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Труды второй международной конференции, посвященной 105-й годовщине со дня рождения Н. В. Тимофеева-Ресовского и 70-летию публикации статьи Н. В. Тимофеева-Ресовского, К. Циммера и М. Дельбрюка «О природе генных мутаций и структуре гена»

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FROM THE MUTATION THEORY TO THE THEORY OF THE MUTATION PROCESS[†]

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"The general theory of inheritance seems to me the same way impossible and unnecessary as the general theory of variability".
K.A.Timiryazev, 1890

"...science is the art of doubt, not of certainty".
F.Ashcroft, 2005

Abstract - The main contributions to the biology made by N.V. Timofeev-Ressovsky and his co-authors (in "three gentlemen paper", 1935) were: radiobiological approach to the mutation process, materialization of the gene as a macromolecule and foundation of molecular biology. These directions appeared as development of the template principle, offered previously by N.K. Koltzov, the teacher of Timofeev. Finally Timofeev formulated his principle of con-variant reduplication, which united two main biological features - inheritance and variations as a single one. Study of primary lesions and repair was added to the theory of mutations from this point of view. Nevertheless, we have no satisfactory definition of mutation so far and even contemporary classification of types of variations is contradictory now. The situation is explainable by the fact that the classification was introduced rather from the phenomenological approach than from the mechanisms underlying the variation phenomena. It is proposed to divide variations for two groups: those connected with replication of genetic material and those connected with expression of genetic information. This classification should be introduced without a-priori division of variations on inherent and non-inherent because the same mechanisms may be involved both in inherent and non-inherent variations, depending upon taxonomic position and stage of ontogenetic development of the organism.

[†] Radiation Risk Estimates in Normal and Emergency Situations / Eds. A.A. Cigna and M. Durante. Springer, 2006. P.15-26.

Keywords: mutation, variation, template principle, primary lesions, repair, variations classification

1. Introduction

The tremendous contribution of N.V. Timofeev-Ressovsky to different fields of biology is very well known. It is partially reflected in the title of our meeting. Now it is 70 years of the "Green Pamphlet" (Timofeev-Ressovsky, Zimmer & Delbrück, 1935) issued by three scientists: a biologist, a physicist and a mathematician, who strongly influenced future development of Biology. Among other consequences of this paper was its influence on E. Schrödinger, who wrote his "What Is Life? The Physical Aspect of the Living Cell" (Schrödinger, 1945). The paper of the three gentlemen should be considered as the first ideological step in development of molecular biology and of molecular genetics in particular.

We have to remember that the main questions discussed in the paper were: the mechanism of mutagenesis and the nature of the gene. These two problems are tightly linked to each other. Moreover their interpretation reflects the very status of genetics during all periods of its history. Both the problems were resolved in the paper in a very stimulating manner for that time. The gene had been identified as a macromolecule and mutation was described as a change in the structure of that molecule.

I am not going to discuss the contemporary problem of the gene here and will consider it only in some aspects, namely in the aspect of mutational variability and in the aspect of general theory of variability. Our modern knowledge of genetic processes and of their molecular mechanisms shows that in understanding of mutations and of the other types of variability (including modifications) we are still far from having a perfect understanding of what they are.

2. Template principle

Interest in the nature of the gene and its variation originated in the works of Timofeev-Ressovsky from the template principle in biology. This principle had been formulated by his teacher N.K. Koltsov (Fig.1) in 1928 in his paper in which he offered a hypothesis of template reproduction of the chromosome (Koltsov, 1936).

From the modern position we shall say that Koltsov's definition of the gene as a protein molecule appeared to be wrong, but the template principle proved to be absolutely right. Finally it was extended by Timofeev as the "principle of convariant reduplication" of genetic material. This principle united the two

processes as a single one: reproduction and variation of the gene. It supposed that the gene reproduction (reduplication) is accompanied by variation of its structure (mutations) and these mutant variants are capable of reproduction and so on. Since that times the template principle became the central one in development of molecular biology (Inge-Vechtomov, 2003).

FIGURE 1 - N.K.Koltsov (1872-1940) (from Soyfer, 2001)

Highly promising in the study of inherent variations was the discovery of radiation-induced mutagenesis in *Drosophila* by H. Müller (1927). Timofeev (in Germany) (Fig. 2) and another coworker of Koltsov – A.Serebrovsky (in Russia) appreciated the meaning of this discovery and utilized the method of induced mutagenesis in their works. Serebrovsky and his coworkers (with H. Müller among them) finally demonstrated the fine structure of the gene in Drosophila in the 1930s (Müller & Prokofyeva, 1934). Timofeev came to what we now call the "Green Pamphlet", working with the same object and utilizing the same method as Müller – registration of recessive lethal mutations in X-chromosome of *D. melanogaster*.

Finally the template principle had been proved by demonstration of genetic functions of DNA, by discovery of DNA structure and the mechanism of its semi-conservative replication. So the substrate of "convariant reduplication" was identified. Later we could see development of the template principle in

Francis Crick's Central Dogma of molecular biology (Crick, 1958). There are many contradictory theses in the contemporary interpretation of the Central

FIGURE 2 - N.V.Timofeev-Ressovsky (1900-1981) Berlin-Buch, 1940 (Timofeev - Ressovsky, 2000)

Dogma, but it is completely valid to consider it as a symbol of template principle in biology. Even the recent discovery of so-called protein inheritance (Prusiner, 1998) may be included in this scheme. This is the prion mechanism of inheritance in lower eukaryotes (namely in fungi). In this mechanism of protein inheritance we again deal with template processes. The difference is that there is a protein template, which does not code the sequence of a daughter molecule, as in nucleic acids, but defines the conformation of the sister protein. Here we deal with inheritance on the level of protein conformation (Inge-Vechtomov, 2003). Today therefore, we can see a triumph of the template principle founded by Koltsov (1936) and extended by Timofeev-Ressovsky.

3. Mutations

An interesting episode accompanied the development of the other achievement, published in the "Green Pamphlet". It is interesting from natural

scientific and from historical perspective. It concerns the very mechanism of mutations. Timofeev, Zimmer and Delbrück first considered gene mutation as a monomolecular reaction, which changed gene structure in accordance with the "treffer teorie", or the target theory, developed by the authors. In accordance with this theory they got a one-hit dependency of mutation frequency from the dose of irradiation. It was a nice generalization for that time. Later on it became evident that the great majority of recessive lethal mutations in X chromosome of *Drosophila*, induced by radiation, were small chromosome rearrangements, predominantly small deletions. They needed two hits, two breakage points to appear. Taking this into consideration, one-hit dependency appeared to be a puzzle from the point of "traffer teorie".

This contradiction was picked up by M. Lobashev, who tried to approach the mechanism of mutation process from the other side. He considered mutation as a result of non-adequate or non-identical repair of genetic material. Even as a result of the repair of the cell as a whole (Lobashev, 1947). It is necessary to say that Lobashev considered protein as genetic material the same way as Koltsov did, and thought that the protein was a substrate of the repair process. Nevertheless he was the first who put together two terms: mutation and repair. He did it in 1946 in his theses of dissertation for the degree of Doctor of Biological Sciences (Lobashev, 1946). Now we know that all three main template processes: replication, transcription and translation include mechanisms of correction or repair (Inge-Vechtomov, 2003). Later it was proven that very often mutations start from primary lesions in DNA and that mutations are fixed in the process of repair as inherent traits of genetic material. Remember "mistakes of three R" (Replication, Recombination, Repair) offered by Jack von Borstel in late sixties of the last century (von Borstel, 1969).

Timofeev-Ressovsky and Lobashev did not know each other until the sixties, when Nikolai Vladimirovich was allowed to visit big cities such as Moscow, Leningrad, Kiev etc. after his sentence to prisoner camp and liberation from it. They met first in the apartment of Daniil Granin, the author of a popular novel "A Bison" about Timofeev-Ressovsky's life. Granin told me the story of meeting of these two scientists. It was a really dramatic situation. These two men were completely different. One of them – Timofeev-Ressovsky had noble roots in his origin, he was a well-educated person, and he was still in Berlin-Buch in 1945.

Lobashev was of completely proletarian origin. He was a very soviet person. He also was a hero, of another novel, written by V.Kaverin – "Two captains", a very popular book in Soviet Union. The part of Lobashev's life – before the University is shown in this book. The dramatic episode of the meeting of the two classics of mutagenesis was presented in our previous paper (Inge-Vechtomov, 2004). Since that meeting they became friends and every

year Lobashev invited Nikolai Vladimirovich to Department of Genetics in Leningrad University (Fig. 3) and we (the students of that period) had unforgettable opportunities to hear his brilliant lectures on population genetics, evolution, mutations and radiobiology. Many of us, students of Lobashev in Genetics Dept of Leningrad University, consider Timofeev as our teacher as well.

FIGURE 3 - N.V.Timofeev-Ressovsky (left) and M.E.Lobashev (right) in Department of Genetics and Breeding of Leningrad State University. Late 1960s. (Archive of the Department)

So, let us get back from history to natural science. These two scientists, very different in their approaches, studied mutation process from different sides and described very important features of it. In spite of time passed and huge amount of information obtained since their time, we are still in a contradictory situation in understanding the very nature of mutations.

Now we at least understand that mutation is not an abrupt event, but it is a multi-step process. We even think about mutagenic pathway, which starts with a step of formation of a primary lesion in genetic material (DNA). The primary lesion is a substrate for several repair systems, which watches for the native DNA structure. Then in the process of repair there may be "mistakes" and the primary lesions are processed to the stable mutations.

Experimental evidence for the existence of the primary lesions in genetic material was shown in the study of photo-reactivation of DNA, damaged by UV light by A. Kelner and R. Dulbecco (1949) and a little more than one decade later it was shown that photo-reactivation is an enzymatic process and its substrates are pyrimidine dimers (Friedberg, 1995).

Now we know that only a few of the primary lesions are processed to true mutations. Also the fraction of the primary lesions, which are processed into mutations, is possibly different for different mutagens. Only a few experimental

systems allow us to score this fraction directly. The first attempt to show it was by M. Reznick and R. Holliday (1971), utilizing the genetic system of nitrate reductase in *Ustilago maidis*. They showed that after UV irradiation without photo-reactivation a fraction of inactive enzyme, which was encoded by the damaged gene, appeared in the cells (Reznick & Holliday, 1971).

The next decade after that we utilized another system in *Saccharomyces* yeast to calculate the ratio of the primary lesions and of the real mutations after UV irradiation in a single locus. It was a system of mating types in *S. cerevisiae*. There are two mating types in haploid yeast: α and β. It was shown that in "illegitimate" crosses α X β(hybrids appear predominantly through phenotypic expression of primary lesions within the locus MAT. These primary lesions express themselves as a transient a-mating type. And after mating these lesions are repaired in more than 99% and the original mating type is restored (Inge-Vechtomov & Repnevskaya, 1989) (Fig. 4). So it was evident that only less than 1% of primary lesions after UV mutagenesis are processed in real mutations. Now we are studying this ratio for different mutagens.

Another intriguing problem of mutagenesis is distribution of mutations and susceptibility to mutagenesis within a cell population. The standard view is that mutability is randomly distributed among cells in genetically homogeneous populations. From this point the probability of multiple mutants should be calculated by multiplication of the probabilities of every mutation event. But it is not the case in some instances.

Sometimes we encounter the phenomenon of so called multiple mutability, which we studied in the 19's, also in yeast. It looks like there may be some cells in genetically homogeneous cell population, which are more likely to undergo the mutation process (Arefyeva & Inge-Vechtomov, 1977). Unfortunately, we could say nothing about the mechanism of this phenomenon. Some kind of similar phenomenon is described and discussed in paper of J.Drake in this issue (2006).

4. Some more problems and contradictions

Besides that we still encounter a lot of contradictions in the classification of not only the mutation types, but even in the general classification of the types of variations. The widely accepted classification of variability is presented on Fig. 5. It is not satisfactory now, though it is what we teach our students. Let us take as an example so-called ontogenetic variability. It includes mechanism of mutations and mechanism of recombination. If we remember differentiation of immunoglobulins we encounter recombinational rearrangements of genetic material and site directed elevated mutagenesis. At the same time we have many examples of modifications – regulation of gene expression in ontogenesis

and such events as genome imprinting and other effects, which we hide now under the term of epigenetic variation. Epigenetics now is nothing more than one more word, which does not clarify the situation, but makes it more complicated.

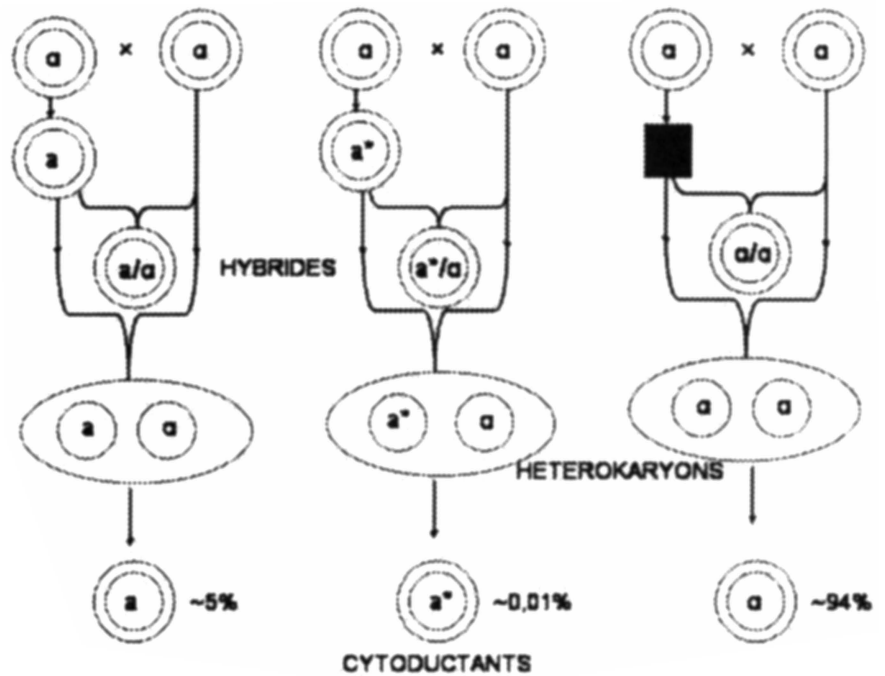

FIGURE 4 - Phenotypic expression of the primary lesions of genetic material. Hybridisation and cytoduction among yeast (*Saccharomyces cerevisiae*) of the same α-mating type. See commentary in the text

Even if we return to the problem of mutation the situation is not simpler. Chromosome mutations or chromosome aberrations usually appear as a result of either illegitimate or ectopic recombination either among non-homologous chromosomes or between different regions of the same chromosome. We can use a popular combination of words – "transposon mutagenesis". In reality "transposon mutagenesis" is an example of combinational variability because transposon insertion is a result of recombination. So a great part of what we used to call mutation in reality is a result of recombination – of the other type of variability.

We may mention also so-called genome mutations, for example polyploidisation. It is a change of cell content (change of copy number) of genetic material. It happens regularly in ontogenetic differentiation of some tissues. These, so-called mutations (genomic mutations) are connected with

disturbance of micro-tubules in the cytoskeletal apparatus of the cell, but not of DNA structure. I am not going to discuss modification of DNA bases (e.g. methylation) which we do not consider as mutations, again trying to hide the problem using the term "epigenetics".

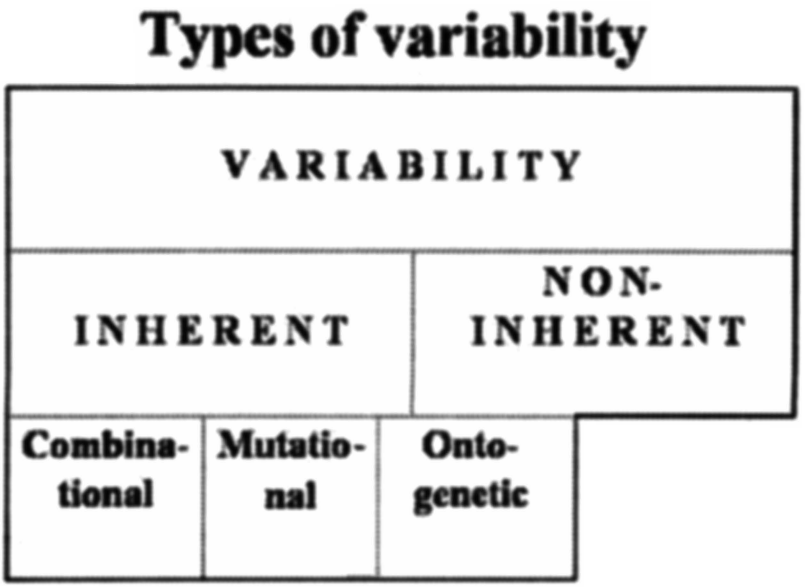

FIGURE 5 - Contemporary classification of variability

So, what is mutation? There is no satisfactory definition so far.

These contradictions are understandable because our accepted classification of variability and of mutations in particular is based historically upon phenomenology, but not on mechanisms, which became evident only later on. Now we understand that the same mechanism may be involved in different phenotypic events in different organisms or at different stages of its development, and a vice versa, since different mechanisms may cause the same phenotypic effect. The most intriguing example is connected again with phenomenon of prions. It presents an example of typical modification of protein molecule on the level of its secondary and tertiary structure, but not of its primary structure. It is a typical modification (non-inherent change) in mammals (Prusiner, 1998). At the same time it is an inherent variation in lower eukaryotes (Wickner *et al.*, 1995). So, to think either of the general theory of mutations or even of the general theory of variations we must start to understand their mechanisms rather than rely on pure phenomenology.

Probably by asking "What is mutation?" we are putting a wrong question. It is well known from the history of science that it is necessary to put a right question to get a proper answer. The history of mutagenesis study gives us a

nice example of this. Remember that H. de Vries (1901) and even S. Korzhinsky (1899) beforehand proposed their mutation (or heterogenesis - S.K.) theory as explanation of evolution, considering mutations as the elementary events of evolution. Now as a consequence of Timofeev-Ressovsky's works we understand that the elementary event in evolution is a change of allele frequency in a population (Timofeev-Ressovsky *et al.*, 1969) de Vries defined mutation as phenotypic variations, but only when we started to discuss mutations as a change in genetic material, was it the first real step toward understanding of mutation variability made. In the same way it was incorrect to ask how organs are inherited from generation to generation. Only when Mendel asked how elementary characters are inherited, was it possible to come to the general theory of inheritance (contrary to Timiryasev's opinion. See the first epigraph to this paper).

5. Prospects

In the same way if we want to understand the very nature of mutations (or of whatever it is) we should return to the general theory of variation. It seems reasonable to me:

(1) to classify different types of variations in connection with mechanisms of template processes. All of them possess a characteristic of ambiguity level. It means that variations are already included in the very mechanisms of template processes.

(2) I would suggest for this purpose to consider only two types of variations: those connected with reproduction (replication) of genetic material and those connected with expression of genetic information (transcription, translation and some other events in processing their products) (Fig. 6).

(3) Whether this or that type of variation would be inherent or non-inherent depends on taxonomic position, the stage of development of an organism and on a specific process in which this or that type of variation would be involved.

It is possible to suggest that only this way we would be able to understand the real nature both of mutation process and of inherent and non-inherent variations.

REPLICATION OF GENETIC MATERIAL ------→ **VARIABILITY**

EXPRESSION OF GENETIC INFORMATION ------→ **VARIABILITY**

FIGURE 6 - Alternative classification of variability, proposed in this paper

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