, biology and biotechnologies will exert a determining influence through their incidences on medicine, chemistry, agriculture, food, energy production and, most importantly, on the protection of the environment.

Biology is the science of the future, certainly by its potential applications but surely also by the fascinating interrogations that remain and that recent advances have themselves uncovered. For the scientists involved in biological research, ours is the most exciting era that this field has ever enjoyed.

One of the most surprising wonders that the living world offers in the present context of the developing knowledge in cybernetics is the extreme miniaturisation of biological information. It can be calculated that all the information necessary to yield the 5 billion men presently living on earth is contained in only 32 mg of DNA, an amount that could easily be contained within a thimble.

How a large quantity of information is stored is certainly an enigma, but the major interrogation ahead concerns the mechanisms that regulate gene activity. The problem is striking even if one considers the operation of only a single cell, the functional unit of all eukaryotic organisms; it becomes even more acute when one meditates upon the immeasurably greater level of complexity involved in the development of the initial cell, the egg, into an embryo.

Indeed, development of an embryo from an egg is probably the most complex problem of modern biology. Development can be conceived as the execution of a genetic programme encoded in the DNA of the egg. The information stored in the DNA involves not only the plan of the future organism and of its constitutive parts but also the means by which the programme will be executed both in time and space. The algorithm according to which the programme unfolds, however, is still unknown. Many congenital abnormalities and diseases which arise later in life, including malignancy, a disorder of the genetic programme regulating growth, result from errors occurring in these processes.

This is why the study of embryonic development is so fascinating. It represents, in fact, a basic and fundamental field of biological sciences, and any progress in this direction is certain to have important consequences, which, hopefully, should benefit humanity. Attracted from the beginning of my studies to biological sciences, it was only at the age of 29 that I turned to research after having taught natural sciences in a lycee for several years. I had the great good fortune to begin my career under the aegis of a

remarkable French embryologist, Etienne Wolff, and it was in his laboratory, well known in the 1950s and 1960s for its work on teratology and organogenesis, that I had the first opportunity to watch the fascinating changes leading, in the avian egg, from the minute mass of the germ to the embryo and then to the recognisable young chicken.

For my Ph.D. thesis, on the development of the digestive tract, I was led to analyse the interactions occurring between the cells of different types and embryonic origins that play a decisive role in liver cell differentiation. The techniques I used for this purpose were cell and organ cultures, but already at this time I was much interested by *in vivo* experimentation and application of microsurgery to the embryo in the egg. I frequently practised excisions and transplantations of defined embryonic territories or destroyed small tissue areas by X-irradiation. The effects of such operations yield interesting information of the developmental relationships existing between different types of embryonic cells during the ontogenetic process. In this respect, the avian embryo is a particularly interesting experimental model. It is, as a higher vertebrate, close to mammals and man, but, in contrast to the mammalian embryo, it is accessible to experimentation in the egg during the full span of development.

It was observed long ago that embryonic development involves movements of cell sheets as well as migrations of isolated cells, and it was felt from the beginning of embryology that such processes played an important role in morphogenesis and organogenesis. These movements are extremely difficult to analyse, especially in complex organisms such as the embryos of higher vertebrates. They are, however, fascinating since, as will be shown later in this talk, the fate of embryonic cells largely depends upon the cellular context in which they happen to find themselves at critical stages of their development. The fact is that when they start migrating, certain embryonic cells have several developmental options ahead of them. The choice they make will be imposed on them by the cellular environment in which they are immersed during and at the end of the migratory process. This choice, however, depends also on the developmental capacities of the embryonic cells themselves. The latter are dictated to them via an intrinsic regulation of gene expression, which, once it has occurred in a given cell, is transmitted to its progeny. Therefore, it is through an interplay between environmental and inherited cues that cell differentiation is achieved.

Embryonic manipulations are among the approaches that can be used to analyse the role and nature of each of these factors. But how can one study rigorously

cell migrations, cell interactions and morphogenetic movements in the extraordinarily complex and ordered unfolding of embryonic development ?

Two years after I moved to the University of Nantes, where I created a course and a laboratory of embryology, I made an observation that changed the course of my work.

A geneticist, Dr. Ernst Bösiger, was working at that time in a laboratory run by the Centre National de la Recherche Scientifique at Gif-sur-Yvette (close to Paris) on hybrid vigour, that is, the selective advantage of hybrids, and using the Japanese quail *(Coturnix coturnix japonica)* as experimental material. His quail flock was so flourishing that he offered fertilized eggs to several developmental biology laboratories, including mine. This is how the Japanese quail has joined the long-used chick as a common animal in embryological studies. It is fortunate that quails and their eggs are appreciated by some “gourmets”, since many laboratories in the world use them now.

The observation that I made in 1968 concerned the structure of the interphase nucleus in quail cells. Chromatin, that is, DNA associated with proteins, in which genetic information is stored, occurs in the nucleus in two spatial configurations (this is true for all eukaryotic cells): one dispersed, *euchromatin*, containing the genes accessible for transcription, and one condensed, *heterochromatin,* where the compact arrangement of the DNA molecule prevents transcription. In most animal species, heterochromatin is evenly distributed throughout the nucleus in small clumps called chromocentres. In quail cells, however, it is condensed in one (although sometimes two or three) large masses that are always associated with the nucleolar RNA, thus making the nucleolus exceptionally large and DNA-rich. As a consequence, any specific staining procedures for DNA allow quail and chick cells to be easily distinguished when they have been experimentally associated in culture or by grafting in the embryo *in ovo*.

The difference between the cells of the two species is so striking that a single quail cell buried within a chick tissue can be picked out at first glance.

The light microscope and a simple staining method for DNA are thus sufficient to analyse the cellular composition of a given tissue where cells of the two species are mixed together, but the electron microscope also reveals very clearly the quail or chick origin of the cells, giving in addition detailed information on their cytoplasmic feature and therefore its type of differentiation.

Why did I alone make this observation when at the same time several other

scientists were working with Dr. Bösiger's quail embryos? I think the prime reason is that the study I was pursuing on hepatocyte differentiation predisposed me to look at the nucleolus very carefully. The experiment which led me to this discovery consisted in investigating whether the cell-cell signalling which takes place between the endodermal and mesenchymal components of the liver was operational when these tissues belonged to two different species. I associated quail hepatic mesenchyme with chick hepatic endoderm. A liver lobule differentiated in the culture dish but the most important result of the experiment was elsewhere : not only had the chick endodermal cell nucleolus increased in size during hepatocyte differentiation, as expected, but the nucleus of the quail mesenchymal cells looked to me abnormally large and I realised rapidly that this was not because they had differentiated in culture, but because of their genetic constitution. This was fully confirmed when I saw that the same characteristic occurred in all embryonic and adult cell types of the quail. I thought of using this particularity of the quail nucleus to devise a cell marking technique enabling cell migrations, morphogenetic movements and cellular interaction to be investigated during embryogenesis. The cell marker provided by the association of quail and chick cells had several advantages over all the labelling techniques previously used: it was simple, natural (i.e., did not involve artificial labelling with dyes or radioisotopes, which all present some toxicity), not diffusible and therefore providing a high resolution, and above all, was stable, being based on genetic differences between the two cell types in question. The fact that I was well trained in microsurgery on embryos allowed me to envisage exchanging definite regions of the embryo of one species for their counterparts from the other species. The cells of each could be recognized unambiguously any time after the association, when morphogenesis and differentiation were completed. This is how the idea of making *chimaeras* came about.

A chimaera is an organism resulting from the association of tissues originating from more than one zygote. In the aggregation chimaeras of mammalian eggs, initiated by Tarkowsky and Mintz, one fuses the blastulas from two mice, for example, and gets a single mouse in which cells from each zygote are randomly assembled. In quail/chick chimaeras, in contrast, chimaerism is established at later developmental stages and can be made to affect any particular territory at the experimenter's will.

Taxonomically, the quail and the chick are closely related. Although they differ significantly in the duration of their incubation period (16 days for the quail, 21 days for

the chick) and their size at birth (10 g for the quail, 50 g for the chick), the chronology of development and the size of the embryo differ only slightly during the first half of the incubation period, when most of the decisive events of embryogenesis occur. This is why viable chimaeras can be constructed between these two species.

The first chimaeras I made were for studying cell migration in the nervous system. In the vertebrate embryo, the nervous system arises from the superficial germ layer, the *ectoderm*. After the formation of a neural groove, its ridges fuse in the mediodorsal line to yield a tubular structure from which all the central nervous system develops. As they join, the ridges form a transient structure, the neural crest, from which a variety of cell types are derived.

One of the interesting features of neural crest cells is that they undergo extensive migration in the developing embryo, at precise periods of time and along apparently defined pathways before settling in specific sites where they differentiate into a variety of structures. Immediately after discovering the quail-chick marker system, I decided to work on the migration of neural crest cells. I was joined by several students, some of whom continue to pursue this study with me. I wish particularly to acknowledge the collaboration of M.A. Teillet, C. Le Livre and J. Fontaine. The experimental procedure I designed to follow the neural crest cell migration *in ovo* was intended to disturb the normal course of development as little as possible and consisted in constructing chimaeras in which defined fragments of the neural primordium were isotopically and isochronically exchanged between embryos of the two species.

This type of graft results in the normal development of the chimaeric birds. If the host is a white Leghorn chicken, the only external sign that it is a chimaera is the transverse strip of quail-like pigmented feathers in which pigment cells originating from the grafted neural crest of the quail have migrated and differentiated, but the feathers here, of course, belong to the chick host.

In this type of chimaera, migration of cells from the graft can be followed easily since they can be distinguished from the chick host cells by the structure of their nucleus. Later on, one can locate them precisely and determine their fate in the embryo as a result of the stability provided by the nuclear marker.

Here, after such a graft, one can see, for example, a suprarenal gland containing a mixture of host and donor cells. By applying two successive treatments to the tissue sections, one can tell which cells are quail and which are chick and then show that quail

cells contain adrenaline, the hormone of the adrenal medulla. This means that quail cells differentiate quite normally in the chick host.

Grafts done at the level of the encephalic vesicle have revealed for the first time the paramount importance of the contribution of neural crest cells to the facial structures. When brain vesicles are isotopically exchanged between quail and chick embryos, the dorso-ventral migration of crest cells into the face and branchial arches can be visualized and their contribution to adult structures made apparent. Crest cells proved to form all the bones and dermis of the face. By transposing these observations to man, one can see which part of the head is derived from the neural crest. Many congenital malformations of the face, ranging from relatively commonplace cleft palates to rarer and more dramatic ones like otocephaly, occur in humans. In the light of the results obtained with the chimaera model, one can understand the genesis of such malformations. For example, in otocephaly it is clear that the defect is due to the absence of migration of crest cells during the first weeks of pregnancy.

From 1970, we concentrated our efforts on the derivatives of the neural crest and the ontogeny of the peripheral nervous system. We found in 1971 that the cells producing calcitonin, a hypocalcemic hormone of the thyroid gland in humans, originate from the rhombencephalic neural crest. At the same time, we established the fate map of the peripheral nervous system, defining the origin of its component cells along the neuraxis. We showed that the neural crest is divided into distinct areas from which definite sets of cellular phenotypes arise. These phenotypes, each defined by particular biochemical markers, and which correspond to particular physiological roles, include sensory neurons, sympathetic, parasympathetic and enteric ganglion cells.

It was then that it occurred to me to devise experiments in which the position of neural crest cells along the neuraxis was changed prior to the onset of migration. For example, the vagal region of the neural crest could be transferred to the truncal level and vice-versa, taking advantage of heterospecific combinations between quail and chick as usual. When this sort of experiment is done, it is found that the neural crest cells take migration routes different from those that they would normally take, but which correspond to their novel position in the embryo, and settle in organs that they would normally never encounter. In fact, the developmental plasticity of the neural crest cells is such that they adjust to their environmental situation. For example, when cells that should normally give rise to the adrenal medulla and produce the hormone adrenaline

are grafted into the appropriate region of the neural axis, they colonize the gut and differentiate into enteric ganglion cells that synthesize acetylcholine and neuropeptides.

It is therefore the milieu in which the crest cells migrate that is decisive in determining their differentiation.

Another more recent experiment, in which quail ganglion cells in the process of differentiation are grafted into certain territories of a younger chick embryo, was also very informative concerning the plasticity of the developing ganglioblasts. In fact, the fate of the grafted cells can be radically modified under the influence of the new embryonic environment they are subjected to. This ability of differentiating cells to change their differentiation programme has been very elegantly documented by Professor Tokindo Okada and his school in the system provided by the chick embryo retina. In certain, well defined, culture conditions, retina cells produce structures, reminiscent of the lens, that actually synthesize crystallins, the characteristic marker proteins of this organ. This phenomenon, called “transdifferentiation” by T. Okada, has considerable theoretical importance, since it evidences the extraordinary capacity of the embryo to adapt itself to environmental changes, an ability which may have played a decisive role in evolution.

This work on the chimaeras has opened new avenues to further research in cellular and molecular biology now in progress in my laboratory, which attempts to elucidate the nature of the cellular interactions driving the differentiation of peripheral neurons. The various cell types of the peripheral nervous system are defined by sets of biochemical markers including neurotransmitters, enzymes and neuropeptides. Surface molecules, some of which play important roles in cellular interactions, have been brought to light and characterized through their antigenicity and the production of monospecific antibodies. By combining in vitro culture techniques and the chimaera system, we have been able to study the cell lineages which, from neural crest precursors, generate several types of differentiated neurons. In these lineages, the regulation of genes responsible for specific differentiated traits can be studied. This work is now in progress with a group of people among whom I wish to acknowledge J. Smith and C. Ziller for their excellent collaboration.

Simultaneously with the research on the nervous system, I have since 1972 worked actively on the development of the *immune system*.

Cell migrations take place not only in embryogenesis, when the hemopoietic

organs are formed, but also throughout the life of the animal, during which intense cell traffic occurs between various hemopoietic compartments of the body. When we started this work, with F. Jotereau and E. Houssaint, at the University of Nantes, the idea that the hemopoietic organs, bone marrow, thymus, bursa of Fabricius and spleen had to be colonized by extrinsic stem cells had already been put forward by Moore and Owen in 1965. These authors had shown, using sex chromosomes as cell markers, an influx of cells into the thymus and other blood-forming organs during embryogenesis in chick embryos joined by parabiosis. However, with this type of cell marker, it was not possible to ascertain clearly the extrinsic origin of the precursors of lymphocytes and other blood cells. In experiments based on the quail-chick marker system, we could trace precisely the embryonic origin of the T and B lymphocytes and other cell components of the thymus and bursa. Dr. F. Dieterlen and her co-workers extended these studies to the other hemopoietic organs and demonstrated that the stem cells originate from intraembryonic blood islands. Thus, after a long controversy, the *hematogenic theory* blood-forming organs in vertebrates was definitively adopted.

Recently, some of the mechanisms of stem cell seeding of the thymus were elucidated when it was shown that the seeding process is cyclic and requires a chemotactic factor produced by the thymic epithelium. The purification of this substance is currently well under way.

Two years ago, part of my research became oriented in a novel direction.

Ever since we had begun to make neural chimaeras, we had wondered whether the birds in which part of the nervous system belonged to a different species would be able to hatch and, if so, whether their motor behaviour would be normal. However, we had always been too busy investigating neural crest migration to follow up this problem. It was, in fact, a time- and effort-consuming project, for it must be realised that constructing such a chimaera takes 30 to 45 minutes in expert hands and that the mortality of the operated embryos at hatching is quite high. It was in 1982 that a Japanese scientist, Dr. Masae Kinutani, associate professor at the University of Ehime in the Department of Anatomy directed by Professor Takashima, and a former student of Dr. Daikoku of Tokushima University, came to our laboratory for a stay that was to last two and a half years. I suggested that she undertake this difficult project. She accepted enthusiastically, and carried it out in a remarkable way. Masae Kinutani learned the necessary microsurgical techniques and got her first bird to hatch after 3 months of hard

work. It must be emphasised that the operation has to be perfectly done for the bird to be able to get rid of the shell and hatch. From that time on, she and others in the laboratory obtained many of these chimaeras, able to run, fly and compete for food like normal birds, thus demonstrating for the first time that functional neural connexions can be established between cells belonging to two different species, the quail and the chick. The molecules involved in the exchange of signals by the cells during neurogenesis and that ensure the setting up of the appropriate neuronal circuitry have not significantly changed during the evolution which led to the divergence of these two species some two million years ago.

But the spinal cord chimaeras were to teach us more.

After a period of time ranging from 3 to 10 weeks, pathological signs appeared in all birds. The graft had always been done at the brachial level, and the first sign of the development of a neurological syndrome was the flaccid paralysis of the wings, followed by the spasmic paralysis of the legs, resulting in the incapacity of the bird to stand.

At this phase of the disease, the grafted part of the spinal cord is the site of an immune rejection process characterized by leukocyte infiltration following rupture of the blood-tissue barrier. T lymphocytes, plasmocytes and macrophages of host origin invade the nervous tissue, inducing demyelinisation and death of neurons and glia. Later, an autoimmune attack of the host nervous system develops through a sensitisation of its own immune cells during graft destruction. This experimental animal has turned out to be interesting in many respects, particularly as a model to study human demyelinating diseases, such as multiple sclerosis and other encephalopathies with which the disease of the chimaeras presents several analogies.

My double interest in the immune and nervous system ontogeny made this model particularly attractive. I realised that it could help in understanding the mechanisms of self-and nonself-recognition, a fundamental function of the immune system that is carried out by the T lymphocytes which differentiate in the thymus. In our experiments, since the graft is introduced into the recipient very early in development, before its own immune system has started to develop, one would have expected it to be recognized as self, that is, “tolerated” just as the other tissues of the host are. Abnormalities in this function of self-and nonself-recognition lead to serious diseases in humans related to the development of autoimmune reactions. In autoimmune diseases

the immune cells of an individual destroy his own tissues and organs.

This is why with C. Martin and a young Japanese scientist, Hiroko Ohki, we decided to produce chimaeric embryos in larger numbers and, instead of grafting the spinal cord, which is a difficult and time-consuming operation, to construct limb chimaeras in which the limb bud of the 4-day chick embryo was replaced by its quail counterpart. Chicks with a quail wing hatch, but this wing is regularly subjected to acute immune rejection from 8 to 10 days of age and is finally autoamputated. Therefore, as for the nervous tissue but even faster, the grafted wing is destroyed by the host's immune cells. Our next goal was then to devise chimaeras in which we could prevent the immune rejection of the graft. And we decided to manipulate the host's immune system during embryonic life. The role of thymus in self- and nonself-recognition has been established using several experimental approaches. However, neither the mechanisms through which it operates nor the cellular components of this organ which are decisive in this process is clearly understood. We have undertaken a series of experiments in which the chick embryo that has had a quail wing grafted onto it receives at the same time a thymic epithelial implant from the donor. The chick is then endowed with two thymuses—its own and the grafted one. The two organs are colonized by the host's hemopoietic cells which differentiate into T lymphocytes. These are "educated" for self-and nonself-recognition in two ways: by the thymus of their own genotype and by that of a different genotype. Such multiple chimaeras survive and, when the thymic graft develops properly, rejection of the wing is prevented.

We are now working at producing such durable chimaeras with a neural tissue graft, with the hope of obtaining similar birds in which pieces of brain from another species have been inserted. This model of quail-chick neural chimaeras could have multiple applications in the study of various biological problems, such as those related to genetic influence on behaviour. How would a chick behave if part of its brain were replaced by the equivalent region from quail?

Therefore one can say that in 1986, a new field of applications for embryonic manipulations, such as can be done in the avian embryo has opened up.

In conclusion, I would like to say that although immense progress has occurred in biology with the conceptual and technical advances of the last few decades, embryonic development still raises problems that the approach of molecular biology alone will not be able to solve. One of the central questions in the development of an

embryo is *morphogenesis*, that is, elaboration of the shape of the organs and of the body. How can the information encoded in the DNA molecule in one dimension be translated into the three-dimensional organization of the organism? The links between the gene and the execution of the genetic programme still remain to be found. Constructing chimaeras along with other manipulations of embryonic cells during development will certainly continue to help in deciphering one of the most central problems in biological sciences: *ontogenesis*.