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Review Article

The Secret of Epigenesis and its Implication for Cell Therapy

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New molecular mechanism for cell differentiation essential for epigenesis is uncovered. The massive transcription of all meiotic chromosomes along the side-elements of the synaptonemal complex has not been explained till now. The messengers transcribed are packed with "translation repressor proteins" into mRNP particles and stored in the ovoplasm, known as "maternal inheritance". The significance of this immense ovoplasmic genetic information was not understood. It is logical to admit that during embryonic development the highly conservative deblocking proteins are transported by the mRNAs to the nascent DNA strands of the corresponding genes. A stable DNA- protein complex is formed, selectively deblocking the genes and transforming the heterochromatin into active euchromatin.

In this aspect the ovoplasmic mRNP particles play the role of morphogenes designed to unlock selectively new genes during epigenesis, which in and of itself represents the molecular mechanism of cell differentiation.

New avenues are thereby introduced, helping to resolve many unsolved questions concerning the biology of cell differentiation. A new kind of in vitro gene engineering will make possible the unlocking of desired genes and create specific cells for the purposes of regenerative medicine.

Key words: epigenesis, cell differentiation, cell therapy.

The molecular basis of cell differentiation is one of the fundamental and most challenging unsolved problems in the biological sciences. A historical survey shows that during the "post genomic era" the efforts of researchers to resolve cell differentiation have been focused on transcriptional mechanisms and substances that switch genes on and off. The Sanger Institute in Cambridge, UK, and Epigenomics, a Berlin-based Corporation have focused their research on gene on/off switches, the main goal of their 'Human Epigenome Project' being to study and map DNA methylation, or 'epigenetic' changes across the entire human genome [5]. David Allis from the University of Virginia believes that the "histone methylation switch" is probably responsible for gene expression or lack of expression [5]. Histone methylation affects genes packed in nucleosomes in the inactive heterochromatin. Such type of gene stimulation for transcription is not equivalent to cell

differentiation [4]. Under normal circumstances a cell has to be already differentiated to respond to stimuli switching on the gene for function.

All differentiated cells possess specific deblocked structural genes in their active euchromatin. These genes must receive a signal to activate the promoter, so that RNA polymerase can begin transcription. At this point, the gene is switched on for *function* or gene expression. After transcription is terminated, the gene is switched off but the cell remains differentiated because the gene is deblocked and ready to respond again to the next starting signal. *Gene deblockage occurs only once during embryonic development and results in DIFFERENTIATION. Activation of deblocked genes in differentiated cells is repeated and results in FUNCTION [4], see Stem Cells and Self Renewal).* "There are two distinct levels of regulation in mammalian somatic cells. One level is concerned with genomic commitment (i.e. determination) and the other level with the expression of tissue specific proteins" [1], p. 247. Hence, *it is critical when considering epigenesis not to confuse gene deblockage and the processes of differentiation with gene on/off switches and the processes of gene expression or function.*

M o r g a n [25] established that gene activity in the earliest embryonic development is influenced by the ovoplasmic heterogeneity. During cleavage, a variety of ovoplasmic substances surround the nuclei and activate specific genes. This process, generally termed nucleo-cytoplasmic induction, has been confirmed repeatedly, as acknowledged by H o p p e r and H a r t [17] who note that " Basically, the interplay between nucleus and cytoplasm is considered to be the force that moves the cell along a specific pathway of differentiation and is thus responsible for development."

Morgan and his followers made tremendous strides in defining the maternal mRNAs in the sea urchin ovocytes [7, 10]. In spite of the large quantities of rRNAs and of other repeated RNA sequences, these investigators established that there are longer transcripts packed with special proteins of unknown chemical composition. These proteins prevent the translation of the messengers in the ovoplasm. These messenger ribonucleoprotein particles (mRNPs), described in the literature as 'masked mRNAs', 'long lived ribonucleoproteins', maternal mRNA and oogenetic messenger RNPs [18, 19, 24, 30, 31], are thought to carry the maternal inheritance. It is very probable that Vogt's fate maps [29] depend on the specific order in which the RNP particles are distributed in the ovoplasm.

The mRNP particles have been separated and analyzed, but the time and mechanism of their origin are not known. D a v i d s o n et al. [11] believe that the mRNPs are translated after fertilization but that other possibilities exist as well, i.e., a regulatory function is considered but has not been found.

During my extensive investigations of ovopoiesis in amphibia, birds and mammals I have become intimately familiar with the details of egg maturation, from oogonium to ovulation and particularly meiosis [2]. Initially, oogonia multiply by simple mitosis, but after a last DNA replication mitotic division stops and the oogonium enters the prophase of the first meiotic (reduction) division, thereby becoming a primary oocyte in leptotene stage. During zygotene and pachitene the duplicated homologous chromosomes fuse as bivalents or tetrads, at which time segments are exchanged between two non-sister chromatids (crossing over), a process responsible for a substantial part of the diversification of the species.

Under the electron microscope, bivalents show a central element and two lateral elements, a structure termed a "synaptonemal complex". The central element is involved in the crossing over. The lateral elements of the synaptonemal complex develop thousands of DNA side loops (as many as 20000/chromosome in some animals, e.g., salamanders), which are involved in active transcription of the entire genome. During the next meiotic phase (diplotene), the bivalents stretch and form "Lampbrush chromosomes" [15], which continue to transcribe all types of mRNAs. This unique phenomenon needs further exam-

ination. Translation does not occur in the ovoplasm, because the mRNAs are packed with special nonhistone proteins found in the nuclei, called *translation repressor proteins* (TRP) or Hox proteins, which prevent translation [1], p. 593. Messenger RNAs packed with TRP do not undergo processing, splicing of introns, or attaching to ribosomes. Instead they are accumulated in large quantities as long-lived messengers (mRNP particles) in the egg cytoplasm.

Some nuclear proteins are synthesized by genes, which contain a homeobox (a sequence of about 180 nucleotide pairs). Homeobox (Hox) containing genes have been found in many species including man [16]. The homeobox sequence has been conserved at the protein level throughout (over) 500 million years of evolution. "These proteins, localized in the cell nucleus suggest direct involvement in the control of gene expression" [1], p. 937.

Recently more data accumulate indicating that Hox proteins cause morphological diversity and contribute to body-plan evolution [16, 24, 26]. However, the exact mechanism remains obscure.

The experiments of De Robertis and his school [6,8] indicate the role of homeodomain proteins in specifying the identity of cells, tissues and body regions. It is in general assumed that these proteins bind directly specific DNA sequences. However, "Gooscoid and bicoid proteins can bind to similar target sequences" [6], but do not have the ability to recognize and bind directly DNA of a given structural gene. The conservation of these special proteins over millions of years is an indication that in combination with mRNAs they participate in a very important process, essential for epigenesis and for the conservation of the cytoplasmic genetic memory.

Two main groups of nuclear proteins have evolved during evolution:

1) Histones (basic) to protect and keep genes blocked.

DNA + histones = nucleosomes = heterochromatin = inactive.

Histones don't recognize gene sequences.

2) Hox proteins (acidic) [16, 26], bind with mRNAs (RNPs), to unlock the genes.

DNA + Hox proteins = no nucleosomes = euchromatin = active.

Chromosome banding clearly shows the localization of hetero- and euchromatin in the condensed metaphase chromosomes. Giemsa C-banding stains the basic histones dark purple, while the acidic Hox proteins in the euchromatin remain unstained.

The rDNA, which is permanently bound with acidic proteins, appears as secondary constriction in the satellite chromosomes 13, 14 ,15, 21 and 22 of the human karyotype [3]. The genes belonging to the housekeeping genome are as well permanently bound with acidic proteins and are located as tiny light segments in the metaphase chromosomes. In interphase nuclei, the heterochromatin appears as dark patches while the euchromatin is dispersed in the nuclear matrix.

Under normal circumstances a structural gene packed with histones cannot be transcribed and belongs to the inactive heterochromatine. It is the complex DNA-Hox proteins that make the gene accessible to RNA polymerase and thus transcribable, as it belongs now to the active euchromatin.

Some attempts have been made to explain the connection between nucleohistones and the acidic nonhistone proteins. The *histone displacement model* of S t e i n et al. [28], for example, proceeds from the assumption that genomic DNA is permanently bound with histones. However, the variety of different models connecting nucleohistones and nonhistone proteins suggests that we are far from understanding the transition from inactive to active chromatin.

The big question is, do histones and nucleosomes exist in the euchromatic synthetically active genes? F r a n k e et al. [14], using the spreading technique of M i l l e r and B e a t t y [23], and electron microscopic observations unequivocally show that the DNA

of actively transcribed regions is not packed into nucleosomal particles. This is a morphological proof that during differentiation acidic proteins replace the histones in an inactive gene, and so prevent the formation of nucleosomes on the level of the newly syntesized gene, which is now deblocked and open for transcription. It is generally accepted that epigenesis is the gradual deblockage of specific genes during embryogenesis.

If histones and nucleosomes are not present in the transcribing genes, methylation and acetylation cannot interact with them, but would interact with inactive histone-bind DNA only. An attempt to explain such interaction [13] does not make clear how the nucleosomes are displaced from DNA and how they get re-formed. Similar mechanism might cause the massive genome transcription of bivalents during meiotic prophase, but further research is necessary.

While observing lampbrush chromosomes in various contexts over the years, I repeatedly wondered why all the genes are transcribed if the egg follicle does not need their products. Why are these messengers packed with specific proteins that prohibit translation? What is the role and significance of the mRNP particles, containing so much genetic information stored in the ovoplasm?

If the genes of sperm are protected to prepare them for the long trip outside the male body, blocked by histones and protamines against extraneous damaging factors, and the genes in the egg nucleus are blocked as well, then it follows that there must be a natural mechanism to deblock the specific genes after fertilization. Then it suddenly flashed upon me that the mRNPs *are designed to deblock their own blocked genes by a positive feedback mechanism. I enjoyed discovering how simple, logical and smart this secret of the Nature is.*

After fertilization the mRNP particles remain inactive, as long as the early embryonic rapid cell cycles are under the control of cyclin-dependent protein kinases [9], and under the high concentration of histones in the ovoplasm. During the rapid replications, the newly synthesized DNA adopts the histones and the genes remain blocked. This control changes markedly as the embryo undergoes gastrulation (species specific differences are possible). The mRNP particles, depending on their ovoplasmic distribution enter the nuclei and unfold to read the sequences of their complementary DNAs at the time of replication. The complementary RNA, synthesized during meiosis recognizes its template and selectively attaches the deblocking proteins (Fig. 1) to the newly synthesized DNA strand forming a stable DNA/ Hox protein complex. Thus the gene passes from a blocked to an unblocked state. Heterochromatin turns into euchromatin, and the gene is permanently deblocked and accessible for RNA polymerase. The embryonic cell, now committed to transcribe and translate the deblocked gene is differentiated.

The role of mRNA as chaperone and mediator gives answer to the question how the deblocking Hox proteins find and bind selectively the corresponding genes.

The mRNP particles stored in the ovoplasm appear now to represent the substrate caring the *CYTOPLASMIC GENETC MEMORY*, or *the MORPHOGENES*, which unlock selectively new genes. In adult somatic cells, nontranslatible mRNPs encoded by the active genes specific for the given cell, maintain the differentiated state in the long term. The same compounds interact with the genes of transplanted nuclei. For instance if a nucleus from a differentiated cell is injected in the cytoplasm in another differentiated cell, it acquires the characteristics of the host cell [1], p.900.

Recent experiments with RNA interference show that "killing the messenger" [12] stops embryonic development [27], and the embryos die. These observations confirm the important role of the mRNPs in cell differentiation during embryogenesis. Moreover the total genome transcription during egg maturation takes on now a new and important significance in understanding epigenesis, for it explains the exact and selective deblockage of genes, which cannot be achieved by methylation or other similar procedures. Instead, *the oocyte displays a remarkable feedback mechanism to selectively unlock new genes*

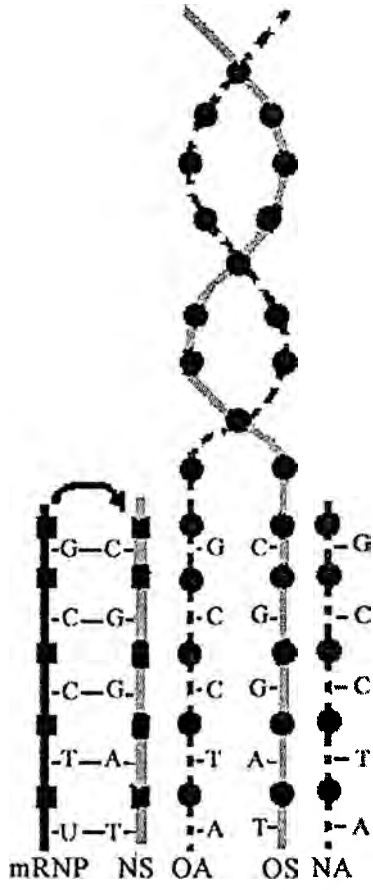

Fig. 1. A model for deblockage of a gene by maternal mRNP
Sense DNA strands — grey; antisense DNA strands — dashline; OA and OS — the old antisense and sense strands; NS and NA —the newly synthesized DNA strands; mRNP — maternal messenger ribonucleoprotein; Black dots — nucleosomes; black squares-mRNA+ deblocking Hox protein complex; a structural gene (NS) is present in the newly synthesized sense strand only; the complementary mRNA recognizes it and the Hox proteins are selectively attached to the gene (NS), which is now permanently deblocked

during development. The attachment of special proteins by mRNA to complementary DNA resulting in gene deblockage indicates that in the addition of the well known process DNA-RNA-protein, a feedback mechanism, namely protein –RNA–DNA exists and is pivotal in the processes of epigenesis. What a wonderful resolution of the theoretical quest against the dogma [21, 22].

Many additional details accompanying the action of the maternal mRNPs need to be elucidated. However, their fundamentally vital role described herein is obvious (albeit not recognized till now), and it explains many previously unanswered fundamental questions regarding the processes of epigenesis.

Given this new conceptual framework, novel avenues of inquiry are possible. Of immediate concern would be the characterization of the specific non-histone proteins in meiotic nuclei that pack the maternal mRNAs and prevent their translation. Once their composition is known, in vitro transcription of given genes in the presence of these proteins will create mRNPs, capable of unlocking the desired genes of cells during replication. With this mechanism in hand, selective transdifferentiation of cells in vitro for use in cell therapy would provide powerful new medical tools.

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