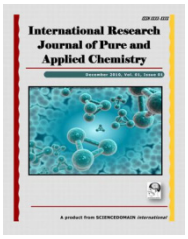

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Isotopic Self - Programming for the Embryonic Cell DNA Methylation Pattern

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Short Communication

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ABSTRACT

This paper is an original attempt to analyze a series of experimental data obtained lately by the author in a course of his long term studies on the probable role of isotopy in the molecular evolution of living systems as well as in the eukaryotic cell differentiation mechanisms. A dependence of the DNA double helix methylation process on the surrounding media / threads isotope composition is in a focus of the concept presented.

Keywords: DNA methylation, DNA replication, C13-isotope effects, cell differentiation;

1. INTRODUCTION

All known biochemical processes involves the fractionation of isotopes (Galimov, 1985). In a separate series of the molecular biology experiments devoted to studies and simulation modeling on the metabolism environment isotopy, a phenomenon of a non-equal methylation of the isotopically different DNA copies has been found. This phenomenon is no doubt significant for making perfectly clear the very nature of the blastomers functional determination. This indicates to the zygote-specific, universal for zygote as well as for a whole biodiversity range, fundamental regularity. A regularity that consists in a non-random, truly regular, distribution of the isotopically different nucleotide forms among the blastomer DNA polynucleotide pairs.

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The role and significance of DNA methylation is been studying and well comprehending (Bird, 2002; Li and Beard, 1993). However the origin of logical forming of DNA methylation pattern is not clearly up to this date.

It is Noteworthy that the free nucleotides pool in any zygote originally contains approx. 11% of the isotopically heavy (Carbon-13 enriched) nucleotides. Naturally the content of ^{13}C in total carbon pool is equal 1,1%. That's why statistically 11% of nucleotides contain ^{13}C because every nucleotide contains 10 atoms of carbon (Galimov, 1985). But in a course of closed, self-isolated, zygote cleavage process – there is no regeneration (no "refilling") of the free nucleotides pool occurrs. The isotopically light nucleotides are the most mobile ones, so they are about to get involved into a DNA synthesis ore actively compared to the isotopically heavy ones. That's why the zygote cleavage kinetic - from one stage to the next one - steadily changes the ratio of isotopically different nucleotide forms to a favor of the heavy ones.

Owing to this, the DNA isotopy patterns should be different at different zygote cleavage stages. Particularly, this difference comes up to its maximal level by the zygote cleavage terminal stages. Due to depletion of the zygote free nucleotides pool, and action of isotopic effects, a ^{13}C isotopic self-organization takes place within the DNA double helix forming polynucleotide chains. This, in turn, leads to the fact that every system of DNA double helix should have an individual, unique, methylation predetermination in all blastomers. This would also mean an individual methylation pattern of all genome.

This idea was presented in some previous research papers, (Ivanov, 2003; Ivanov and Volkov, 2006a; Ivanov and Sevastyanov, 2006b; Ivanov, 2006c; Ivanov and Galimov, 2007a; Ivanov, 2007b; Ivanov, 2009; Ivanov, 2010) and shows a possible way to the explanation of the fundamental biological phenomenon of the functional differentiation of cells, when differences between uniform cells appear while the chemical structures of the DNA of these cells are fully identical (the well known problem of the nature of blastomere determination and gene expression).

The idea is oriented toward the isotopy of DNA molecules. During DNA replication, the isotope composition of a new daughter DNA thread differs from the isotope composition of the parent thread because of the kinetic and thermodynamic isotope effects that accompany the enzymatic synthesis of DNA in a closed zygote division system. Substitution of isotopes slightly changes the length of bonds, interbond angles and molecular fragment stretching and bending vibration frequencies (Melander and Saunders, 1980). The daughter DNA therefore slightly differs conformationally from the parent DNA. Every repeated reduplication cycle creates new DNA molecules with different isotope compositions of partner threads. It follows that the conformations of these DNA molecules should also be different.

As a result, repeating reduplication events produce a broad spectrum of DNA molecules whose chemical compositions are identical but conformations different. It is assumed that this collection of conformations is responsible for methylation process and the differentiation of those cell types that are present in the organism.

To find out whether or not the idea presented in (Ivanov, 2003; Ivanov and Volkov, 2006a; Ivanov and Sevastyanov, 2006b; Ivanov, 2006c; Ivanov and Galimov, 2007a; Ivanov, 2007b; Ivanov, 2009; Ivanov, 2010) is capable of explaining the differentiation of cells, one question must be answered: does the methylation status of the two-thread DNA molecule depend on the difference in the isotope composition of each of the threads constituting it?

2. MATERIALS AND METHODS

2.1 Sample Preparation

Isotope effects are weak in the synthesis of DNA, and, strictly, we cannot predict their contribution to the isotope composition of the daughter DNA thread. This problem can therefore be solved only experimentally. A scheme of the experiment is shown in Fig. 1.

Using standard polymerase chain reaction (PCR) method (Glik and Pasternak, 2002) it were synthesized different isotopic forms of DNA. During the experiments it was used ordinary types of nucleotides and isotopically heavy ones.

Activity of ^{3}H-tracer was determined using liquid scintillation spectrometry method on Tri Carb 2800 TR “Perkin Elmer” by standard procedure (Sapozhnikov et al., 2006).

A certain amount of identical DNAs were synthesized by PCR. This amount was divided into two equal parts. Equal amounts of a mixture ATGC nucleotides were added to both. However, nucleotides containing usual light isotopes (98.9% ^{12}C and ~100% ^{14}N) were added to the first (control) portion, whereas nucleotides of the same quantitative composition but containing heavy isotopes (98% ^{13}C and 99% ^{15}N) were added to the second (test) synthesized DNA portion. Next, both control and test samples were subjected to two polymerase chain reaction cycles. A scheme of DNA transformations during these operations is presented in Fig. 2.

3. RESULTS AND DISCUSSION

The initial DNA comprised usual light-isotope threads denoted by L. After all operations, two-thread DNA molecules were composed of light-isotope nucleotides in the control sample; they are denoted by LL. In the test DNA sample, part of threads contained light isotopes (L), and the other part were heavy-isotope threads (H); these are threads synthesized anew from nucleotides containing heavy isotopes.

When threads combine to produce double helical DNA molecules, three kinds of DNA molecules are formed in the test sample, LL, LH, and HH.

The performed experiment showed following isotopic effect in DNA molecule – different isotopic forms of DNA which are chemically identical will have different methylation status.

In these experiments, the isotopic nonequivalence of partner threads of DNA was introduced artificially, by the addition of nucleotides containing heavy isotopes. As was mentioned above, the native biosynthesis of DNA under the action of DNA polymerase creates isotopically nonequivalent threads because of isotopic effects, both kinetic and thermodynamic. Schematically, a sequence of thread isotopic ordering events is shown in Fig. 3.

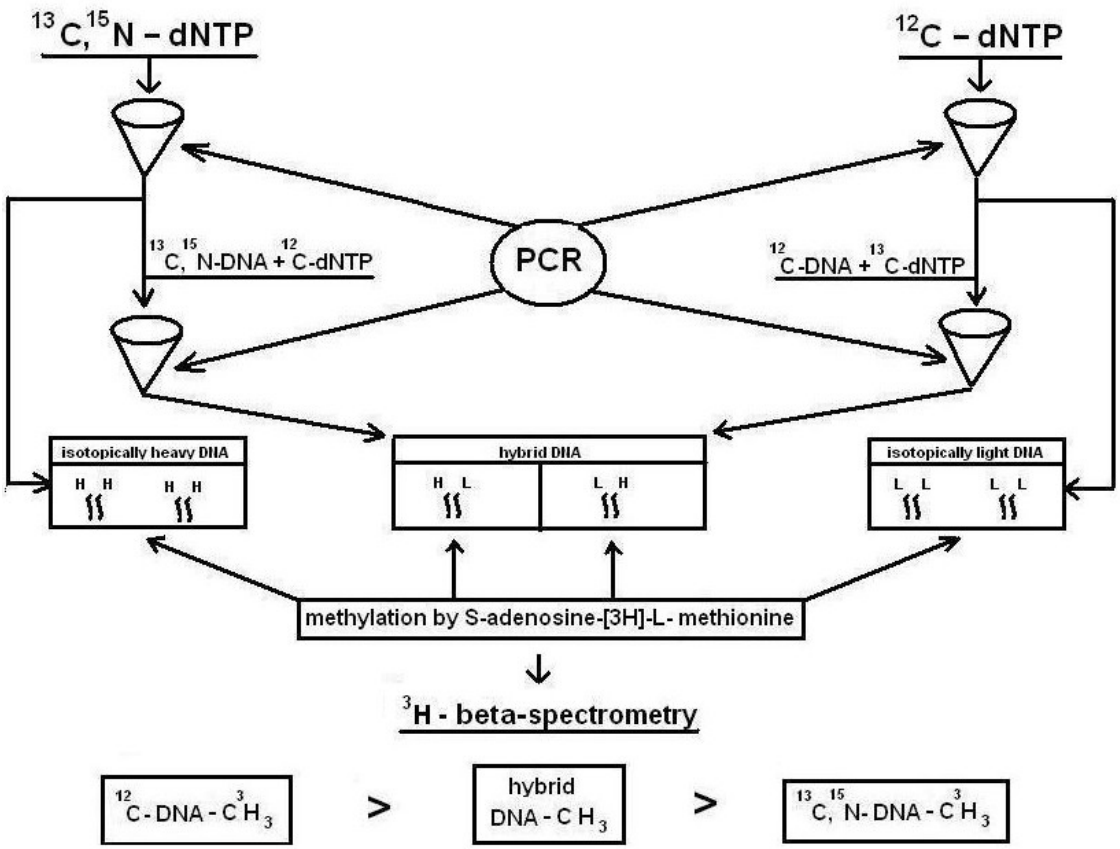

Fig. 1. Scheme of the analysis of DNA synthesis and methylation

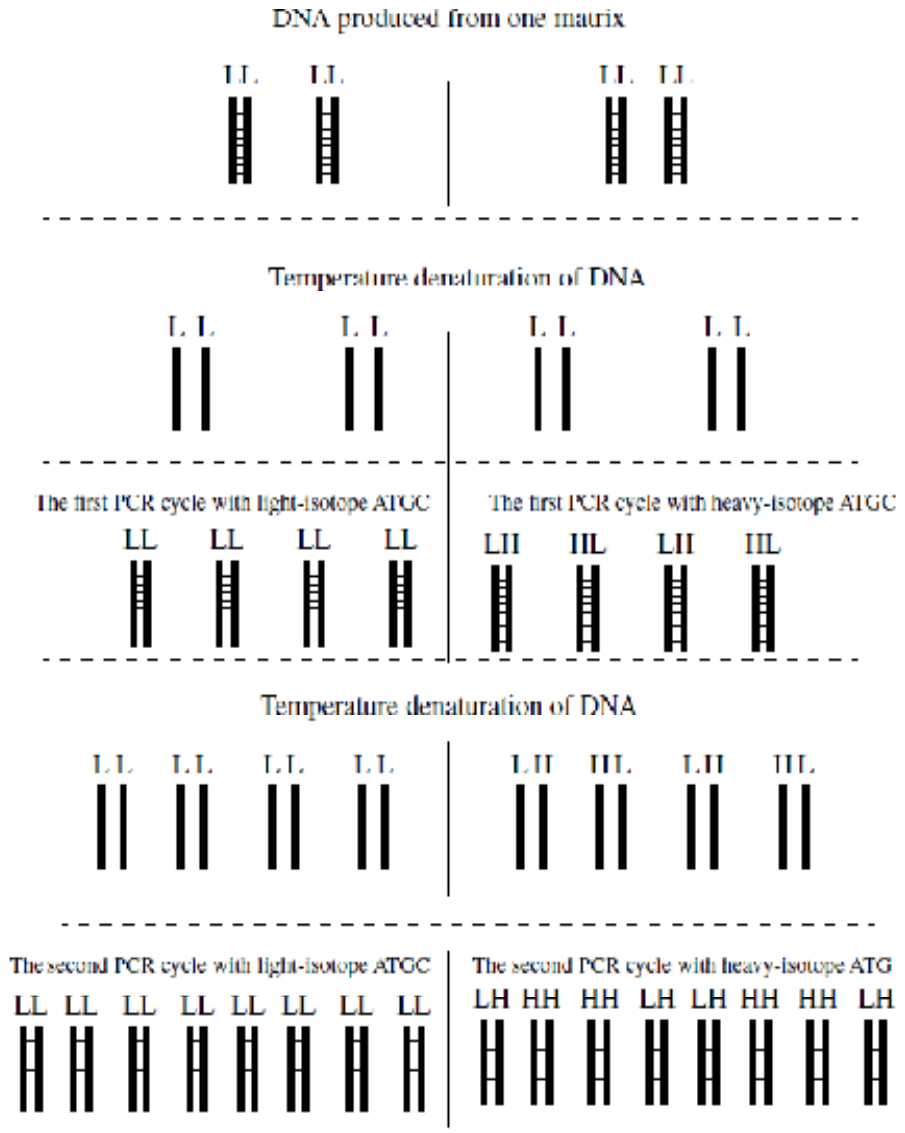

Fig. 2. Scheme of DNA transformations during synthesis

As a result it is formed the composition of DNA molecules with a broad distribution of isotopic nonequivalence of nucleotide threads. Upon that each DNA molecule will have individual methylation pattern.

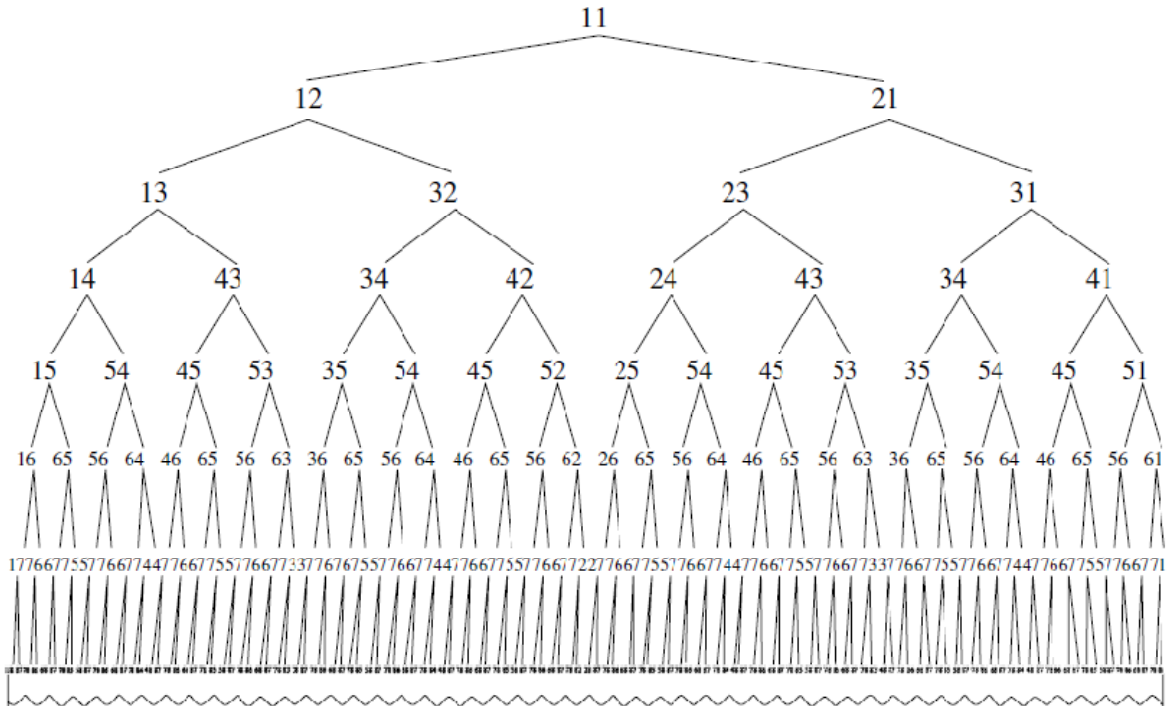

Fig. 3. Conventional numbering of DNA threads in zygote division.

The scheme shows how dynamical and successive transformation of the isotopy of the cytoplasm of blastomeres isotopically orders DNA threads during the whole zygote division process
(11, initial zygote DNA threads, and 12, 21, etc. are pair DNA threads of blastomeres).

4. CONCLUSION

Thereby for the first time it has been found out new phenomenon – influence of isotopy of DNA on the pattern of methylation for different isotopic forms of DNA. Since they are naturally formed during the process of embryonic fission in system which is closed for nucleotides, the explanation of the origin of the self-ordering of DNA blastomers methylation becomes clear. As a consequence it can be explained the origin of self-regulation of individual gene expression for embryonic cells too, that is actually the origin of ontogenesis self-programming. Probably this regularity is own distinctive for all multicellular biodiversity.

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