DEGENERATIVE JOINTS

VOLUME 2

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DEGENERATIVE JOINTS
Volume 2

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PREFACE

Osteoarthrosis can be treated as a condition rather than as a disease. It occurs with increasing frequency in the elderly. Being "osteoarthritic" does not necessarily mean being ill or disabled.

Many clinicians know that the amplitude of an osteophyte is not necessarily correlated with the patient's impression of pain. We are often confronted with a severely modified, but asymptomatic joint anatomy.

Many of the biochemical or roentgenological features of this "condition" can be interpreted as a response of the joint to a number of impacts increasing with time and they reflect the natural defence mechanisms of a continuously loaded joint.

This defence mechanism is probably as important as the aggressive "breakdown" processes described in literature. During this symposium attention was focused on factors influencing repair in joint tissues.

Genetic control of specific cell function, the influence of ageing and cell division on cell function were discussed. Structures, turn-over, intermolecular organisation and physiological role of matrix components were reviewed. Various mediators, cell-cell, matrix-cell and tissue-tissue interactions were themes of discussion. The reliability and the possibilities of different in vitro and in vivo models were subject of criticism.

It was a pleasant and interesting task to edit the flow of ideas that emerged from this meeting.

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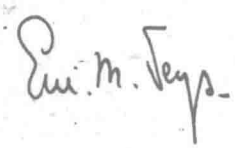
Last but not least the lively and informal way in which the different participants discussed and criticized each other's results stimulated the organisers to think about a next workshop in the city of Ghent.

Eric M. VEYS



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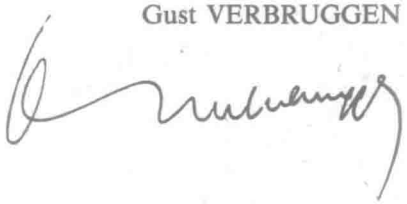

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"De vissersvrouw" by Constant Permeke
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CELL DIFFERENTIATION AND GENE EXPRESSION: A BRIEF OVERVIEW

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CONTROL OF GENE EXPRESSION

In bacteria such as Escherichia coli growing under normal conditions, at most 10% of the genes are expressed at any given time. However, all genes are potentially active and can be switched on by appropriate signals. The control mechanisms which keep most of the genes silent nearly all act at the transciptional level. Functionally related genes are clustered in single control units called operons. Most of these operons are under negative control; they are kept transcriptionally inactive by a repressor. Upon induction, the latter becomes reversibly inactivated. These repressors are usually highly specific for a given operon. There are also examples of positive control mechanisms, operons switched on by the appearance of a positive control element called an activator. The latter can be highly specific for a given operon or can have pleiotropic effects such as, for example, the cyclic AMP binding protein which can activate a number of transcription units when the glucose concentration becomes low and cyclic AMP accumulates.

In the case of the yeast Saccharomyces cerevisiae we are still dealing with an undifferentiated, monocellular system. However, yeast is already a eukaryote with a topological separation between genetic information and its expression as a messenger RNA in the nucleus, and the execution of the program, namely translation, which occurs in the cytoplasm. Although the DNA content of yeast and, hence, the genetic complexity is only 4-fold that of a bacterium, we see that the regulatory networks are already considerably more complex and sophisticated. Nevertheless, practically all genes are still potentially active and can be switched on by the proper internal and/or external conditions. However, there is already one important exception, namely, a form of primitive differentiation which fixes the cells in an α or an a mating type. The cells, as well as their progeny, are committed to a given mating type and the switch from one type to the other involves a rearrangement at the DNA level.

This possibility of a given cell lineage to be committed to a selected genetic program has further evolved in, for example, the Trypanosome parasite. This parasite evades the immune defense system of the host by sequentially switching expression of surface antigen genes. This switching to a new surface antigen expression again involves fairly irreversible rearrangements at the DNA level.

Obviously, if differentiation of an animal involves rearrangements at the DNA level, the somatic cells are no longer pluripotent. Indeed, except for the very

primitive forms, it is not possible to regenerate an animal from a single cell as it is often possible in plant systems (even with the higher plant species). When is pluripotency lost in embryogenesis and what is its molecular basis? The most important experiments which were addressed to this question were carried out by the British biologist J. Gurdon. He took oocytes from Xenopus laevis, inactivated the nucleus, and reintroduced a nucleus from tadpole gut cells. These transnucleated oocytes now could give rise to fully viable tadpoles and frogs. Hence, the actively growing cells of the tadpole gut were still pluripotent and potentially able to express the entire genetic program. However, similar experiments in which the nuclei were provided by gut cells from the adult frog failed. Thus, in developing from the tadpole to the adult, some irreversible step apparently had taken place at the chromosomal or DNA level, but the molecular mechanism of this irreversible event still escapes us.

Similar experiments have later been carried out in mammalia. For instance, it has been possible to carry out transnucleation experiments in mouse oocytes. This was successful with nuclei coming from the blastomere stage of the mouse embryo, which means again that at least in this early stage in embryogenesis, all cells were still pluripotent. However, nuclei coming from the next stage in embryogenesis could not be used successfully for transnucleation. Hence, we can conclude that very soon in embryogenesis an irreversible step (or more likely a program of successive steps) takes place. Hence, the genetic program of development and differentitation can be seen as a whole series of sequential dichotomies, resulting in more and more differentiated and committed cell lineages. As far as can be determined, a full complement of DNA and, hence, of genetic information is present in any single somatic cell of the animal or of a human. However, the expression of a given gene in a differentiated somatic cell is under two constraints, developmental control and environmental control.

DEVELOPMENTAL CONTROL

As explained above, embryogenesis and later development can be seen as a series of irreversible steps taking place in the cell lineage. These steps have profound consequences for the further expression of the genetic program and presumably involve rearrangement at the DNA level, but the proof for this at the molecular level is not known. There is, however, one example, where the DNA rearrangement leading to terminal differentiation is known, namely, in the formation of functional lymphocytes. With some oversimplification, we can say that the B-cells are responsible for the diversity of humoral antibody formation while the T-cells, including helper, suppressor and cytotoxic forms, recognize antigen in a cellular context by having a matching diversity of T-cell receptors. We know now that both

the diversity of B-cells (which each secrete a different antibody) as well as the diversity of T-cells (which each recognize a cell-presented antigen) exert their committed phenotype as a result of irreversible rearrangements at the DNA level.

I doubt whether a terminally differentiated cell can return to an earlier stage of its lineage. The only apparent exception is upon malignant transformation; in this case, silent genes, among which oncogenes are the most important, become highly expressed, either in a normal or mutated form. Although this is a new field which still has to mature, it is known that this oncogene expression can be due to irreversible rearrangements which have taken place in the formation of the cancer cell. The first reported case was the Philadelphia translocation in myeloid leukemia. Following up on this hint, it is now believed that at least 20%, if not more, of solid tumors in humans have experienced DNA translocations and rearrangements, often resulting in expression of oncogenes like c-myc or c-ras.

ENVIRONMENTAL CONTROL

In a normal somatic cell, a limited number of genes are constitutively expressed. These code for proteins and other factors which take care of the cell's housekeeping functions. In addition, a limited number of genes, usually different in each type of differentiated cell, can be induced by outside signals. The latter can be of a fairly aspecific nature such as, for example, butyrate which interferes with the deacetylation of histones and, in so doing, causes a general tendency towards a resting stage or a more fully differentiated stage. Dimethylsulfoxide (DMSO) has a somewhat similar effect, presumably by changing membrane properties. For example, Friend cells are erythroleukemic cells which can be induced by DMSO to a more differentiated program in which globin in now synthesized. Another example is the human promyelocytic leukemic line HL60 which can be switched to differentiate into a myeloid type cell by treatment with DMSO or butyrate. Alternatively, upon treatment with phorbol ester, the HL60 cells become terminally differentiated into macrophage-like cells. 5-methyl C-residues in CG sequences, present in promoter regions, also have an effect on gene expression; upon treatment with a specific analog, namely 5-aza-cytidine, some dormant genes may become activated.

In addition to these fairly aspecific treatments which presumably are related to chromatin structure in potentially active regions of the chromosome, more specific induction of a gene or a small set of genes is often possible by external factors. For example, in many cells, glucocorticoids can induce a number of functions. Heavy metal ions (zinc, cobalt) can switch on metallothionein genes, etc. Another example is the induction of interferon-β in fibroblast cells upon treatment with double-stranded RNA. These specific inductions can now be studied by genetic engineering approaches and in these examples, it has been shown that the regulatory elements responsible for gene expression are found upstream from the gene and its promoter.

By selective removal of DNA sequences upstream from the gene, the latter becomes unresponsive to the inducer (Tavernier et al., 1983). Undoubtedly, this upstream DNA control element must be recognized by a specific protein which so far is unknown. However, one protein which is a very interesting control element, has been studied in detail. This is an oncogene, namely the T-antigen coded by the virus SV40. Very interestingly, T-antigen can switch off the SV40 early promoter by binding to the promoter region (and in so doing it negatively autoregulates itself). In addition, SV40 T-antigen is an essential positive control element for late gene expression allowing the synthesis of the viral structural proteins in monkey cells.

A number of important regulatory proteins act on cells from the outside and have a positive or negative proliferation activity (autocrine and exocrine control). The positive control elements are growth factors such as epidermal growth factor, platelet-derived growth factor (which also can be an oncogenic protein), insulin, somatomedin, etc. Interferons, by definition, have an antiviral activity and they act on the cell by induction of a limited set of genes, perhaps as many as 20 or more. The natural function of interferon-γ is presumably not antiviral but more immunoregulatory. It has an antiproliferative activity on many types of cells and it switches on a number of genes, some but not all correspond to the genes switched on by interferon α or β. Like the other interferons, IFN-γ enhances HLA expression but, furthermore, it has the unique property of specifically switching on the Ia or the DR HLA antigens, even in cells such as endothelial cells which are normally Ia^- (Collins et al., 1984). Such cells are not only Ia^+ but also functionally immunocompetent. They can present antigen to the T-cell system. In this way, IFN-γ can play an important role in a local inflammation where it causes and immune activation cascade.

This control of gene expression by extracellular factors brings us to a different and higher hierarchy of control exerted by extracellular factors. For example, interferon-γ may be induced (and secreted) in lymphocytes by interleukin 2, which is itself produced as a result of stimulation of helper T-cells by antigen and interleukin 1. The latter is secreted by macrophages activated by interferon-γ. In this way, the regulatory circuit is closed and may be self-enhancing until other factors dampen the response. This gives a glimpse of the complicated communication network between cells which, of course, as in the case of inflammation, can easily be distorted at one point or another in the network.

In order to understand such networks in molecular detail, one needs to dissect them such that the individual steps can be studied. For this, the mediators have to be available in an absolutely pure form. This can now be done through genetic engineering, involving the cloning of the gene responsible for synthesis of the factor, its manipulation and its expression in appropriate systems such as Escherichia coli.

Human interferon-γ can be expressed in E. coli at a 30% level which means that 30% of all proteins present in E. coli are in fact human interferon-γ; Simons et al., 1984). This interferon-γ is now in clinical trials in Europe, the United States and Japan. Also, human interleukin 2 has been cloned and expressed in bacteria (Devos et al., 1983) and is currently in clinical trials.

What a molecular biologist needs for such studies is either a pure factor from which an amino acid sequence can be derived, or else, a very sensitive biological assay. Starting from there, he can identify the gene, clone it, and determine its structure. Subsequently, it can be expressed in bacteria or other appropriate systems. In this way, pure products can be made available both for study of the complex control networks between cells, and for studying the expression of genes (e.g., by using the cloned gene for quantitation of specific mRNA by hybridization) and occasionally such genetically engineered products may be useful for therapy.

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