ISSN 0233-7657. Biopolymers and cell. 2007. Vol. 23. ISS 3. Translated from Ukrainian.

# Mutagenesis induced by integration processes and evolution of nuclear genome

**L. L. Lukash**

The Institute of Molecular Biology and Genetics
150 Zabolotny Str., Kyiv 03143, Ukraine

imbig@serge.relc.com

*Mutational variability induced by large-scale reorganizations of genetic material such as MGE transpositions, integration or disintegration of exogenous nucleotide sequences of viral and non-viral origin, changes in chromosome set or individual chromosomes, chromatin diminution and its role in the evolution of nuclear genome are discussed.*

*Key words: mutagenesis, chromosome, chromatin diminution*

Nowadays, the question of mutagenesis are brought forward among the majority of scientific investigations due to the fact that anthropogenic pollution of the environment lead to intensive accumulation of mutations in human genome, which is out of the control of natural selection. Some forecasts say that it may affect essentially the numbers of inherited and somatic diseases, lifetime duration, and possible progeny. Genetic instability, induced by factors of different origin, is considered to be the main mechanism in the complex multi-staged process of malignant retrogression of human somatic cells [1].

The use of genetically modified products in everyday life and recombinant DNAs included as parts of viral vectors as novel pharmacological gene therapeutic preparations [2] unveiled the problem of genetic consequences of the introduction of allogenic biological factors into the human organism.



**Mutations as inherited changes of genetic material.** The capacity to change genetic material – to mutate – is the universal capability of the living beings – from viruses and microorganisms to higher plants, animals, and humans. Mutational variability is the genetic variability which involves changes in genotype as a result of mutations. The disorders occurred may affect the nucleotide sequences in a DNA molecule, cause rearrangements of chromosomes, and result in increase or decrease in numbers of some chromosomes or sets of chromosomes. The most common definition of the notion of mutation was provided by Zhimulev in his book *General and Molecular Genetics* – "mutational variability is the inherited changeability of genetic material" [3].

Many principles have been applied to classify mutations. Usually, the mutations are classified not in their phenotypic manifestations but in the pattern of changes in genetic apparatus. Inge-Vechtomov singled out three types of mutations as follows: i) genetic or

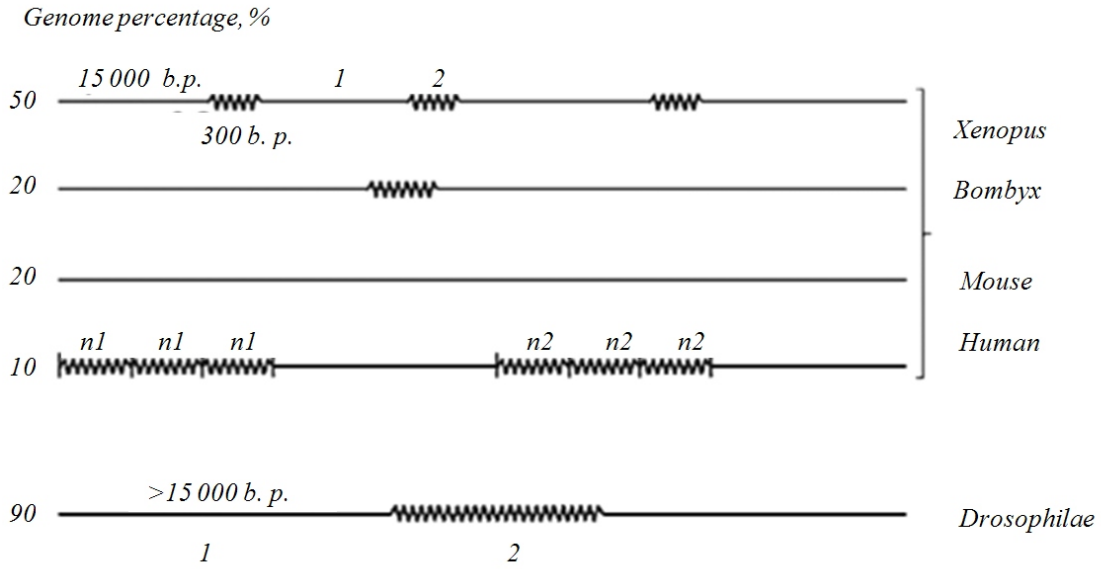


Fig.1 System of two types of interchange of nucleotide sequences of different repeatability [13]

point mutations, ii) changes in the structure of chromosomes or chromosomal rearrangements, iii) changes in the sets of chromosomes [4]. Gershenson's monography presents mutations in four basic groups: i) changes in the sets of chromosomes (or genomic mutations), ii) changes in numbers of some chromosomes (aneuploidy), chromosomal rearrangements (chromosomal aberrations), iv) genetic (point) mutations [5, 6]. Many authors still adhere to these classifications, though these classifications had to be revised long time ago due to the essential enrichment of the ideas on mutations with the new and significant data [3].

Certain level of genetic stability is "encoded" into the structure of the cell genome and is dependent on the work of mutator and anti-mutator genes, various regulatory genetic elements, responsible for the basic processes in the matrix, *i.e.* replication, recombination, reparation, modification, and restriction [7–10]. As a rule, spontaneous mutation in prokaryotic and eukaryotic organisms is specific for its low frequency ($10^{-5}$–$10^{-8}$), owing to the effective work of enzymes with corrector and reparative functions, and is hereditarily fixed feature. Zhestyannikov in his book *DNA Reparation and its Biological Significance* states that the value, inversely proportional to the frequency of spontaneous mutations, apparently, characterises the level of genetic stability of a biosystem [7].

As the mutations are considered to be the source of genetic variability of the population, there has been relatively constant rate of spontaneous mutations, specific to a certain genus or type of the cells, secured evolutionarily as the necessary condition of growth and adaptation [11]. This genetically secured feature has been maintained at the certain level by the systems of protection of the organism. For instance, the formation of anti-body-forming cells, genetic instability is maintained at the same level throughout the organism lifetime, yet it is strictly restricted by some regions of corresponding genes.

The application of natural genomes (*e.g.* onco-viruses) along with the development of gene engineering method of DNA-constructions made it possible to influence the mutation rate in cell populations [12]. All facts presented allow stating that the possibility of regulation of mutational variability at the level of cell as well as at the level of organisation of the living.

The chief specificity of eukaryotic genetic material, comparing to the prokaryotic one, is the presence of surplus DNA and some nucleotide sequences of different repeatability in heterochromatin regions [13]. Heterochromatin regions possess the series of features, which make them different euchromatin

ones. The relative compacting constancy and capability to conjugation are among the main ones. The ideas on the significant role of heterochromatin regions in the genome evolution are commonly known [3].

Fig. 1 presents the scheme of two types of interchange of nucleotide sequences of different repeatability. The paradoxes of eukaryotic genomes can be listed: i) disagreement of genome sizes and location of species on the evolutionary step-ladder, ii) significant differences in the sizes of genome and the contents of surplus DNA in closely related species, living in the same conditions. The latter testifies in favour of the fact that the genome size of closely related origin-wise species remained inconstant throughout the evolution. How is it then that their genomes became bigger and/or smaller so fast?

Many different hypotheses have been stated regarding the role of surplus DNA, the comparative analysis of which brought up the conclusion that surplus DNA performs neither encoding nor regulatory function. For some time surplus DNA was called egoistic, parasitic, and even junk, till the moment its possible role in evolution of genomes began to unveil. This kind of supposition was put forward by Akifjev more than 30 years ago [14].

To the author's opinion, the idea of surplus DNA as "evolutionary melting pot", where new structural genes and the regulatory sequences are matured, as it has been proposed by Swedish biologists Edstrцm, seems to be the most appropriate. Some of the ideas on this issue have been proposed by Russian geneticist Serebrovsky in 1920's. It is severely doubted that the new gene could be formed at the expense of mutational rearrangement of the old one if the latter is presented by a singular copy. In case when there is the surplus of genetic material in the form of doubled (duplicated) genes, then one of them may not function. With the period of time this "silent" gene accumulates gene mutations, having changed it so much that this gene presents itself a new structural or regulatory gene. At these conditions this gene may start functioning, forming a new protein product and, consequently, new feature. Yet this process is very slow, which can not be explained by fast change in genome evolution.

Therefore, having underestimating not the role of genetic mutations and chromosomal aberrations, we have decided to focus on mutations, occurring in the course of large structural reorganisations of genome, as they may be of special value for accelerated evolution.

Primarily, these large structural rearrangements include:

a.) insertions and deletions of nucleotide sequences at the integration of mobile genetic elements (MGE), viruses, which transform the DNA;

b.) macromutations – changes in the number of sets of chromosomes, appearance and elimination of some chromosomes, chromosomal rearrangements, chromatin diminution (the loss of genetic material during the formation of somatic cells out of germ cell lines).

Thus, current paper will present the discussion on mutational variability, occurring during large structural rearrangements of genetic material, such as MGE transpositions, integrations and disintegrations of exogenic nucleotide sequences of viral and non-viral origin, the changes on the level the whole chromosomal apparatus and separate chromosomes, chromatin diminution, and the role of this variability in the evolution of nuclear genome. There will be some examples of evolution of cell genomes connected with incorporation of adenovirus DNA, exogenic transforming DNA, changes in sets of basic and additional chromosomes in some animal species and the loss of genetic material present in germ line cells.

**MGE systems as factors and objects of evolution.** The comparison of fragments of exogenic DNA, introduced into the cell by various methods, with MGE, discovered by McClintock in 1956 and investigated intensively ever since, is considered to be reasonable [3]. Hersin considered almost any nucleotide sequence, surrounded by DNA repeats, capable of becoming a mobile element [8]. At the same time it has to be noted that different MGE types may be contributed by exogenic DNA into the cell system, as well as they can be activated in cell genome as a result of transfection and genomic stress [15–17].

Among the biological factors, capable of destabilising cell genome, MGE and oncogenic viruses are distinguished the most. MGE relocations evidently play the important role in both the process of normal development and the differentiation of stem cells into the specialized ones and in malignant transformation of tis-

sues [15]. Not in vain the oncogenes are sometimes called "molecular robots", capable of destabilising the cell genome and changing the programme of cell genome towards the malignant transformation.

Notably, biological factors of regulatory effect on mutagenesis also include nucleic acids, hormones, regulatory and enzymatic functions, vitamins, and various cell metabolites [18]. Both MGE and viruses influence the mutagenesis via the expression of genes of their own and the activated cell genes [19]. However, this article deals with the other aspect of the influence on the mutagenic process and evolution, namely, the consequences of the integration of exogenic nucleotide sequences into cell DNA.

Insertions of MGE into the encoding zones of genes result in disordering or rapid change in their functions, due to the direct damaging of encoding nucleotide sequence of genes and the influence of punctuators (promoters, terminators, enhancers) on reading-in processes. The mutations are especially numerous in prokaryotes of high density of encoding sequences in genome [16, 17].

Meanwhile, in the genomes of higher eukaryotes, where encoding sequences are as numerous as islands in the ocean (3–5%), MGE are most commonly incorporated into the nucleotide sequences of surplus DNA [15–17]. Insertions of MGE into non-encoding areas (spacers, introns, flank regions, *etc*.) result in lighter consequences – intensification or decaying in activity of neighbouring genes, changes in their regulation. However, the appearance of an additional MGE-belonging enhancer, in the close proximity to functional cell gene, is capable of activating this gene rapidly. The enhancers can increase the level of transcription of the neighbouring genes, at the distance up to 5 000 b.p., in ten and hundred times.

MGE localisation pattern for every case is relatively stable and the sites, available for insertion, are, evidently, "remembered by molecular memory" for a long time [17]. It may be considered as the significant component of the mechanism of determination of quantitative features. Generally, these systems contain the following: 1.) oligogenes (the genes of main effect), necessary for the formation of the feature; 2.) polygenes, each of them is not a necessary component of the formation of the feature, yet together they are capable of altering its expression; 3.) MGE, modifying and intensifying the activity of the nearby oligogenes and polygenes.

Critical stress conditions of existence are often connected with the population's beating through "the bottle-neck stage", which may result in either massive extinction or adaptation to new ecological niches according to the founder's principle [16, 17]. MGE are considered as specific receptors of stress signals from internal or external environment, which also start systemic outbreaks of genetic variability, up to the establishment of new genetic homeostasis in the critical periods of evolution of the populations. The forms, induced at these conditions, may be the founders of new populations with distinctively changed phenotype in accordance with limited quantitative or qualitative features. It is not excluded, however, that the changes in MGE localisation patterns are one of the mechanisms of the formation of species.

Gene and functional sites of retroposons are subjected to the intensive mutagenesis, as their genetic material goes through the RNA-stage in the course of replication, mutation possibility at this stage is $10^{-3}$–$10^{-4}$, which is ten, hundreds times higher than the possibility of mutation of genes in the eukaryotic DNA.

MGE systems are specific for the following functions:

serve the source of insertional variability of genes;

influence the appearance of quantitative and qualitative features;

response to external stresses, temperature fluctuations, increases of transpositional variability;

response for the changes of localisation patterns of numerous MGE at the same time during the selection according to some features.

To some extent, these features are specific to MGE of different objects, namely, yeasts, drosophilae, plants, mammals. Mutations may occur not only in sites of incorporation of MGE under the influence of transposition-providing enzymes in instable cell systems but also in many other loci [8].

**Evolution of cell genetic material during the integration of viruses and DNA transformations.** Genomes of oncogenic viruses, especially DNA-containing ones, are much more complicated organisa-

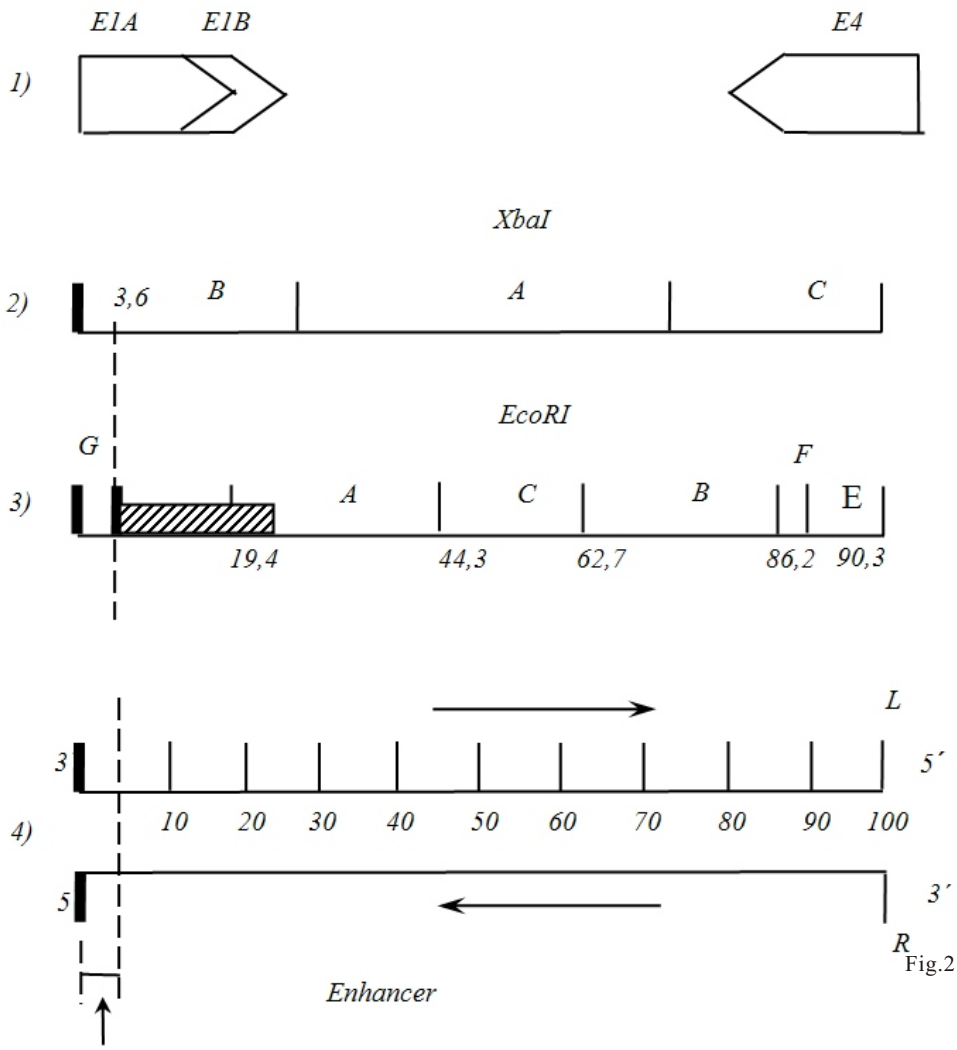


Fig.2 Restriction card of BAV3 genome

tion-wise than genomes of MGE. Fig. 2 presents the restriction card of genome of a typical adenovirus (bovine adenovirus, type 3). Genome of adenovirus is represented by a linear DNA molecule with long direct repeats at the ends. Operons E1A and E1B, comprising the transformation regions, responsible for malignant transformation of cells, are localised on the left end of genome, the right end locates the early E4 region, which controls the process of transformation proper. The analysis of the integration of this one and some other DNA-containing viruses into the chromosomal DNA revealed several stages of establishing of the integrated state and malignant transformation of cells [20–24]. The main stages can be presented as follows:

establishment of integrated state and manifestation of structural instability;

stable transformation, lasting for ten and hundreds of cell generations;

inactivation of nucleotide sequences as a result of methylation and their consequent elimination.

The mathematical modelling of manifestation of mutagenesis in the system of adenovirus-cell revealed two intensive increases in frequencies of mutations – the first peak, manifestated moderately, coincides with the period of temporary expression; the second one coincides with the period of structural instability at the establishment of integrated state (Fig. 3) [25]. Fig.4 presents the series of the investigated events in adenovirus-cell system in time.

The resulting mutagenic effect of exogenic mutagen is determined by both the interaction intensity, the possibilities of mechanisms of protection of the cell,

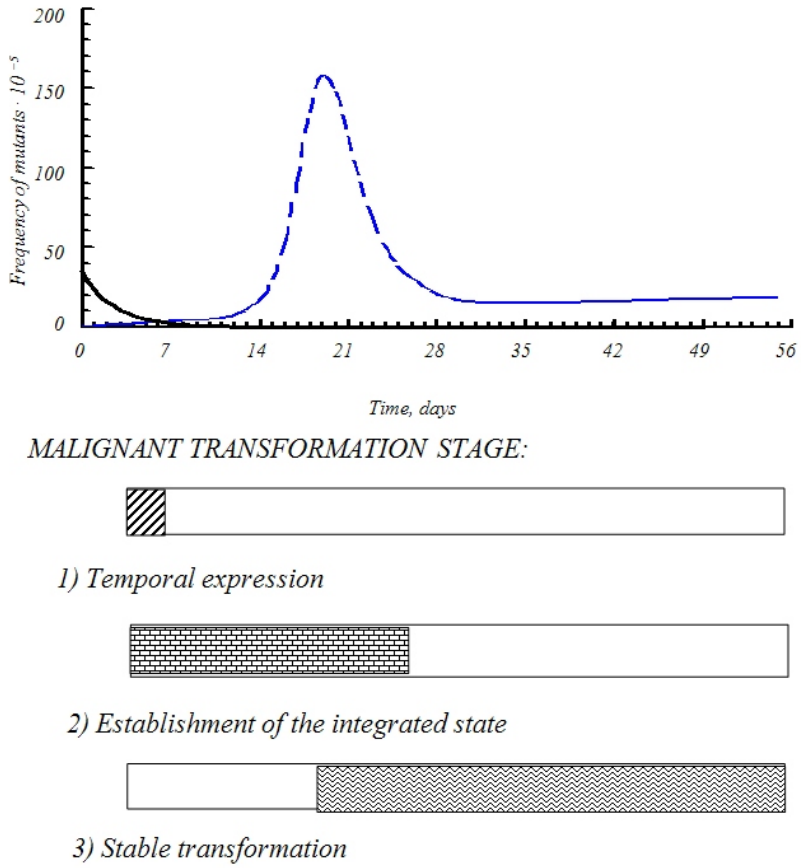


Fig.3 Modelling of mutagenesis and malignant transformation after the introduction of E1 adenovirus (E1A+E1B) transforming region into the cells

the specificities of intercellular environment, and the metabolism. Evidently, gradual decrease in the frequencies of the induced mutants in cell populations in time (Fig.3) reflects the dynamics of mutagen removal of the cell and the effect of reparative as well as some other mechanisms of protection.

Free virus DNAs are fast to arrange and disarrange [21], which, apparently, determines the short-lasting time of early mutagenic effect. Functional inactivation (as a result of methylation) and removal of the incorporated exogenic DNA molecules take more time and mainly determined by their structural and functional specificities and reparative capabilities of cell systems [22]. The increase in the level of induced mutations long after transfection (4–7 weeks), evidently, reflect the dynamics of inactivation and removal of viral nucleotide sequences and the death of mutable cells, "overloaded" with mutations of different genes (Fig. 3). It is commonly known that cells eliminate viral nucleotide sequences with the period of time – it starts with the elimination of the fragment of the older sequence and then spreads to the early regions of oncoviral genome [23]. As a result of some malignantly transformed cells there are almost no viral nucleotide sequences at all. Similar effects have been observed in the experiments on transgenic animals [26–29].

The possibility of incorporation of induced mutations to incorporate into allogenic nucleotide sequences of cell genome has been confirmed by the *in vivo* experiments on both mice and drosophilae [26–29]. The series of experimental works by Gazaryan *et al.* on transgenic mice with recombinant construction pBR322, containing provirus Rous sarcoma, present the integration in several cases of plasmid nucleotide sequence into the mouse genome [26–28].

However, there is not always a link between insertion and mutation. Sometimes complete loss of viral nucleotide sequences resulted in the change of the mutant phenotype. Authors have come to the conclusion that transfection can be considered to be the reason of

induction of cell MGE transpositions, resulting in locus-specific mutations.

The progeny of tumorous and transformed cells, obtained using oncoviruses, showed the rearrangements as a result of consequent recombination acts during the establishment of the integrated condition [20]. The place of incorporation of viral and cell genomes contains the rearrangement act, which includes deletions, insertions, and tandem duplications of the integrated fragment. Chromosomal DNA, flanking the integration site, contains the regions, homologous to viral DNA, which may take part in the recombination acts, resulting in establishment of the stable condition. Some new and big deletions of cell genome occur sometimes in the course of recombination (up to $3 \cdot 10^3$ nucleotides).

The study on the mechanisms of integration of DNA-containing viruses showed their genomes to be incorporated into both strands of cell DNA – into both newly synthesised and the matrix one [20]. This process may acquire the pattern of homologous recombination with subsequent insertion of the exogenic nucleotide sequence. In adenoviruses, the integration may be performed using short homologous regions between viral and cell sequences similarly to the site-specific recombination [21–24], however, the details of this process remain unveiled. The incorporation may take place using the non-homologous recombination, providing the integration of MGE (this is the basic mechanism for retroviruses) [8].

Thus, insertional mutagenesis, caused by the incorporation of viral genomes into the viral DNA is basically connected with the stage of structural instability during the establishment of the integrated condition. Regardless of the absence of distinctive specificity of incorporation of DNA-containing oncoviruses, the regions of moderate repeats are the most preferable for the realisation of integration processes. The region of integration is usually located close to the place of transition of the unique structure of cell genome into the repeated sequences [20, 22–24]. Predominant incorporation of oncoviruses into *Alu*-rich regions has been shown. T-antigen SV40 and early adenovirus proteins were shown to "recognise" the point of replication of *Alu*-sequences as well as it does the corresponding element pf viral DNA.

The incorporation into the unique chromosomal DNA takes place with the significantly lower probability. Therefore, the mutagenesis, induced by viruses in the unique cell genes may be determined by the integrational events to a smaller extent, than the mutagenesis in the heterochromatin region.Heterochromatin is the place of induction of chromosomal rearrangements [30].

Besides the insertion-deletion mutagenesis, some point mutations in the wide range of loci are induced in the integration region under the influence of early regulatory proteins [18]. The system of early virus genes of adenovirus is of interest due to the fact that it provides the example of maintaining the processes of both malignant transformation and mutagenesis. The transformation region (operons E1A and E1B) stimulate the transcription of numerous cell targets, responsible for the replication of DNA and the stimulation of cell proliferation, and the early region of E4 suppresses the accumulation of cell mRNA (Fig.5). The same E4 region blocks the appearance of the second, more distinct, peak of induced mutagenesis at the joint introduction into the cells with transformable E1 region or operon E1B (Fig.3, 6).

Summarizing on the mentioned-above – it is possible to distinguish three destabilizing factors in the oncovirus-cell system [12]. The first factor of destabilisation is the expression of early regulatory genes, responsible for the stimulation of DNA replication and malignant transformation of cells. The second factor is the reprogramming of the cell genome (change in activity and mutation of cell genomes and regulatory elements, MGE transposition) under the influence of expression of viral genes and their integration into cell DNA. The additional factor, increasing the possibility of mutations, is the changeover of reparative and other DNA-binding enzymes to the interaction with molecules of genetic matrix of exogenic origin.

Numerous works on the transition of allogenes show that almost any DNA can integrate with point cell genome [31–37]. Mammalian somatic cells are capable of consuming the enormous numbers of molecules of exogenic transforming DNA. Importantly, however, in what micro-surrounding and what regulatory elements are used to introduce the transgene into the cells. If exogenic DNA molecules are inactivated and/or do not

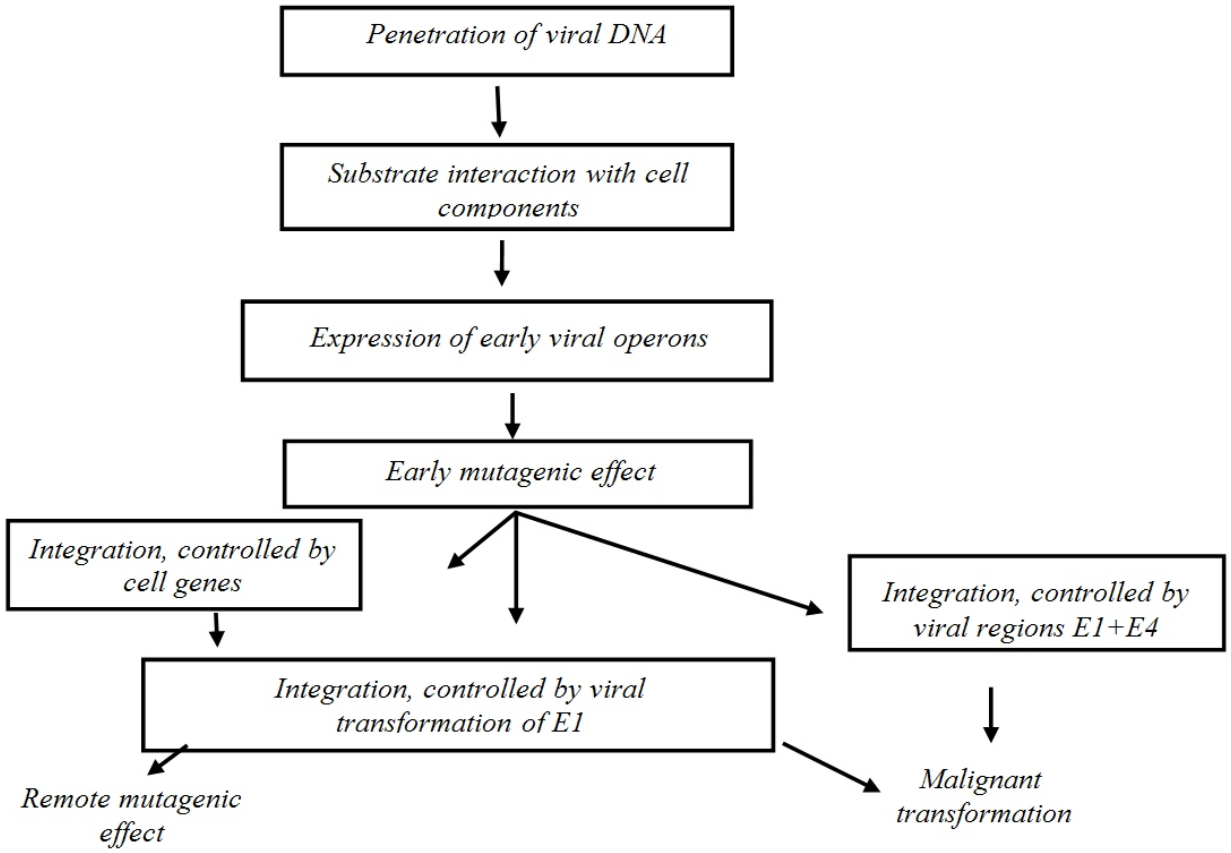


Fig.4 Sequence of integrational and mutational events in adenovirus-cell system

contain genetic elements or genes, influencing the integration, then this process is performed by means of cell enzymatic system only. The technique of transposition of cells into mammalian somatic cells is important too. The mutation frequency in the case of DNA introduction using the method of microinjections is higher, than that of the retrovirus infection, which is, evidently, connected with the specificities of integration mechanisms.

The possibility of induced mutations at the integration of allogenic nucleotide sequences into the cell genome was demonstrated in the case of mammalian somatic cells in the culture [31–37] and in the in vivo experiments on mice and drosophilae [26–29]. As it has been noticed above, transgenic mice, containing recombinant construction with incorporated Rous sarcoma provirus, wash shown to be incorporated into plasmid nucleotide sequence in mouse genome [26]. However, the direct connection between insertions with mutations has not always been revealed, therefore, authors make the conclusion that transfection results in induction of transpositions of cell MGE, inducing locus-specific mutations.

The process of integration of transforming DNA into cell heterochromatin can be divided into the same stages of evolution of exogenic nucleotide sequences as at the incorporation of DNA-containing oncoviruses: 1.) temporary expression of transforming genes and the formation highly-molecular complexes, pecelasomes; 2.) establishment of integrated condition and structural instability; 3.) stable genetic transformation; 4.) functional inactivation and structural disintegration of allogenic nucleotide sequences of genome cells.

The condition of integration stabilises after the whole series of rearrangements and selections in a long time after transfection. The level of insertions in syngeneic animals is app. 8%, which is very close to the results, obtained for retro-viruses in mammalian somatic cells [26–29].

More and more data on insertional mutagenesis during the transgene integration, causing the disorders

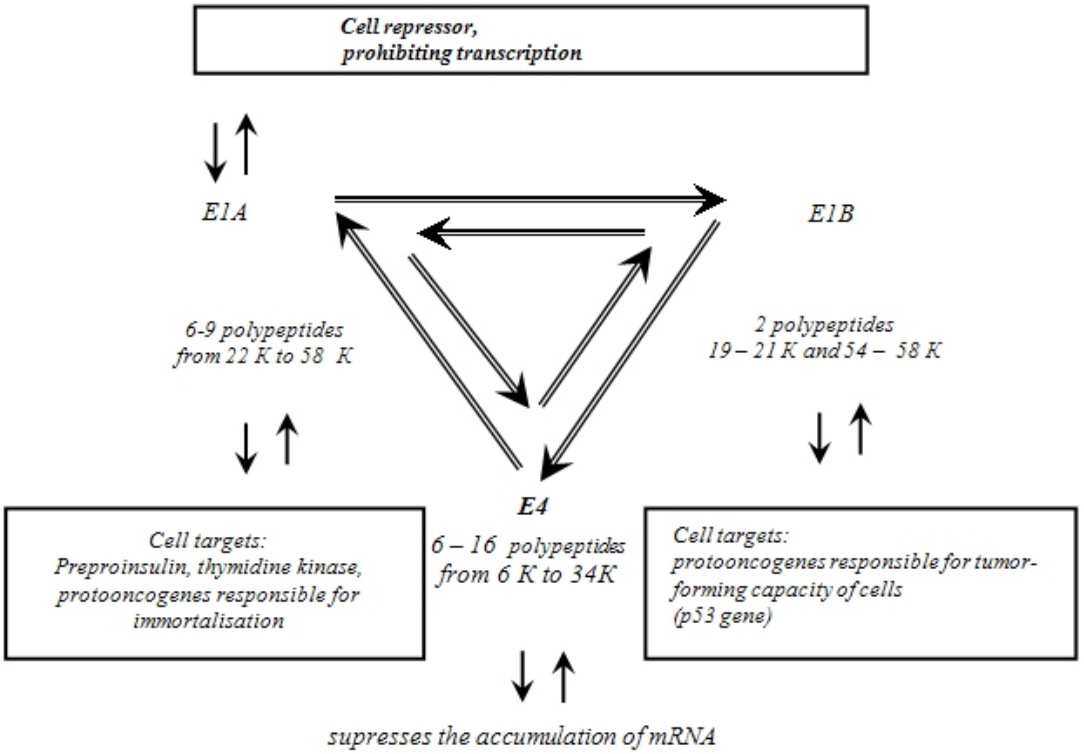


Fig.5 Integration of early operons of adenovirus and their influence on the expression of cell genes

in genes, involved in embryogenesis and cell differentiation, is being presented [33]. Some cases were specific for determining the gene, the disorders of which during the provirus integration, resulted in mutation. Thus, the retrovirus incorporation into the collagen gene resulted in embryonic death on the 6$^{th}$ and the 12$^{th}$ day [34]. Another insertional mutation is caused, apparently, by the penetration of transgene into the region of genome, responsible for morphogenesis of extremities in mice [35].

The difference of exogenic DNA as "living" mutagen from physical and chemical mutagens is in the capability to be "grafted-in", which increases significantly the time of its presence in the cell. The process of sort of investigation-and-culling of allogenic material is concluded with the formation of new highly-molecular genetic structures – pecelasomes – which are capable of involving into the acts of recombination with chromosomal DNA [31–37]. During the capturing of centromere by pecelasome, there may be formed a new mini-chromosome on its base. Sometimes, newly formed mini-chromosomes are integrated into the host chromosomes and become a part of cell genome. In some cases allogenic DNA remains the independently replicated structure.

The presence of chromosomal regions (the regions of concentration of *Alu*-repeats), sensitive to the influence of transforming DNA, was determined similarly as well as in the experiments on viruses. The analysis of the data bulk revealed that it is *Alu*-elements that may be the "hot spots" of recombinant and mutational events, as well as to be the mediators of homologous and non-homologous recombinations [38, 39].

**Evolutional development of chromosomal apparatus.** Comparative cytological analyses revealed the significant role in the species-formation in plants is played by polyploidisation. However, polyploidy in animals resulted in disorders of chromosomal mechanism of gender determination, that is why this process was not so important in their evolution; almost all not

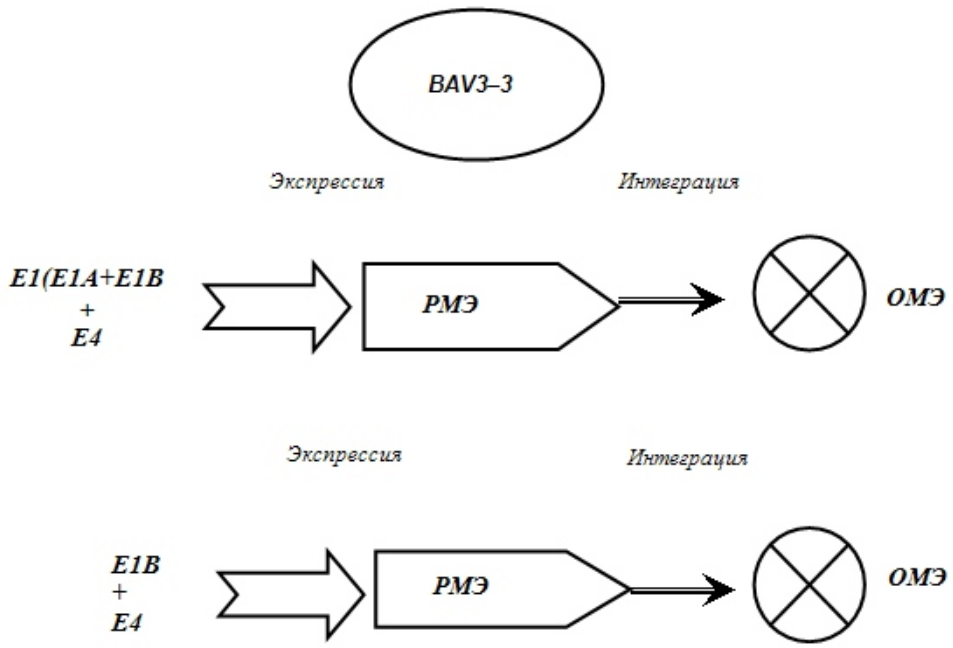


Fig.6 Regulation of mutagenesis by the system of early operons of adenovirus

numerous polyploidy species are multiplied parthenogenetically. The evolutionary significance of various chromosomal rearrangements – duplications, inversions, translocations – has been demonstrated [5]. At the same time, the special role of duplications as the basic means of increase in population and diversity of genes in the course of evolutionary development of organisms.

The set of chromosomes – karyotype – is the reliable characteristic of belonging to a certain species of animals or plants. It is true for the majority of species, yet for not all of them. Many animals and plants are specific for specific for additional sets of chromosomes (so called, B-chromosomes) along with the basic sets (A-chromosomes) (Fig.7). The sizes and the forms of the former may differ, depending on the representatives of the same species. For examples, the karyotype of Asian wood mouse (*Apodemus sylvaticus*) consists of 23 pairs of autosomes, sex chromosomes, and nine B-chromosomes [40].

The application methods of molecular genetics allowed to determine that B-chromosomes consists 100% of surplus DNA, while all B-chromosomes possess common DNA repeats. Later on, their affinity in some DNA-repeats with A-chromosomes has been revealed.

It has been revealed that near-centromeric regions of B-chromosomes contain the repeats, affined with the sequences, located in the near-centromeric regions of autosomes and in the dense blocks of sex chromosomes. More detailed analysis of these regions by DNA-probes revealed something more beyond the affinity of A- and B-chromosomes. When the A-chromosomes preparations were applied with B-chromosomes labelled probes, it has been noted that the label has been observed not only in centromeric regions but also in the arm of autosome, yet the staining index was many times lower.

The use of labelled probes allowed discovering one more interesting specificity of B-chromosomes, namely, B-chromosomes are isochromosomes. Such chromosomes with absolutely identical arms occur due to the rearrangements in cancer cells, as well as *in vitro* cultivated cells, yet they hardly ever occur in the norm. Regarding B-chromosomes of wood mouse – all most all of them are isochromosomes. Staining of wood mouse chromosomes using B-chromosomal probes showed that the genome of this species contains at least three types of repeats as follows: 1.) localised in near-centromeric regions of B-chromosomes and autosomes, as well as in two regions of sex chromo-

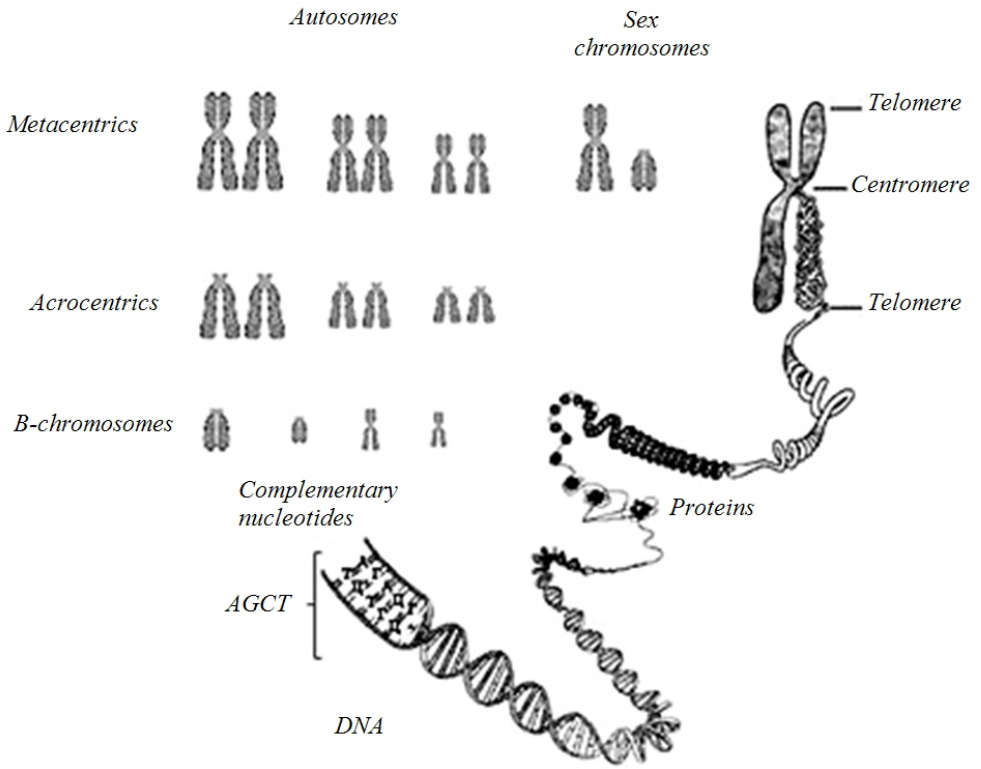


Fig.7 Organisation of basic and additional chromosomes of mammals [41]

somes; 2.) comprising the bulk of the arms of B-chromosomes and present in A-chromosomes in smaller amounts; 3.) detected at the ends of some B-chromosomes.

The analysis of sets of chromosomes of close species allowed to put forward the supposition of hypothetic picture of appearance and evolution of B-chromosomes (Fig.8). Initially, some MGE entered near-centromeric sequences of autosomes and then, having captured centromeric and telomeric sequences of host DNA, became chromosomes. The presence of centromere allows participating in the process of cell division along the main chromosomes, while telomeres protect the ends of newly-formed chromosomes from destruction. As soon as B-chromosomes occurred, they have attracted some other MGE, present in the cell. Terminal repeats were integrated the last into B-chromosomes; the impossibility of their dense packing in the moment of division testifies in favour of their extra-chromosomal origin. It has been showed also that some B-chromosomes contain some genes, responsible of synthesis with ribosomal RNA. It is also possible that, having captured some other useful genes, they will turn into A-chromosomes with the period of time? As not all of the wood mouse species have got the B-chromosomes, it is supposed that they inherited them from a common ancestor not the B-chromosomes proper, but the capability to form them *de novo*.

The scheme presented describes the appearance of new chromosomes and, consequently, the expanding of genome. Yet the question still remains, how can genome decrease rapidly in size?

Possible answer for this question is provided by the data of chromatin diminution. The notion of chromatin diminution in *Ascaridae* was discovered by T. Boveri in 1887 [3]. It has been shown that differentiation of cell on of germ track and the soma are accompanied by the partial loss of genetic material at early embryonic development (chromatin diminution, elimination of chromosomes) is rather common in the nature [3, 13, 41–43]. Chromatin diminution is, to some extent, present in some species of ascarids, cyclops, infusoria, chiggers, slivers, lepidopterans, flies, and fishes. The loss of nucleus by the erythrocytes during the differentiation in human may be considered as the extraordinary case of diminution.

Chromatin diminution corresponds to the concept of macromutations on the level of chromosomal pheno-

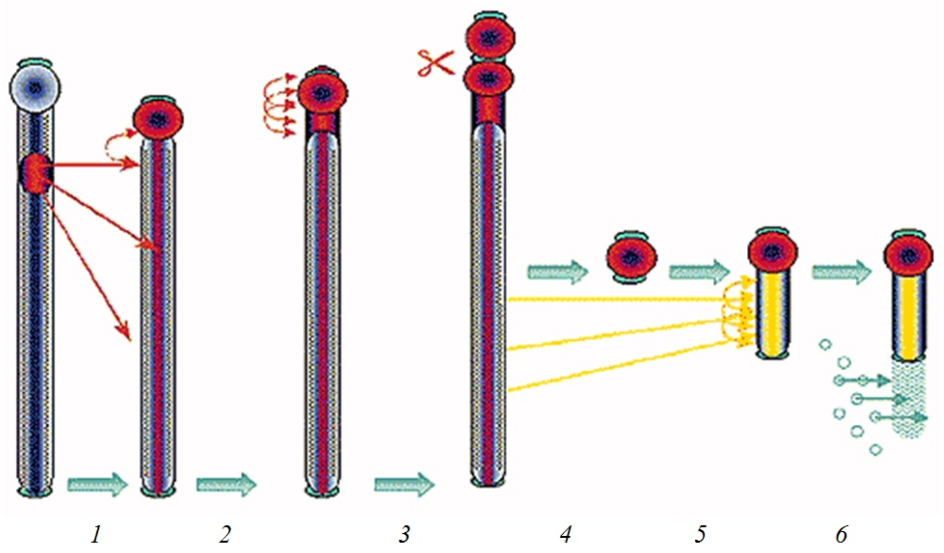


Fig.8 Origin and evolution of additional B-chromosomes of Asian wood mouse (*Apodemus sylvaticus*)

type [3]. Chromatin diminution in the listed-above organisms is, on the one hand, the classical form of total reduction of the genome during ontogenesis at differentiation of cells, while, on the other hand, it is the possible model for similar genome transformations of eukaryotes in the course of evolution. The latter is the proper reason for dwelling upon this issue in current review.

As it has been shown in dipterans, elimination of certain chromosomes is one of the phenomena of diminution; yet, it is quite possible, that no significant molecular rearrangements of the structure of chromosomal DNA takes place [43].

Chromatin diminution out of embryonic somatic cells in *Cyclops kolensis* is specific for the record DNA quantity for multi-cellular organism (94%) [13]. It is not much lees than in the case of hypotricha – the absolute record. However, the loss of the bigger part of DNA has no effect on the number of chromosomes – it remains constant – 22 (the same number as in the cells of germ track). What conclusions can be made regarding the role of surplus DNA for the investigation of chromatin diminution in cyclops?

First of all, only 6% of chromosomal DNA is enough differentiation, histogenesis and body formation of in the case of *C. kolensis*. This implies 94% of DNA contains neither genes nor regulatory sequences, necessary for individual development of a given species.

Secondly, chromatin diminution is quantitatively strictly repeated in the course of every reproduction cycle of cyclop (10 years of observations), which is possible only if germ track cell DNA is not affected by diminution and is preserved as long as the species exists.

Therefore, the DNA, removed from chromosomes during the diminution can not be considered to be the "junk" one. Moreover, it is considered as surplus for somatic cells only, and not for the cells of germ track. The process of diminution is a vivid example of natural gene engineering. The main consequence of the diminution phenomenon is in the fact that the genes, taking part in the individual development, can be neither lost nor damaged, *i.e.* stability of genetic material has to be maintained at a very high level.

The diminution enzymes cut and link back the chromosomal DNA. They perform these functions faultlessly, as the disruption sites are strictly defined (Fig.9). Such gene engineering operations are far away from those available to the scientists manipulation-accuracy-wise, performed by cell enzymes. Once mutations occur, embryonic development of this gene is going to stop only when the period of functioning has come.

Another variant of fault cutting is the chromosomal rearrangements, which are also lethal. However, the number of spontaneous chromosomal rearrangements in the course of early development of cyclops is strikingly small – app. 100 times smaller than in human lymphocytes, classical object for testing of chromo-

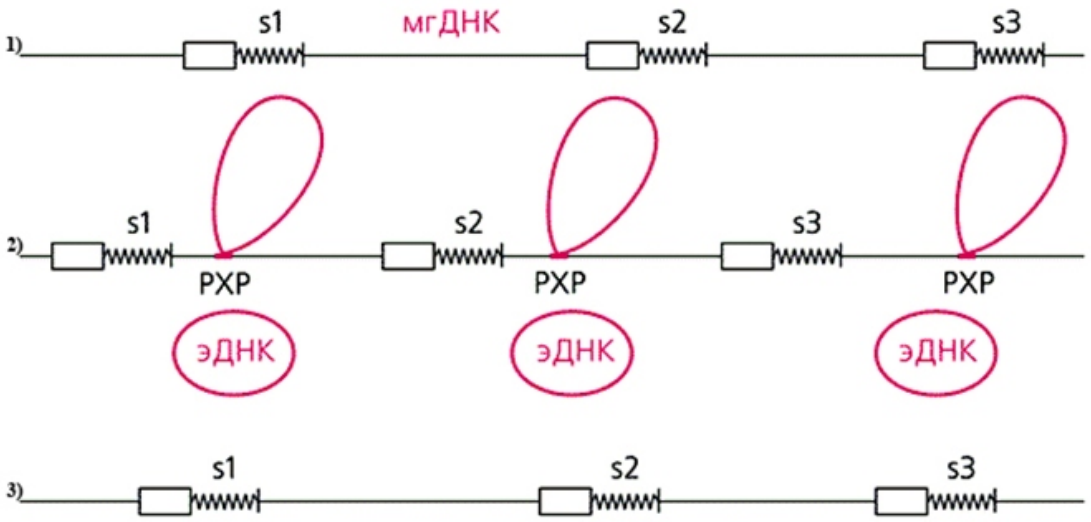


Fig.9 Scheme of diminution of chromatin in cyclops [13]

somal mutations. This fact testifies in favour of the presence of a powerful regulatory system against mutations, acting, at least, in the time when the level of enzymatic activity in the cell is very high. The latter has to "attack" the specified DNA regions only. Is any new regions, sensitive to these enzymes, occurred as a result of mutations, they would have been cut out and deleted of the genome. But if only one cell, which would be parent for the development of not somatic line but the germ line, was subjected to diminution, then it would thread not the animal unit only, but for the species in general, as it would inevitably resulted in infertility of the organism with non-programmed loss of genetic material.

The regions of cut-out DNA are gathered in granules surrounded by firm membrane. Molecular and genetic analysis revealed DNA to contain repeated nucleotide sequences of high homology. The formation of these sequences is related to the concert evolution and two reasons for their formation are considered: the first one – recent derivation of repeats from the main ancestral; the second – recombinational events. If the homologous regions are located in the nucleus in such a way that recombination may take place, newly occurred mutations will be eliminated as a result of recombinational acts and homologues will be retained [41, 42].

What is the designation of surplus DNA, eliminated after diminution? According to Akifjev *et al.*, the only consistent explanation of the role of surplus DNA in the germ track cells is that surplus DNA creates a unique picture of the species and is considered to be the factor of its genetic isolation [13, 41]. If this picture had not been kept throughout the generations, synopsis of homologous chromosomes in meiosis would have been distorted and the progeny would become aneuploidy (*i.e.* aliquant number of chromosomes to the haploid one) with no possible chances to survive.

Heterochromatin is one of the most studied DNA components. The functions of heterochromatin in the cells are diverse and are the issue of special importance [3]. The cases of heterochromatin diminution reviewed in wide range of organisms reveal conclusively that the functions of the major part of heterochromatin may be limited by the germ track cells. The higher is the difference in molecular structure of non-encoding regions, the higher is the possibility of disorders in conjugation of homologues with all consequences followed. This is the key factor to stop hybridisation of close species (along with other isolating mechanisms).

The investigation performed show that chromatin diminution requires coordinated work of the clusters of dozens of genes, *i.e.* this process is under tough genetic

control. Essentially, chromatin diminution at the level of chromosomal apparatus matches the notion of regulated macromutations [3]. It is possible that the change of gene mutation programme is accompanied by the initiation of regulatory processes of mutational and recombinant variability [10].

Regardless of the fact that chromatin diminution has occurred and secured in only some organisms, it may be a good example for modelling of similar transformations in eukaryotic genome in the course of evolution. This example allows approaching the problem of decreasing genomes in the evolution of close species. Thus, if in the process of chromatin diminution, the cells subjected to removal are those of DNA, parent to germ track and the process is not accompanied by the lethal event, as this process involves non-encoding DNA only, than all gametes of this organism will acquire new reduces genome. The whole series of individual organisms will acquire it, which will result in creation of the isolated group. Supposedly, this was the way that evolution of organisms, which secured chromatin diminution, has gone through. The notion of diminution is applicable for systematisation of Cyclops, as the differences in morphology between close species are very small.

Other species obtained different ways to inactivate, *i.e.* to "calm down" the surplus DNA. Around 50% of human genome genes are known not to take part in gene expression at all. The whole regions are often unreachable for transcription due to highly-compact arrangement of chromosomal regions. The way of changing the genetic material in this case is different, but the result remains the same – isolation of some genome regions [13].

Thus, the following conclusions can be made:

The systems of MGE of eukaryotic genomes are the sources and the mechanisms of insertional variability, they influence the expression of qualitative and quantitative features, change the patterns of MGE localisation in accordance to features and stress effect as a response to the external selection. MGE perform the role of some sort of receptors of stress signals, which initiate the outbreaks of transpositional variability in the critical periods of evolution, resulting in the transformation of homeostatic species norm.

The cells are capable of forming new macromolecular complexes, consisting of DNA-repeats, which are incorporated predominantly in the instable heterochromatin regions (*Alu*-repeats in human genome).

Autonomous macromolecular complexes (pecelasomes) may capture centromeres and transform into mini-chromosomes, which will be later developed into independent genetic structures.

Macro-mutations at the interaction of main cell and additional chromosomes (as in the case of A- and B-chromosomes of wood mouse) may the source of farther genome evolution. Deletions of the repeated nucleotide sequences out of heterochromatin regions may result in fast genome decrease (as it happened in the cases of evolution of some species, which secured the mechanism of chromatin diminution).

Significant reorganisations of genome in the course of integration processes are likely to be accompanied by the increase in the rate of point mutations in the wide range of loci under the influence of enzymatic systems of activation, which provide the basic matrix processes, *i.e.* replication, recombination, reparation, modification, and restriction.

*Л. Л. Лукаш*

Мутагенез при интеграционных процессах и эволюция ядерного генома

Резюме

*Рассмотрены вопросы мутационной изменчивости, вызываемой крупными структурными перестройками генетического материала, такими как транспозиции мобильных генетических элементов, интеграция или дезинтеграция экзогенных нуклеотидных последовательностей вирусной и невирусной природы, изменения хромосомного набора или отдельных хромосом, диминуция хроматина, и роль такой изменчивости в эволюции ядерного генома.*

*Ключевые слова: мутагенез, эволюция, ядерный геном, гетерохроматин, мобильные генетические элементы, хромосома, диминуция хроматина.*

REFERENCES


1. *Кордюм В. А*. Опухоль – как она видится сегодня с позиций молекулярной генетики // Биополимеры и клетка.–2001.–**17**, № 2.–С. 109–139.
2. *Глазко В. И*. Генетически модифицированные организмы: от бактерий до человека.–Киев: Мин-во образования и науки Украины, 2002.–210 с.
3. *Жимулев И. Ф*. Общая и молекулярная генетика.–Новосибирск: Сиб. университетское изд-во, 2003.–480 с.
4. *Инге-Вечтомов С. Г*. Генетика с основами селекции.–М.: Высш. шк., 1989.–С. 290–369.
5. *Гершензон С. М*. Основы современной генетики.–Киев: Наук. думка, 1983.–240 с.
6. *Гершензон С. М*. Мутации.–Киев: Наук. думка, 1991.–111 с.
7. *Жестяников В. Д.* Репарация ДНК и ее биологическое значение.–Ленинград: Наука, 1979.–286 с.
8. *Хесин Р. Б*. Непостоянство генома.– М.: Наука, 1984.–472 с.
9. *Алекперов У. К*. Антимутагенез.–М.: Наука, 1984.–100 с.
10. *Cervantes R. B., Stringer J. R., Shao C., Tischfield G. A., Stambrook P. G*. Embryonic stem cells and somatic cells differ in mutation frequency and type // Proc. Nat. Acad. Sci. USA.–2002.–**99**.–P. 3586–3590.
11. *Шапиро Н. И., Варшавер Н. Б*. О темпе спонтанного мутационного процесса в соматических клетках млекопитающих и о некоторых вопросах, с ним связанных // Генетика.–1976.–**12**, № 7.–С.132–149.
12. *Лукаш Л. Л*. Регуляция изменчивости генома соматических клеток млекопитающих под влиянием экзогенных биологических факторов // Біополімери і клітина.–2004.–**20**, № 1–2.–С. 93–105.
13. *Акифьев А. П.* Избыточная ДНК – генетическая квадратура круга? // http:/vivovoco.rsl.ru/VV/JOURNAL/NATURE/10_04/EXCESS.HTM – А. П. Акифьев // Природа.–2004.–№ 10.
14. *Акифьев А .П*. Молчащая ДНК и ее роль в эволюции // Природа.–1974.–№ 9.–С. 49–54.
15. *Гвоздев В. А*. Подвижная ДНК эукариот. 2. Роль в регуляции активности генов и эволюции генома // Соросовский образоват. журн.–1998.–№ 8.–С. 15–21.
16. *Ратнер В. А., Васильева Л. А*. Индукция транспозиций мобильных генетических элементов стрессовыми воздействиями // Соросовский образоват. журн.–2000.–№ 6.–С. 14–20.
17. *Ратнер В. А., Васильева Л. А*. Мобильные генетические элементы (МГЭ) и эволюция геномов // http://macroevolution.narod.ru/ratner1.htm – мобильные генетические элементы.
18. *Лукаш Л. Л*. Дестабилизация клеточного генома под влиянием экспрессии ранних регуляторных генов онковирусов // Цитология и генетика.–2002.–**36**, № 2.–С. 68–80.
19. *Лукаш Л. Л*. Биологические мутагены: их влияние на стабильность эукариотических клеточных систем // Вісн. українського тов-ва генетиків та селекціонерів.–2003.– № 1.–С. 62–81.
20. *Агеенко А. И*. Онкогены и канцерогенез.–М.: Медицина, 1986.–Т. 256.–152 с.
21. *Попов Л. С., Горбунова Л. В., Варшавер Н. Б*. Интеграция ДНК ОВ40 в геном клеток и вирусный мутагенез // Генетика.–1986.–**22**, № 9.–С. 2213–2219.
22. *Doerfler W*. Uptake, fixation and expression of foreign DNA in mammalian cells: the organization of integrated adenovirus DNA sequences // Curr. Top. Microbiol. Immunol.–1982.–**101**.–P. 128–188.
23. *Kuhlmann J., Doerfler W*. Loss of viral genomes from hamster tumor cells and nonrandom alterations in patterns of methylation of integrated adenovirus type 12 DNA // J. Virol.–1983.–**147**.–P. 631–636.
24. *Schultz H. M., Doerfler W*. Detection of cellular DNA at site of viral DNA insertion in the adenovirus type 12-induced mouse tumor CBA-12-1-T // Nucl. Acids Res.–1984.–**12**.–P. 4959–4976.
25. *Лукаш Л. Л., Лукаш С. И., Задорожный В. Ф*. Математическая модель динамики мутагенеза, индуцированного фрагментом ДНК аденовируса, в клетках млекопитающих // Биополимеры и клетка.–1996.–**12**, № 3.–С. 7–16.
26. *Газарян К. Г*. Микроинъекции генов в зиготы и эмбрионы: интеграция в геном и генетические эффекты // Успехи соврем. генетики.–1985.–№ 13.–С. 75–88.
27. *Тарантул В. З., Кузнецова Е. Д., Газарян К. Г*. Характеристика участков генома трансгенных животных, прилегающих к интегрированным последовательностям чужеродной ДНК // Молекуляр. биология.–1989.–**23**, № 4.– С. 1036–1040.
28. *Набирочкин С. Д., Габитова А. Б., Бегетова Т. С., Газарян К. Г*. Индукция нестабильных мутаций у *Drosophila melanogaster* микроинъекцией ДНК онкогенных вирусов в полярную плазму эмбрионов. Малигнизирующий эффект онковирусных ДНК // Молекуляр. биология.–1991.–**24**, № 5.–С. 783–789.
29. *Gordon J. W*. A foreign dihydrofolate reductase gene in transgenic mice acts as dominant mutation // Mol. Cell. Biol.–1986.–**6**.–P. 2158–2167.
30. *Akifyev A. P*. Mechanisms of the production of chromosomal aberrations in eukaryotic cells // Physiol., General. Biol. Rev.–1995.–**10**.–P. 1–56.
31. *Томилин Н. В*. Генетическая стабильность клетки.–Ленинград: Наука, 1983.–196 с.
32. *Palmiter R. D., Brinster R. L., Hammer R. E*. Dramatic growth of mice develop from eggs microinjected with methallothionein-growth hormone fusion genes // Nature.–1982.–**300**.–P. 611–615.
33. *Wilkie T. M., Palmiter R. D*. Analysis of the integrant in Myk-103 transgenic mice in which males fail to transmit the integrant // Mol. Cell. Biol.–1987.–**7**.–P. 1646–1655.
34. *Harbers K., Kuehn M., Delins H., Jaenish R*. Insertion of gene leads to embryonic lethal mutation in mice // Proc. Nat. Acad. Sci. USA.–1984.–**81**.–P. 1504–1508.
35. *Hanahan D*. Heritable formation of pancreatic B-cell tumors in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes // Nature.–1985.–**315**.–P. 115–122.
36. *Бобрышева И. В., Барон Е. М., Варшавер Н. Б*. Плазмида *pSVc-myc-1* индуцирует генные мутации и хромосомные аберрации в культивируемых клетках китайского хомячка // Цитология и генетика.–1993.–27, № 4.–С. 51–56.
37. *Бобрышева И. В., Варшавер Н. Б*. Характеристика мутантов, индуцированных онкогеном *c-Ha-ras1*, и природа мутагенного действия онкогена // Генетика.–1995.–**31**, № 12.–С. 1598–1604.
38. *Шахмурадов И. А., Капитонов В. В., Колчанов Н. А., Омельянчук Л. В*. Эволюция повторов Alu: динамика распространения в геноме // Генетика.–1989.– **25**, № 9.–С. 1682–1689.
39. *Лукаш Л. Л., Швачко Л. П., Костецкая Е. В*. Мобильные генетические элементы в процессах мутагенеза, рекомбинации и злокачественной трансформации

клеток человека // Биополимеры и клетка.–1996.–**12**, № 2.– С. 7–19.
40. *Рубцов Н. Б., Бородин П. М*. Эволюция хромосом: от А до В и обратно // http:/vivovoco.rsl.ru/VV/JOURNAL/NATURE/03_02/NATI.HTM. – Н. Б. Рубцов // Природа.–2002.–№ 3.
41. *Акифьев А. П., Худолий Г. А*. Мутагенез и генетический гомеостаз у высших организмов // Вестн. РАМН.–1993.–№ 1.– С. 3–9.
42. *Акифьев А. П., Гришанин А. К., Дегтярев С. В*. Диминуция хроматина – ключевой процесс для объяснения парадокса размера генома эукариот и некоторых механизмов генетической изоляции // Генетика.–2002.–**38**.– С. 595–606.
43. *Гришанин А. К., Шеховцов А. К., Бойкова Т. В., Акифьев А. П., Жимулев И. Ф*. Проблема диминуции хроматина на рубеже XX и XX1 веков // Цитология.–2006.–**48**, № 5.–С.379–397.

УДК 577.21+575.857
Надійшла до редакції 24.05.07