Letter to the Editor

From molecular genetics to functional genomics and physiome

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Shortly after the Conference in Banbury (1997), held under the generic "Genomics to physiology and beyond - how do we get there?" a representative group of American physiologists funded a new journal of functional genomics, "Journal of Physiological Genomics" (1999). Since physiology has to be redefined and completed, as integrative biology, Victor Dzau [1] considers the increase in contacts between physiologists and geneticists indispensable for the correlation between the classical knowledge in the field of normal and pathological physiology and those newly emerged within functional genomics and proteomics. Such contacts become increasingly necessary when gene transfer procedures bring along the possibility to alter functional features based on the respective protein, in fact to change the genomic potential [2]. This new trend is represented by the cognitive and applicative achievements in cellular and molecular biology, which lead the way to the biotechnological research and genetic engineering, where molecular cloning is now a wide used instrument for the study of proteins and even for the industrialized production of many substances. In just three decades we went from DNA cloning and mono-

clonal antibodies to the development of a wide variety of medical applications of molecular biology investigations, for both diagnostic and therapeutic purposes. One important step ahead is the recent deciphering of human genome, providing new perspectives for the detection, pathophysiology and gene therapy of various pathological conditions. Important progress regarding the put on view of human genetic vices has already been made in some inherited or acquired diseases: cardiovascular, neurological, endocrine, hematological, immunology, rheumatic, tumor and others. Their reproduction in transgenic animals brought new elements of knowledge and interpretation of their complex pathogenic mechanisms and also on the therapeutic level, leading the way to gene therapy in some pathological conditions [3].

The cloning performed so far using various animals (sheep, cows, goats, pigs, rats, mice, etc.), still presenting some genetic vices, should not be extended to humans. Mainly on ethical but also on technical reasons, for now the only therapeutic cloning foreseen involves human-pig cloning (considering metabolic similarities), for the breed of animals to be used as donors in transplant surgery. Human asexuate reproduction and fertilization by cloning are highly controversial and subject of debate for scientists and the large public as well.

On the functional level, genetics correlates have been established in various tissues and

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organs for the majority of endogenous signaling molecules (chemical mediators, circulating and local hormones, cytokines, growth factors, etc.) and their receptors (plasmalemmal, cytosolic and nuclear), as well as for some of the plasmalemmal channels allowing the passage of major ions (Na^+ , K^+ , Ca^{++} , Cl^-), showing their roles in the accomplishment of the various forms of cellular activity. Meanwhile, descending from the cellular level to the sub-cellular and molecular ones, the research in structural and functional genomics contributed substantially to the understanding of mRNA and protein synthesis and of the modulation of gene expression by extracellular signals influencing protein biosynthesis.

Just a few of the many examples are given below to illustrate the contribution of molecular biology to the genomics of physiological sciences.

Cognitive acquisitions in functional genomics

The molecular biology detailed both the storage and transmission of hereditary traits and their impact on the subsequent biochemical and morpho-functional cellular characteristics. Special attention was given in the last years to the functional concept of gene expression regarding the ultra-structural and enzymatic relevance of various proteins and other biomolecules. This has brought along precious knowledge on membrane receptors, ionic channels and their interaction and generally on the phenomena of ionotropic and metabotropic transduction of extracellular signals, involving phospholipases, G proteins, second messengers (cAMP, cGMP, IP_3 and DAG, calcium ions, etc.) and protein-kinases (Ser/Thr, Tyr), generating amplified cell responses.

Certain mutations in the genes encoding ionic channel proteins (receptor- or voltage-dependent) alter their activation/inactivation kinetics. Such structural changes with functional consequences involving the plasmalemmal conductance for major ions (Na^+ , K^+ , Ca^{++} , Cl^-) have been identified, and experimentally reproduced in transgenic animals, in the heart, skeletal muscle, kidney, central nervous tissue, opening the way for research on the diseased conditions resulting from

the respective "channelopathies" [4].

In the heart muscle for example, three types of channelopathies have been detected so far, causing QT interval prolongation or ventricular arrhythmia, as a result of single mutations involving Na^+ and K^+ channels. Remodeling of gap junction organization and connexin expression as a result of correspondent mutations is a common feature of human heart disease conditions in which there is an arrhythmic tendency [5]. Other mutations under study are those of Cl^- and K^+ channels in skeletal muscle involved in myotonic manifestations and those in vascular wall of hypertensive subjects. In the case of arterial hypertension it has been shown that certain genetic alterations of membrane ion fluxes or of endothelial release of nitric oxide are accompanied by an increase in vascular smooth muscle tone, hence the vasoconstriction and increased blood pressure [6].

A complex relationship between calcium entry from the extracellular space and calcium release from reticular stores has been described, involving not only the already classical calcium-induced calcium release, but also the modulation of plasmalemmal calcium channels by the reticular calcium load; capacitative calcium entry, meaning activation of calcium influx by store depletion and inactivation at normal reticular load. On the other side, an important fraction of cytosolic calcium is permanently taken up by the mitochondria (to maintain the activity of redox enzymes, hence the level of oxidative phosphorylation) and by the nucleus, involving an ATP-ase and inositol - tetraphosphate [7]. As an intracellular messenger almost indispensable to all forms of cellular response, cytosolic calcium increases during cell activation as a result of the intensification of both the influx and the release from the endoplasmic reticulum, involving IP_3 and from mitochondria, involving cyclic ADP-ribose [8]. In addition, mutations in genes encoding presenilins proved to sensitize neurons to apoptosis by different mechanisms e.g. increased caspase-3 activation, oxyradicals production and calcium signaling dysregulation [9].

In the modulation of calcium channels, nitric oxide (NO) also participates, as a chemical messenger (very diffusible through lipid membranes), by activation of the guanylate cyclase - cGMP

system and by other mechanisms. NO donors as L-arginine, nitro-glycerine or sodium nitropruside inhibit calcium entry to the cytosol, while NO-synthase inhibitors activate it [10]. Through this mechanism NO participates (together with oxygen-containing free radicals) in the basal vascular tone and also to ischemic preconditioning and myocardial cytoprotection [11].

In turn, molecular biology research on membrane receptors substantially contributed both to the establishment of their chemical structure and to a more profound understanding of the physiopharmacological mechanisms for the actions of bioactive substances. Both in the case of ionotropic or metabotropic membrane receptors and of cytosolic and nuclear ones, the genetic expression, chemical structure and transduction system of the specific cellular response-generating information have been made clear. Also, it has been precised that while the ionotropic receptors are polymers coupled with an ionic channel, that will induce fast cellular responses, the metabotropic receptors are monomers coupled with a transduction membrane protein (G protein), which generates slow effects, with the participation of the second messengers.

Important progress has been made concerning the plurality and heterogeneity of the receptor types and subtypes, according to their chemical structure, genetic origin and affinity for the physio-pharmacological ligands.

The study of various receptor types and subtypes as information transducers has meanwhile lead to the delineation of a new field in medicine, the membrane receptor pathology. One of the examples to be given is that of the insulin receptor, representing one of the most studied molecules concerning both the structural-biochemical intrinsic features and the resulting effects, at the cellular and subcellular level, including the chronology of molecular events triggered by the hormone-receptor interaction. It is well known that insulin is one of the very first peptide hormones to be synthesised by genetic engineering and for which the molecular structure of the specific receptor and the three steps of hormonal information transmission to the effector cells have been clearly established.

On the other hand, the identification of genes that modulate "death receptors" like Fas [12] pro-

vide new opportunities for therapeutic approaches. Neuronal apoptosis for instance, triggered by anti-Fas antibody is enhanced by dexamethasone [13]. Human dendritic cells generated from adherent peripheral blood mononuclear cells after *in vitro* stimulation, express Fas antigen and can undergo apoptosis upon triggering of Fas by monoclonal antibodies [14].

An increasing interest has also been shown for the inhibition of protein synthesis using antisense oligonucleotides. Their inhibitory properties rely either on their binding on the nuclear DNA double helix (polypurine-polypirimidine oligonucleotides), forming an inactive triple helix or on the hybridisation of mRNA, thus removing its capacity of translating the nuclear information to the ribosomes. In both circumstances the antisense effects of the respective oligonucleotides are accompanied by a certain degree of stimulation of the ribonuclease inactivating mRNA [15]. These can be observed both *in vitro* in cell cultures and *in vivo* in the whole organism, resulting in a molecular blockade with functional alterations similar to those induced by pharmacological agents endowed with inhibitory actions on specific receptor proteins. Antisense inhibition proved useful especially when specific antagonists are not available (drugs or antibodies). Thus, antisense oligonucleotides became a new pharmacological research tool used to interfere the chemical processes generating cellular responses.

Other examples can be given to demonstrate the new integrative concepts of genetic mechanisms involved in the normal and pathological states. Such a concept, called physiome, has already been elaborated for the identification, quantification and integration of all vital components of a tissue or organ, from molecule to function.

The integrative concept of physiome

Benefiting from the cognitive and applicative progress in molecular biology of the cell, physiology undergoes a natural fulfillment of its classical orientation by the functional genomics research. Such a goal is nowadays accomplished by the integrative concept of physiome [16]. For example, depicting the integrated knowledge on gene expression, structure, metabolism and functions

of the cardiac muscle [17] for this new integrative orientation in physiology, the term of cardiome emerged.

The PHYSIOME project launched by FEPS [2,18], although may look ambitious, has taken up and extended the basic ideas from the cardiome to other sectors of cardiovascular function (micro-circulation, arterial blood pressure, etc.). This line also encompasses the research referring to the structure and functions of proteins generated by the genome, which are the base of the new concept of proteome [19,20], as the proteic molecular expression of the genome. As a branch of functional genomics, the research on proteome is focused on the separation, identification and characterisation of cell proteins at the molecular level. By this way the molecular biology research enters a new era, called post-genomic [21].

Getting to the molecular level, research in functional genomics and proteomics deepen, enlarge and integrate the content of classical notions in normal and pathological physiology [22].

In its turn, the causal-analytical and integrative orientation of physiology, imprinting functional traits to the research in molecular genetics, opens new perspectives for both the future studies on human genome and for the development of the integrative concept of physiome. This attenuates the reductionism tendencies of molecular biology and extends the integrative view from the genome and the cell to the main tissues and organs, conferring a new content to the classical notions of homeostasis and homeostatic equilibrium, which extend now their covering area down to the molecular level.

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