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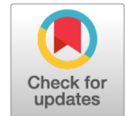
Research Article



M.E. Lobashev's physiological theory of the mutation process and the formation of contemporary views on mutational changes in genetic material

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ABSTRACT
Changes in mutation rates can significantly impact population size and its genetic structure, leading to the emergence of new traits and species. At the same time, the destabilization of genetic material is the main cause of hereditary and oncological diseases and aging. M.E. Lobashev was the first to point out the connection between mutations and repair. He introduced the concept of a premutation state or primary lesion of genetic material and suggested that mutagenesis is a physiological process in which mutations occurs during the repair of damaged genetic material due to non-identical restoration of its structure. The theories of M.E. Lobashev laid the groundwork for understanding the causes and mechanisms of inherited changes in genetic material, which have been experimentally confirmed in studies of replication, repair, and recombination. It is now known that mutations arise through a multistep process over time, due to ambiguity of one of template processes — DNA synthesis. Recent research made it possible to establish the physical nature of primary lesions and mutations, to develop various methods for their identification, and estimate the impact of primary lesions and mutations in the phenotype formation.

Keywords: mutations; primary lesions; DNA repair; replication; recombination.

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Научная статья

Физиологическая теория мутационного процесса М.Е. Лобашёва и формирование современных взглядов на мутационные изменения генетического материала

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АННОТАЦИЯ
Изменение частоты мутагенеза существенно влияет на численность и генетическую структуру популяции и, как следствие, приводит к появлению новых признаков и видов. В то же время дестабилизация генетического материала является основной причиной наследственных и онкологических заболеваний, а также старения. Первым на связь между мутагенезом и репарацией указал М.Е. Лобашёв. Он сформулировал понятие о предмутационных повреждениях генетического материала и предположил, что мутагенез — это физиологический процесс, в основе которого лежит нетождественная репарация поврежденного генетического материала. Теория М.Е. Лобашёва заложила основу для понимания причин и механизмов наследственных изменений генетического материала и позже была экспериментально подтверждена при исследовании репликации, репарации и рекомбинации. Согласно современным представлениям, мутации возникают в результате многоэтапного процесса вследствие неоднозначности одного из матричных процессов — синтеза ДНК. Современные исследования позволили установить физическую природу первичных повреждений и мутаций, разработать различные методы их идентификации и оценить влияние первичных повреждений и мутаций на формирование фенотипа.

Ключевые слова: мутации; первичные повреждения; репарация; репликация; рекомбинация.

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BACKGROUND

The first efforts to explain and comprehend the causes of sudden heritable genetic changes (mutations) began in the late 19th and early 20th centuries. The term "mutation" was coined by Hugo de Vries as part of the mutation theory he formulated between 1901 and 1903 [1, 2]. Prior to 1925, there were no successful attempts to increase the occurrence of mutations intentionally. During this time, geneticists focused solely on spontaneous mutations, which led to the belief that the mutation process was independent of environmental factors [1]. The hypothesis that mutations occur regardless of environmental factors was tested by the work of G.A. Nadson and G.S. Filippov in 1925–1926. They demonstrated that the frequency of new hereditary forms increased in lower fungi treated with radium rays. The observation led to the successful creation of several stable races of fungi [3]. H. Muller's work in 1927 showed the influence of X-rays on mutagenesis in *Drosophila melanogaster*; he also developed a quantitative approach to account for recessive lethal mutations in the X chromosome of fruit fly [4, 5]. The conclusions of Muller were confirmed by S.C. Gager, A.F. Blakeslee, and L.J. Stadler, who demonstrated the influence of X-rays and radium on mutagenesis in durum, corn, and barley [6–8]. N.V. Timofeev-Resovskii was able to induce somatic mutations by exposing eggs and young larvae of fruit fly to X-rays [9]. Subsequent research demonstrated that X-ray, gamma, and ultraviolet (UV) radiation induce various hereditary changes in genetic material, including gene mutations and chromosomal rearrangements, in both germ and somatic cells. The frequency of these induced genetic alterations depends on the radiation dose.

At the same time, the discovery of chemical mutagenesis took place. In 1928, M.N. Meissel induced mutations in yeast using chloroform and other chemical compounds [10]. In the 1930s, V.V. Sakharov, M.E. Lobashev, and F.A. Smirnov demonstrated that iodine, acetic acid, and ammonia caused the increase of recessive lethal mutations in the X chromosome in fruit fly. In 1946, potent chemical mutagens were discovered: ethylenimine by I.A. Rapoport in the USSR and nitrogen mustard by J. Robson and S. Auerbach in England [1, 11, 12]. The list of mutagenic factors has since expanded considerably and now includes tens of thousands of substances with mutagenic activity. The number of new genotoxic factors continues to grow every year.

The discovery of induced mutagenesis allowed for the study of its mechanisms. One of the initial theories regarding the causes of mutations suggested that natural background radiation was the primary source of spontaneous mutations. However, it was found that natural background radiation could only account for approximately 0.1% of all spontaneous mutations in *Drosophila* [1]. In 1935, N.V. Timofeev-Resovskii, C. Zimmer, and M. Delbrück proposed the single-hit theory based on studies of radiation mutagenesis in *Drosophila*. According to this theory, mutations occur through random fluctuations of atoms or through external energy sources, such as ionization or excitation from a radiation quantum hitting the gene [9]. The gene was conceptualized as a block of atoms in which a mutation occurs due to an instantaneous rearrangement of atoms or dissociation of bonds at the moment of ionization [9].

However, this model did not explain all the experimental data available at the time. In particular, the effect of temperature on the frequency of mutagenesis. G. Muller, N.V. Timofeev-Resovskii, and L.J. Stadler noticed that in the range 15 to 29°C, a 10°C increase resulted in a threefold increase in the mutation frequency [9]. P.K. Shkvarnikov and M.S. Navashin observed that the mutation rate increased with prolonged heat treatment of resting seeds of *Crepis capillaris* [13, 14]. In the years 1933–1939, Y.J. Kerkis published a series of papers demonstrating the connection between exposure to low temperature and mutation frequency [15–17]. In 1935–1936, M.E. Lobashev conducted research showing the influence of both constant and changing temperatures on the formation of lesions induced by X-rays in *Drosophila* germ cells [18]. M.E. Lobashev, in his research conducted during 1935–1936, pointed out that the hit principle, which views gene changes as a single molecular event, cannot describe the occurrence of chromosomal aberrations and small deletions. These events cannot be attributed to a single quantum of radiation hitting a gene; rather, they require multiple simultaneous local changes in the same region of the chromosome. Given the low probability of the simultaneous occurrence of several changes and the high frequency of chromosomal rearrangements induced by X-rays, the hit theory fails to convincingly explain the mechanism behind the appearance of chromosomal rearrangements [18, 19]. This led to the proposal that the mutational process may be influenced by physiological factors [20]. Further research provided evidence that mutations occur as a result of processes that require time rather than at the moment of ionization of gene atoms by an energy quantum [1]. The above observations led to the hypothesis that mutations must be preceded by a reversible pre-mutation state that can either lead to a mutation or vanish. This idea was further developed in the works of M.E. Lobashev.

M.E. LOBASHEV'S PHYSIOLOGICAL THEORY OF THE MUTATION PROCESS

In the 1940s, Mikhail Efimovich Lobashev was the first to propose the idea of the connection between mutagenesis and the repair process, as well as the existence of pre-mutational (primary) lesions in genetic material. These ideas formed the basis of M.E. Lobashev's physiological hypothesis of the mutation process, formulated on the base of the works of D.N. Nasonov and V.Y. Alexandrov, as well as his own experimental data. Nasonov and Alexandrov discovered that exposure to damaging agents in plant and animal cells leads to reversible changes, resulting in increased tissue sorption towards certain dyes [21]. The authors of this work concluded

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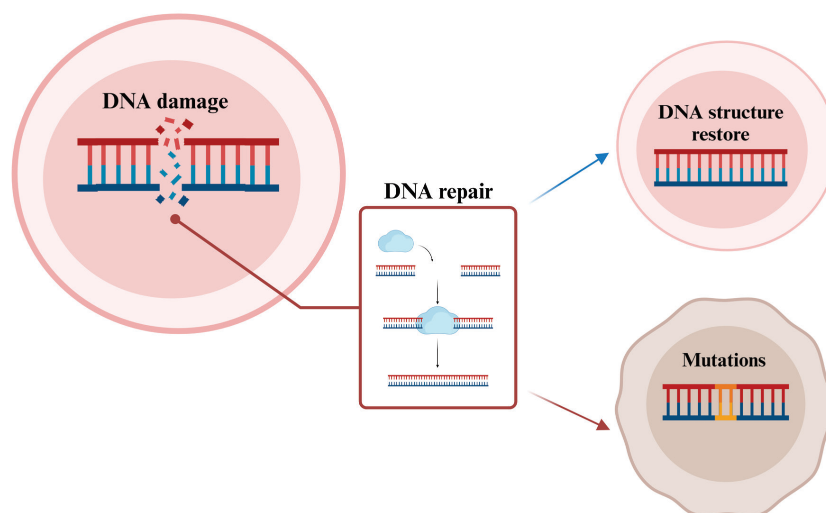

Fig. 1. The relationship between mutations and repair
Рис. 1. Связь между мутациями и репарацией

that various factors cause a non-specific reaction in the cell with a characteristic set of changes and hypothesized that reversible denaturation of cytoplasm proteins underlies the reversible changes [21]. Lobashev suggested that changes in the amount of dye adsorbed by tissues indicate the degree of cell damage and allow for the assessment of the sensitivity of cells to various damaging agents. He confirmed this hypothesis through experiments in which frogs and mice, previously kept under varying temperature conditions, were exposed to high temperatures [18]. Additional experiments were conducted on the neutral-rot staining of *Drosophila* germ cells exposed to high temperatures and X-ray radiation [18]. It was shown that these exposures result in a reversible increase in the sorption properties of the cytoplasm of germ cells, and the recovery of the cell to its initial state depends on the depth of exposure and the conditions under which it occurs [18]. Based on these experimental data, M.E. Lobashev proposed a new hypothesis to explain the mechanisms of mutations, suggesting that reversible lesions of cellular structures underly mutational changes represent a response of living systems to adverse changes in environmental conditions. Mutations occur when the living conditions deviate significantly from the optimal conditions, exceeding adaptive reactions to changing conditions [18]. The frequency of mutations depends not only on the extent of cell damage but also on the cell's ability to repair, defined as the rate of repair processes after the cessation of agent action [18]. The main principles of M.E. Lobashev's hypothesis are outlined in his dissertation for the degree of Doctor of Biological Sciences, "On the nature of the action of external conditions on the dynamics of the mutation process", and in the article "Physiological (paranecrotic) hypothesis of the mutation process" [18, 22], they also were reviewed in several publications [23–26].

Thus, the physiological hypothesis of the mutation process connected the concepts of mutation and repair for the first time, leading to the development of new ideas about the mechanism of mutation generation as a process, revealing that lesions in genetic material caused by mutagens do not necessarily result in a mutation. In the 1940s, when M.E. Lobachev formulated his theory, the role of DNA as a carrier of genetic information has not yet been established. Despite being based on the idea of the protein nature of genetic material, the physiological hypothesis revealed that the general principle of denaturation-repair of macromolecules also applies to DNA. The formation of a mutation is a complex physiological process that occurs during the repair of damaged DNA through non-identical repair. DNA repair can be inaccurate, leading to a mutational change or accurate, resulting in the restoration of the original genetic material structure (Fig. 1). The global scientific community only came to understand the connection between mutations and repair in the 1960s [27].

MODERN VIEW ON THE MUTATION PROCESS

When Lobashev proposed the physiological hypothesis of the mutational process, nothing was known about the physical nature of pre-mutational and mutational changes in genetic material, neither the molecular mechanisms through which primary lesions are fixed as mutations. Answers to these questions emerged in the second half of the 20th century. They arose due to mastering methods of induced mutagenesis, deciphering DNA structure, and discovering the molecular mechanisms of repair, replication, and recombination. After these discoveries, the connection between mutations and repair was once again established, not in the form of a hypothesis but as a proven mutational theory.

It became clear that mutations occur in two steps [28]. In the first step, a primary DNA lesion appears, which exists in the cell for some time. The second step involves the

conversion of the primary lesion into an inherited change in genetic material due to error-prone repair. Thus, any mutational change, whether spontaneous or induced by various factors, is preceded by a primary lesion of genetic material. These views, consistent with Lobashev's physiological hypothesis, received an important addition. By the end of the 1960s, it became clear that not only repair but also replication and recombination play an important role in fixing primary lesions as mutations. In the 1960s, von Borstel defined mutation as an error of three Rs — replication, repair, and recombination Later, mechanisms of temporary resistance to DNA damage were discovered, such as post-replicative recombinational repair and translesion synthesis, which are also important sources of mutational changes in genetic material. All these processes — replication, repair, recombination, and temporary tolerance to damage — are united by the fact that at a certain stage of each of them, the template-dependent DNA synthesis occurs. If DNA synthesis is impaired, it may result in changes in the sequence or quantity of DNA or, in other words, lead to the appearance of inheritable changes in genetic material (mutations). Thus, the rate of the mutational process mainly depends on the accuracy of template-dependent DNA synthesis, which is significantly reduced by primary lesions.

It is now known that primary lesions are changes in the chemical structure of DNA. DNA can undergo temporary changes, known as primary lesions, due to natural chemical instability of the DNA molecule, ultraviolet and ionizing radiation, reactive oxygen species, metabolic intermediates, exogenous chemicals, replication errors, error-prone repair, and other exogenous or endogenous factors [29–32]. Both the nitrogenous bases and the sugar-phosphate backbone of DNA are susceptible to damage (Fig. 2). The nitrogenous bases can undergo deamination, methylation, formation of adducts, and pyrimidine dimers. For instance, cytosine, adenine, and guanine can be converted to uracil, hypoxanthine, and xanthine through deamination. Additionally, spontaneous hydrolysis of N-glycosidic bonds can lead to the formation of apurinic and apyrimidinic sites (AP sites). Damage to the sugar-phosphate backbone can result in single- and double-strand breaks, as well as cross-linking of DNA strands [29, 31, 33]. Every day, approximately one million primary lesions occur in each human cell, such as 50,000–200,000 AP sites and 10,000 to 86,000 oxidative lesions in mammals [34, 35]. Despite this high level of damage, the observed rate of spontaneous mutations is much lower than expected. In bacterial genomes, the mutation rate ranges from 1×10^{-8} to 1×10^{-10} per cell division, and in mammals, from 1×10^{-5} to 1×10^{-6} per gamete, equating to 1 to 10 mutations per million gametes [36]. This indicates that only a small fraction of primary lesions results in inherited changes, while repair systems eliminate most primary DNA lesions without a trace [37, 38].

Primary DNA lesions are able to interrupt the replication of genetic material and the expression of genetic information. They can reduce the accuracy of replication, serve as signals to trigger repair systems, an essential step of which is homologous and non-homologous recombination, and can lead to the arrest of cell divisions [30, 31]. Primary DNA

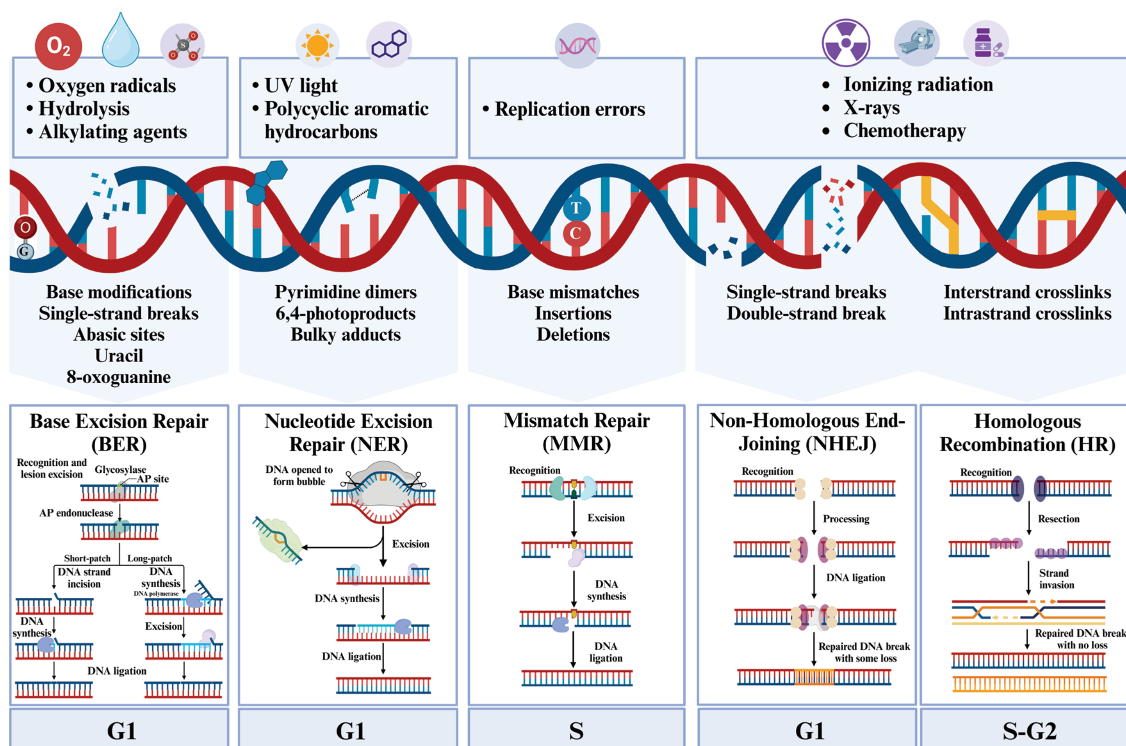

Fig. 2. Primary DNA lesions, repair mechanisms, and the cell cycle stages during which the corresponding repair systems are active [39]
Рис. 2. Первичные повреждения ДНК, механизмы репарации и стадии клеточного цикла, на которых активны соответствующие системы репарации

lesions contribute to the aging and the development of many diseases, such as cancer, neurodegenerative diseases, and fetal intrauterine defects [32, 34, 40–42]. Therefore, pro- and eukaryotic organisms have various repair systems and mechanisms of temporary DNA damage tolerance (Fig. 2). DNA repair may take a relatively long time and occurs in several stages. Different repair systems are responsible for repairing lesions of varying chemical structures. Generally, the initial stage of repair includes the excision of the damaged DNA site and the subsequent feeling of the resulting gap by a DNA polymerase. If double strand brakes, the most severe DNA lesions, occur, then homologous recombination and non-homologous end joining of two DNA molecules are also potential ways of repair. Often, intermediate products of incomplete repair serve as a substrate for subsequent repair steps. Thus, during the repair of lesions, interconversion may happen, which can impact the expression of damaged genes to different extents. For example, when modified bases are eliminated during base excision repair (BER), DNA glycosylase excises the damaged bases to form AP sites, which have been observed to have a lifespan of up to 190 hours at physiological temperature and pH, as demonstrated *in vitro* using bacterial phage PM2 [43]. The formation of AP sites can result in replication fork arrest and must be eliminated before replication to prevent cell death. Eliminating AP sites can cause single-strand breaks, which in turn trigger the formation of double-strand breaks and recombination (Fig. 3) [44, 45]. Double-strand breaks can lead to the loss of chromosomes or parts of chromosomes and, if inaccurately repaired through recombination repair or direct end joining, can result in gene mutations and chromosome rearrangement (Fig. 3) [30, 46, 47].

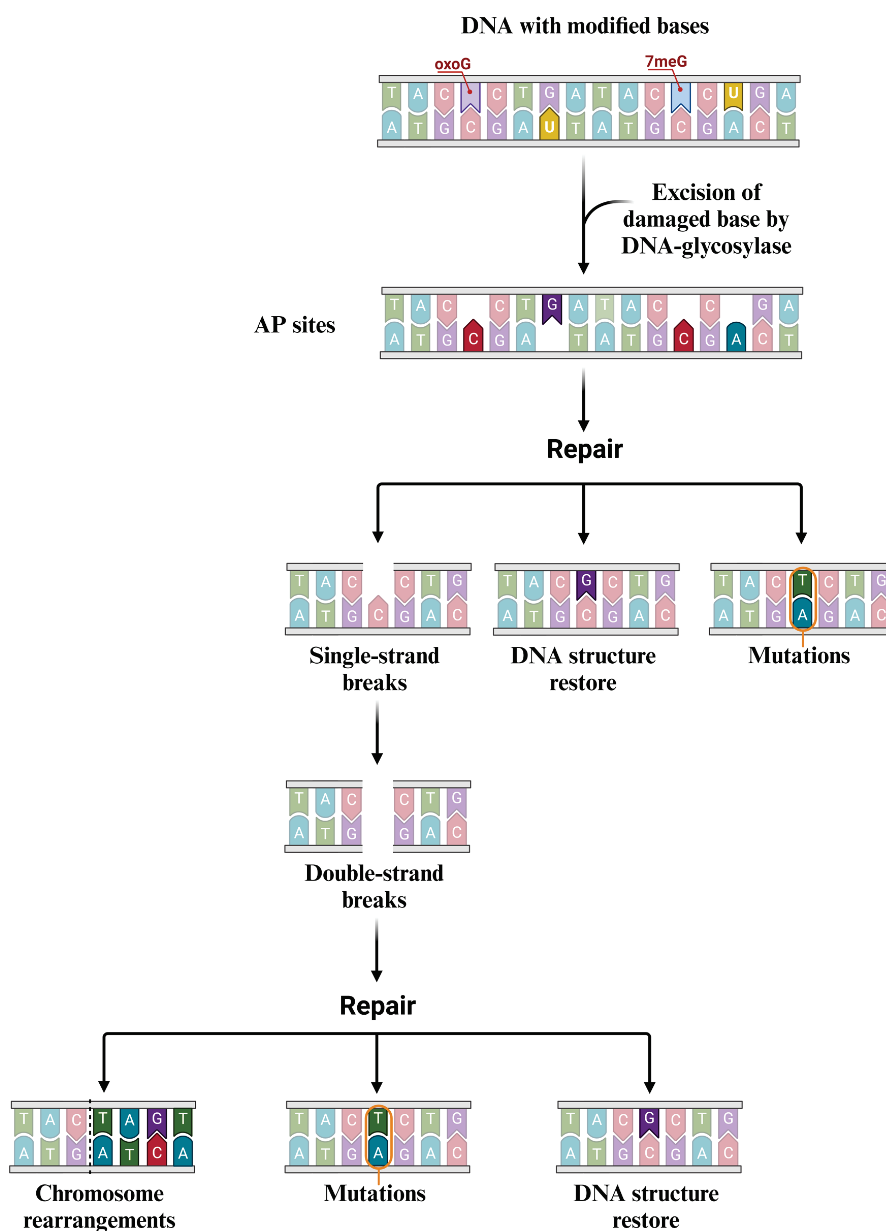

Fig. 3. Interconversion of primary DNA lesions during repair
Рис. 3. Взаимопревращение первичных повреждений ДНК в процессе репарации

Primary lesions	Mutations
Changes in the chemical structure of DNA	Alterations in the sequence or amount of DNA
Arise spontaneously or induced by chemical and physical factors	Result of error-prone DNA synthesis during replication, repair and recombination
Block replication and transcription, recognized by repair enzymes	Do not block replication and transcription, not recognized by repair enzymes
Temporary non-inherited changes	Inherited through generations
Able to induce phenotype changes, decrease cellular viability, promote aging and diseases, source of genetic variability Primary lesions are often precede to mutations	

Fig. 4. Main similarities and differences between genetic material alterations of two types — primary lesions and mutations
Рис. 4. Сходство и отличия между изменениями генетического материала двух типов — первичными повреждениями ДНК и мутациями

The involvement of a specific repair system in the DNA structure repair process is determined by both the type of damage and the cell cycle stage [48]. For instance, repairing double-strand breaks by non-homologous ends joining is only effective during the G1 stage. At the same time, homologous recombination works during either the S or G2 stage, and base or nucleotide excision repair systems are effective during the G1 stage (Fig. 2).

Thus, depending on the type of DNA damage and the cell cycle stage, primary lesions can be channeled through various repair pathways, leading to two possible outcomes of DNA damage repair. The repair can either fully restore the primary DNA structure and its sequence or, in the case of error-prone repair, result in gene mutations or chromosome aberrations, which are implicated in the development of hereditary and cancer diseases [30, 40]. For a full understanding of the importance of physiological factors for the occurrence of mutational changes, it is important to emphasize the main differences between primary lesions and mutations. Therefore, when discussing mutagenesis mechanisms, it is important to keep in mind that primary lesions are damages to the chemical structure of the DNA molecule, while mutations are changes in the sequence or amount of DNA resulting from error-prone repair of damaged DNA. Primary lesions are not inherited, and while mutations may be transferred through generations, both may influence the phenotype of the carrier (Fig. 4).

For a primary lesion to become a mutation, it is necessary for the DNA carrying the lesion to go through a stage of template-dependent synthesis as a part of replication, repair, recombination or translesion synthesis process due to occasional errors by DNA polymerases. Thus, the accuracy of a DNA polymerase involved in synthesis determines whether the lesion will become a mutation and its probability. Here we should stop and outline an important addition to the physiological hypothesis of the mutation process, which does not contradict it, but reinforces the idea of mutagenesis as a physiological process. Mutations can also occur during DNA synthesis on an undamaged template, due to the limited accuracy of the DNA polymerase involved in this process. In pro- and eukaryotes, at least 20 different DNA polymerases have been described that are involved in genome duplication, repair, reactions of temporary resistance to DNA damage, and in recombination processes. The fidelity of known DNA polymerases differs by several orders of magnitude. The most accurate replicative DNA polymerases (Pol III in *E. coli*, or Pol δ and Pol ε in eukaryotes) insert incorrect nucleotides opposite normal undamaged bases at a frequency 1 per 10^6–10^8 polymerized nucleotides. The fidelity of other DNA polymerases, usually involved in synthesis of short stretches of DNA during repair or replication, is several orders of magnitude lower. The fidelity of DNA polymerase can depend on various factors such as the presence and ratio of DNA precursors (deoxynucleoside triphosphates), the presence of damage in the template DNA or replication protein, the state of chromatin or transcriptional status of the region where DNA synthesis occurs, the efficiency of post-replicative repair of mismatched bases, and others [49]. Although mutations can occur during the replication of undamaged DNA, lesions significantly increase the frequency of mutagenesis.

The genetic consequences of primary DNA lesions are well-documented, and the impact of mutations (gene, chromosomal, and genomic) on altering phenotypic traits

is established and undisputed [29, 30, 36, 40]. However, much remains unknown about how primary lesions affect an organism's phenotype before they become inherited changes. The connection between temporary damage to a specific gene and changes in a particular trait is poorly understood. It can be theorized that primary DNA lesions can result in phenocopy of mutations by disrupting the expression of genetic information. For example, the presence of a double-stranded break or other lesion in the structural or regulatory part of some gene should interfere with the expression of this gene and naturally reflect on the organism's phenotype. This is particularly likely to occur in non-dividing differentiated cells, where damage can persist for extended periods because replication does not occur in the non-dividing cell and the frequency of mutation fixation is low compared to dividing cells. For example, in non-dividing mammalian cells and in bacteria *Escherichia coli*, uracil, (a result of cytosine deamination in DNA), and 8-oxoguanine (product of guanine oxidation), lead to the formation of an aberrant transcript (mRNA) of the luciferase reporter gene [50–52]. Conversely, in non-dividing cells without DNA damage, the expression of the luciferase reporter gene results in the production of normal protein. The damage in the coding sequence of the luciferase gene causes errors in nucleotide inclusion during transcription, resulting in the production of mutant mRNA molecules. This leads to the formation of numerous aberrant transcripts and their subsequent translation, ultimately producing a large quantity of abnormal protein. The accumulation of 8-oxoguanine, induced by oxidative stress, has been observed in the DNA of neurons, both in the nucleus and mitochondria. Its levels increase with age and in patients with neurodegenerative diseases like Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis. It is believed that the buildup of abnormal proteins resulting from the transcription of DNA containing 8-oxoguanine and the subsequent translation may lead to a change in the characteristics of neurons [42]. Therefore, the transcription of damaged DNA can lead to phenotypic alterations in non-dividing cells [42, 53, 54]. Even in dividing cells, primary DNA lesions can potentially impact gene expression despite these lesions significantly disrupting essential cellular processes such as replication. However, it is unlikely for primary lesions to persist in the cell for an extended period of time, as normal cellular functioning and genome duplication can only occur under conditions of relatively low levels of primary lesions. The significance of primary DNA lesions in gene expression during crucial stages of tissue differentiation in embryonic development is demonstrated by the development of morphological changes in *D. melanogaster* [55, 56]. It has been shown, that in yeast *Saccharomyces cerevisiae* primary lesions lead to transient changes of mating type [37, 48, 57–62]. Therefore, there are instances in the existing literature that support the potential for primary lesions to result in observable phenotypic effects, although the mechanisms behind this process are not well comprehended.

APPROACHES AND METHODS USED TO DETECT DNA LESIONS

The lack of data on the impact of primary DNA lesions on the phenotype of organisms may be due to underdeveloped systems for studying the independent phenotypic effects of primary lesions, rather than the resulting mutations and chromosomal abnormalities. In genetic toxicology, there are numerous tests for detecting various chromosomal aberrations and mutations through observable phenotypic changes (such as changes in coloration of microorganism colonies, body or eye color in *Drosophila*, development of antibiotic resistance, the appearance of auxotrophy, among others) [63]. Currently, comprehensive methods have been developed to assess the genetic risk of different chemical and physical factors, allowing for the detection of mutagens and carcinogens in various test subjects [11, 63–67]. The primary criteria for genetic activity in these test systems include the frequency of gene mutations, conversion, and reciprocal recombination, chromosomal aberrations, sister chromatid exchanges, non-disjunction in mitosis, as well as an increase in the frequency of abnormal spermatozoa [63, 64, 66]. The most extensively studied species of bacteria, fungi, and animals, along with human peripheral blood cells, mouse bone marrow, and fibroblast cell lines, are utilized as biological objects [63, 64, 66, 67].

Primary DNA lesions can be identified using physical and chemical methods, depending on the type of the lesions. Often these methods involve the step of cell lysis for following DNA extraction [68]. For example, the DNA comet assay is a widely used and accurate method for detecting single- and double-strand breaks in eukaryotic cells [69, 70]. Various modifications of this method enhance its sensitivity and broaden its application [71]. Single- and double-strand breaks can also be detected using the terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assay [68, 72]. Another sensitive method for detecting double-strand breaks is based on the use of fluorescent antibodies to the phosphorylated histone gamma-H2AX (γ-H2AX) [73–75]. In contrast to the DNA comet method, which is most effective at detecting a high amount of DNA breaks and fragments within cells, the detection of phosphorylated histone H2A has a high level of accuracy in identifying single DNA breaks within the cell nucleus. The cytological detection of DNA fragmentation can be achieved through the micronucleus test, which involves the formation of micronuclei from acentric chromosome fragments that result from structural DNA breaks not entering the cell nucleus during cell division [76, 77].

Oxidative DNA lesions, mainly 8-oxoguanine, can be detected using gas chromatography coupled with mass spectrometry (GC-MS), high-performance liquid chromatography with electrochemical detection (HPLC-EC), and high-performance liquid chromatography with electrospray ionization and tandem mass spectrometry (HPLC-MS/MS) [78, 79]. HPLC-EC is a precise and sensitive method that can

determine the percentage of modified bases in hydrolyzed DNA [78]. However, it is labor-intensive and requires multiple measurements for each sample and a large amount of test material for reliable results [79]. GC-MS is less accurate than HPLC-EC and requires nucleotide derivatization, which can cause oxidative damage to nitrogenous bases and overestimate the level of damage in the cell. The most accurate and sensitive method currently available is HPLC-MS/MS, which can measure low levels of DNA damage without lengthy sample preparation, reducing the risk of artifactual damage. This method is more automated compared to GC-MS and HPLC-EC [79]. HPLC–MS/MS can also detect apurinic/apyrimidinic sites, cyclobutane-pyrimidinic dimers, 6–4 photoproducts, and DNA adducts [80, 81].

Currently, several reliable methods are available for quantifying various primary DNA lesions in a cell. However, these methods typically involve the step of lysis of the cells being studied, which hinders the analysis of the fate of primary lesions and the study of their impact on phenotype. Compared to the previously mentioned methods, the alpha-test enables the assessment of primary DNA lesions through changes in cell phenotype and the detection of consequences of DNA damage repair at a specific locus [48, 57, 59]. This test can identify various genetic events that lead to temporary or hereditary changes in mating type α → a in heterothallic strains of yeast *S. cerevisiae.* The alpha-test stands out for its capability to differentiate between hereditary mutations and temporary DNA lesions. With the alpha-test we have studied the influence of mutations in DNA repair genes, such as inactivating translesion synthesis DNA polymerases (Polζ, Polη, and Rev1), mismatch repair (*pms1*), base excision repair (*ogg1*), and homologous recombination repair (*rad52*), mutations in DNA polymerase ε, as well as mutagens causing specific DNA lesions like UV light, 6-*N*-hydroxylaminopurine and camptothecin [48, 57, 58, 60, 61]. Using the alpha-test, we also studied the ability of primary DNA lesions to pass through the cell cycle stages [48]. Our findings indicate that phenotypic expression of primary DNA lesions in the alpha-test depends on the type of primary lesions and the stage of the cell cycle in which this lesion occurred.

CONCLUSION

The development of modern ideas about mutagenesis as a complex process, intricately linked to environmental conditions and regulated by numerous internal factors, has a lengthy history and continues to evolve. A pivotal moment was the recognition of the connection between the rate of mutagenesis and intracellular processes such as repair, replication, and recombination, all of which involve DNA synthesis. These concepts gained widespread acceptance in the 1960s after the first experimental evidence on the molecular basis of heredity and variability emerged. The terms “mutation” and “repair” were first mentioned together in the works of M.E. Lobashev and his co-authors in the 1930s and 1940s, long before the discovery of the structure of genetic material. M.E. Lobashev’s physiological theory of the mutational process suggests that most inherited changes in genetic material are preceded by primary (premutation) changes in genes, which can either be fixed as mutations or eliminated through repair. This theory has been extensively supported by experimental evidence. Primary DNA lesions, during error-prone repair, often lead to chromosomal rearrangements and gene mutations, which can cause hereditary and oncologic diseases in humans. However, not all primary lesions result in inherited changes; repair systems eliminate most of them without error. The time it takes for a primary lesion to be eliminated can be quite lengthy. The process of repairing double-strand breaks in yeast *S. cerevisiae* can take from 2 to 8 hours, corresponding to 1 to 4 cell cycles under optimal conditions [82]. Different lesions during their existence in DNA disrupt transcription and replication to varying degrees and thus impact the expression of genetic information. While this possibility has been demonstrated in several studies [42, 50, 52–54], the mechanism and details of this process have not been thoroughly investigated. Identifying the molecular nature and temporal parameters of the existence and elimination of primary lesions is crucial for understanding general mechanisms of hereditary and modification variability.

ADDITIONAL INFORMATION

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