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CHANGES OF PARADIGMS IN THE LIFE SCIENCES

1. PARADIGMS AND DEVELOPMENT OF SCIENCE

Among both laymen and active scientists an opinion prevails that the development of science occurs predominantly by the accumulation of knowledge and a gradual improvement in the picture of nature due to the application of new methods and techniques. The breakthroughs, such as the Big Bang theory in cosmology or deciphering of the Biological Code in the life sciences, are treated as rare exceptions. However, a closer scrutiny of almost any scientific discipline points to a noncontinuous process of development and the appearance of paradigms which are constantly subjected to verification as postulated by Kuhn (1970) in the original theory of „scentific revolutions". As observed Życiński (1983), great scientific revolutions shaking the foundation of the whole of science are rare indeed in contrast to mini- or micro-revolutions concerning respectively some fields of science (e.g. physics) or disciplines (e.g. nuclear physics).

The broad concept of the paradigm, as a model of knowledge generally accepted by the scientific community, is an inherent part of Kuhn's theory. As pointed out by Życiński (1983), in the theory of scientific revolutions the paradigm appears to be a „master-key word" used ad libitum to solve various difficulties in different contexts, and thus it is not surprising that Kuhn's critics found over 20 meanings of the word paradigm used in his first book. Kuhn himself later defined paradigms as models or patterns of studies, models containing a set of principles related to ideas, methodology and even metaphysics (Charlesworth, 1982). However, in the arts and humanities the paradigm is often related to the tradition transmitted by historical models (Barbour, 1984). To avoid terminological controversy the paradigm will be used here in the sense
of a scientific theory, or a set of generally accepted theories, the meaning of which extends beyond a narrow field of studies and which carries some philosophical implications.

Paradigms are employed by scientists living in a given period of time as a map or quide in solving small research problems, and thus observations not fitting the current paradigm are treated with suspition. In this sense the definition of scientific laws at any given moment of development in a particular discipline is the result of their acceptance by scientists themselves. Charlesworth (1982) states that „scientists do not perceive nature

directly: rather they perceive it through the spectacles provided by the scientific community at a particular time".

With a certain degree of oversimplification the history of science encompasses for Kuhn the following steps (Charlesworth, 1982):

1. the appearance or emergency of a new way of interpreting and studying scientific phenomena which becomes generally accepted;
2. the detailed elaboration of this new approach (this stage may be regarded as „normal science");
3. the gradual accumulation of „anomalies" or facts which cannot be explained within the accepted paradigm;
4. the depreciation of accepted theories and emergence of a new outlook which is accepted by the scientific community (scientific revolution phase).

This cyclic process may be repeated many times in every scientific discipline when abrupt changes and the emergence of new theories are observed. In the extreme interpretation the consecutive paradigms are just the result of a social agreement within the scientific community, but the majority of scientists accept the thesis of progress: new theories are not only different but also better since they describe nature more accurately.

Kuhn illustrates his thesis on scientific revolutions by breaktroughs in astronomy, physics and chemistry, giving as examples the appearance of Copernican heliocentric theory, replacement of the phlogiston theory of combustion by Lavoisier's theroy of oxidation, or Newton's theory of absolute time and space by the relativistic theory of Einstein. However, the life sciences, i.e. biology and medicine, belong to the field of research where during the last decades we have witnessed both macro- and mini-revolutions leading to a re-evaluation of current theories and the emergence of new paradigms. Here I would like to present three examples of such changes in biological theories, although their importance and stage of development are clearly different.

2. THE MACROREVOLUTION OF THE GENETIC CODE

A short paper published in Nature in 1953 by two virtually unknown young scientists (Watson and Crick, 1953) brought about a major change in our understanding of life processes. Although Watson and Crick proposed only

a structural model of the DNA molecule, the implications of this model were profound for genetics, evolution or the regulation of metabolism. Since the moment of elucidation of the DNA structure the focal point of biological studies shifted from the mechanistic model of the cell towards information transfer.

The „central dogma of molecular biology" formulated by Crick in 1958 and updated in 1970 (Crick, 1970) assumes the complementary nature of linear structures of nucleic acids and proteins constructed by means of a four- or twenty-letter alphabet: the replication of DN A preserves the template, while the executive information runs via the synthesis of RNA (transcription) and synthesis of proteins (translation). This strictly one-way transfer of genetic information was soon found to be an oversimplification requiring corrections, some of them being presented as broken arrows in Fig. 1:

- direct protein synthesis on the DNA template is a phenomenon of little importance taking place only in special conditions;
- renewal of RNA is limited to some viruses but it breaks the rule of absolute DNA supremacy;

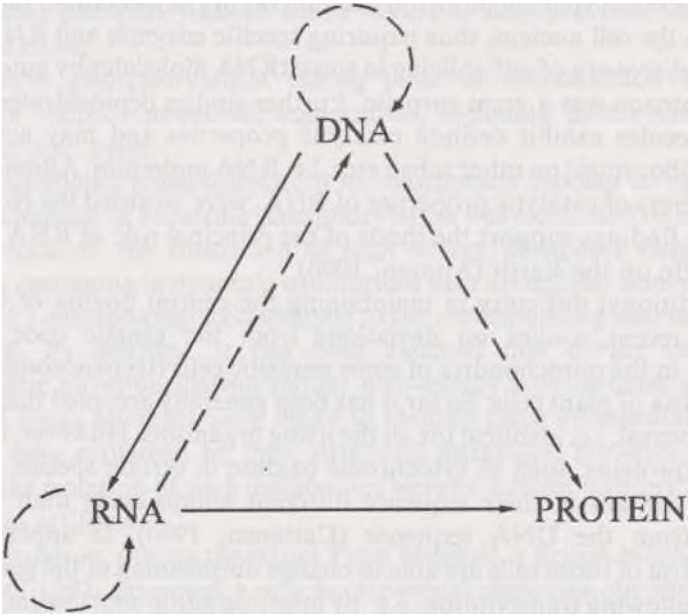

Fig. 1. Transfer of information between macromolecules. Solid arrow indicate principal routes, dashed lines - possible transfers (after Crick, 1970).

- the dashed arrow from RNA to DNA illustrates a process occurring in retroviruses, including HIV (the virus responsible for AIDS), and was for a time regarded by Temin and Baltimore as almost incompatible with the paradigm of molecular biology (cf. Crick, 1970). Although the action of reverse transcriptase is at present a side track in the transfer of genetic information it bears important biological implications, suggesting among others the priority of RNA in the early stages of the evolution of life.

A further difficulty for the central dogma arises from the discovery of the non-continuous structure of many genes. In the late seventies it was found that the majority of structural genes in eukaryotic cells contains intervening non-coding sequences (introns), which are removed after transcription by splicing in the process of mRNA maturation. The existence of introns and exons is not quite compatible with the initially proposed strict colinearity of DNA and coded proteins, but it helps to understand how the diversity of protein molecules has evolved (Dorit et al. 1990). Moreover, RNA maturation may occur in various ways leading to a situation when two different proteins are formed using information encoded in the same stretch of DNA molecule. The synthesis of two types of immunoglobulins, membrane-bound and free antibodies, may serve as an example.

The process of splicing or intron excision occurs in specialized nucleoprotein particles in the cell nucleus, thus requiring specific enzymes and RNA. For that reason the discovery of self-splicing in some tRNA molecules by autocatalysis in certain protozoa was a great surprise. Further studies demonstrated that some RNA molecules exhibit definite catalytic properties and may act almost as enzymes (ribozymes) on other substrates, i.e. RNA molecules. Altman and Cech, the discoverers of catalytic properties of RNA, were awarded the Nobel prize in 1990; their findings support the thesis of the principal role of RNA in the early stages of life on the Earth (Altman, 1990).

An additional difficulty in maintaining the central dogma of Crick arises from the recent studies on deviations from the genetic code, deviations discovered in the mitochondria of some parasitic cells (trypanosoma) and in the mitochondria of plant cells. So far it has been generally accepted that the genetic code is universal, i.e. identical for all the living organisms. However, it was found that some proteins, such as cytochrome oxidase in certain species of plants or protozoa, contain in their sequence different amino acids than it might be expected from the DNA sequence (Cattaneo, 1990). It appears that the mitochondria of those cells are able to change the meaning of the genetic code in the stage following transcription, e.g. by inserting additional uridine nucleotides (Cattaneo, 1990). This proces has been named „RNA editing" and although it is probably limited to a few cell types only, its existence challenges the current dogma.

In my opinion, however, the various exceptions to the principal thesis of molecular biology on the one-way transfer of information from linear structures
of DNA to proteins, briefly reviewed above, are not sufficient to abandon or drastically revise the accepted paradigm, although Altman (1990) regards the genetic information in DNA and RNA as equivocal. In the scheme of development of science proposed by Kuhn molecular biology is still in the stage of accumulation of new data.

3. THE CHEMIOSMOTIC THEORY AS A MINIREVOLUTION IN BIOCHEMISTRY AND BIOPHYSICS

The generation and utilization of energy in the cell have been the subject of extensive studies which have led to the discovery of adenosinetriphosphate (ATP) in the late thirties and its acceptance as the key intermediate. ATP has been regarded as the universal carrier of energy required for performing such biological processes as muscle contraction, transport of metabolites by biological membranes, generation of electric impulses during nerve transmission, or the generation of light in fireflies.

Already in the early fifties it became clear that ATP may be generated in three principal ways:

- substrate phosphorylation, which occurs in such processes as glycolysis and fermentation;
- oxidative phosphorylation taking place in mitochondria during the oxidation of various metabolic compounds, including intermediates of the tricarboxylic acid cycle;
- photosynthetic phosphorylation in chloroplasts exposed to light.

The mechanism of substrate phosphorylation was elucidated relatively early and it depends on the formation of high energy phosphate esters in some metabolites remaining in dynamic equilibrium with the cellular pool of ATP; the oxidation of 3-phosphateglyceraldehyde to the corresponding acid may serve as an example. By analogy, it has been assumed that during oxidative or photosynthetic phosphorylation similar high-energy intermediates are created. For several years attempts were made to identify these intermediates and the difficulties were explained by their structural instability. However, numerous reports on the isolation of such metabolites were on a closer scrutiny found to be experimental artifacts.

In this situation, during the sixties Peter Mitchell, a British biophysicist who died in April 1992, proposed a new controversial chemiosmotic theory, which depreciated the role of ATP and emphasized the importance of biological membranes in the generation of energy during oxidative electron flow (Mitchell, 1979). The basic assumption of Mitchell's theory is the anisotropic character of biological membranes leading to the appearance of electric potentials and differences in the concentration of hydrogen ions on the two sides of the

12 membranes in active mitochondria and chloroplasts. This „proton motive force" of Mitchell may be directly utilized by a cell for osmotic work, active transport of metabolites by membranes and even mobility of cilia in the bacterial cell wall. On the other hand, ATP is generated as a side reaction during the decrease of the hydrogen ion gradient when the ions are passing via the enzymatic complex of membrane-bound ATPase. In other words, as a result of electron transport in mitochondria and chloroplasts the membrane becomes „energized", whereas ATP formation is a secondary, albeit biologically important, process.

The chemiosmotic theory was initially treated with suspicion by scientists supporting the idea of chemical intermediates and only in 1978 did Mitchell receive the Nobel prize. His theory may also be regarded as the sucess of biophysics and its supremacy over classical biochemistry, and this fact explains the initial scepticism of traditional scientists. The beauty of Mitchell's theory depends among other features on its universal character: the same proton motive force functions in bacteria, plants and animals. Several aspects of Mitchell's theory remain elusive and are being extensively studied, including molecular mechanism of ATP synthesis. Many researchers believe that the mystery of energy transformation in the cell is related to conformational changes of specific membrane-bound proteins. But all these problems appear secondary and marginal now that the bold chemiosmotic theory has opened new challenges and new possibilities for contemporary biochemistry and cell biology.

4. THE CYTOKINE NETWORK AS A MICROREVOLUTION IN CELL BIOLOGY AND ENDOCRINOLOGY

Before closing my remarks on the relatively fast changes of views in the life sciences I would like to give an example from my own field of research. For the last 15 years our team in the Institute of Molecular Biology at the Jagiellonian University, has been involved in studies of the regulation of the synthesis of the so-called „acute-phase proteins", which are synthesized at greatly increased rates as a result of infection, injuries, tissue necrosis (such as myocardial infarction) and also in neoplastic diseases. The inflammatory response elicited by various noxious stimuli leads to the activation of macrophages, fibroblasts and other cells which start to produce regulatory protein molecules known as interleukins, or more correctly, cytokines. At present at least a dozen cytokines are known but their list is not generally accepted because of great confusion in nomenclature. Cytokines in vivo are not only responsible for changes in the synthesis of acute phase proteins but elicit broad systemic effects such as fever, leukocytosis and the production of antybodies by immunocompetent cells.

Identification of cytokines has become possible thanks to the application of model systems of tissue culture: on one side hepatocytes isolated from rat liver

(or established tumour lines deriving from human liver) which produce acute phase proteins and on the other, cultured macrophages or fibroblasts producing cytokines. As a result of cytokine action drastic changes in the synthesis of acute phase proteins take place and they can be accurately measured with immunological techniques estimation of messenger RNA for individual proteins in liver cells. Thus it is possible to quantify the synthesis of acute phase proteins induced by natural or recombinant cytokines added to a hepatocyte tissue culture. The situation is highly complex since individual acute phase proteins may respond differently to individual cytokines or their mixtures (Koj, 1989). We are beginning to understand the molecular mechanisms of these phenomena since acute phase proteins are now regarded as a classical model for studying the regulation of gene expression in animal cells. But here I would like to point out two characteristic properties of cytokines as transmitters of metabolic signals (Hilton, 1992):

- Redundancy of action - different cytokines may give similar biological effects in the target cells: e.g. interleukin-6 (IL-6), leukemia inhibitory factor (LIF), interleukin-11 (IL-11) and oncostatin-M (OSM) all evoke similar changes in acute phase protein synthesis in hepatocytes. This redundancy may guarantee that a biologically important homeostatic response (acute phase protein synthesis) will take place even in the absence of one or another of the cytokines.
- Pleiotropy, i.e. ability to act on various target cells which respond in a different way according to their phenotypic differentiation (IL-6 and LIF act on both hepatocytes and the cells of the immune system). Pleiotropy emphasizes the complex character of the acute phase reponse which probably represents, from the point of view of evolution, the organism's oldest defence mechanism before the development of the immune system.

It is interesting that redundancy and pleiotropy is characteristic also for some neurotransmitters, thus indicating certain similarities between the immune and nervous systems.

It should be added that cytokines also act on specialized endocrine glands (hypophysis) and this is reflected by changes in the level of various hormones, mainly glucocorticoids, and the rearrangement of many metabolic processes during the acute phase response. Until recently, hierarchic organization of endocrine glands and specialized secretion of regulatory molecules (hormones) was a biological paradigm. Insulin, a classical hormone transported by the blood, is produced solely by specialized cells in the pancreas (endocrine secretion), whereas acetylcholine is produced locally in the motoric synapses (paracrine secretion), and some tumours produce growth factors stimulating their own proliferation (autocrine secretion) (Sporn and Roberts, 1992).

Cytokines exhibit the features both of local cellular growth or differentiation factors, and of typical hormones transported by the blood and acting on distant target cells, and thus do not fit into the classical definitions. In the biomedical sciences the term „cytokine network" is now being used, similarly to the

 hormonal or endocrine networks. Future studies will elecidate the importance of the cytokine network for the control of metabolic processes in health and disease, but already today we are witnessing a new approach to the mechanism of hormonal regulation and intercellular signalling.

5. CONCLUDING REMARKS

The examples described above on the progress in molecular biology, biochemistry and cell biology confirm the thesis on the non-linear development of the life sciences. Although it is not easy in all cases to establish an idealized scheme of the „normal science" and „revolution" depicted by Kuhn, breakt- roughs in scientific reasoning are often observed. Moreover, if scientists neglect the occurrence of such drastic changes progress and the emergence of new original theories may be delayed. Experimental verification of such theories is often accompanied by changes in the methodology of research and the conclusions may extend well beyond the discipline in which such a breakthrough is taking place.

LITERATURE CITED

Altman, S. (1990) Enzymic cleavage of RNA by RNA (Nobel lecture).
Angew. Chem. Int. Ed. Engl. 29, 749-758.

Barbour, I.G. (1984) Mity, Modele, Paradygmaty, Kraków: Znak.

Cattaneo, R. (1990) Messenger RNA editing and the genetic code. Experientia, 46, 1142-1148.

Charlesworth, M. (1982) Science, Non-science and Pseudoscience. Burwood, Victoria: Deakin University Press.

Crick, F.H.C. (1970) Central dogma of molecular biology. Nature (London) 227, 561-563.

Dorit, R. L., Schoenbach, L. and Gilbert, W. (1990) How big is the universe of exons. Science, 250, 1377-1382.

Hilton, D. J. (1992) L1F: lots of interesting functions. Trends in Biochemical Sciences, 17, 72-76. Koj, A. (1989) The role of interleukin-6 as the hepatocyte stimulating factor in the network of inflammatory cytokines. Ann. N.Y. Acad. Sci. 557, 1-8.

Kuhn, T. S. (1970) The Structure of Scientific Revolutions. University of Chicago Press.

Mitchell, P. (1979) Compartmentation and communication in living systems. Ligand conduction: a general catalytic principle in chemical and chemiosmotic reactions. Eur. J. Biochem. 95, 1-20.

Sporn, M.B. and Roberts, A. B. (1992) Autocrine secretion - ten years later. Ann. Int. Med. 117, 408-414.

Watson, J. P. and Crick, F.H.C. (1953) Molecular structure of nucleic acids. Structure for deoxyribose nucleic acid. Nature (London) 171, 737-738.

Życiński, J. (1983) Jeżyk i Metoda. Krakow: Znak.