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DETECTION OF THE DNA PRIMARY STRUCTURE MODIFICATIONS INDUCED BY THE BASE ANALOG 6-*N*-HYDROXYLAMINOPURINE IN THE ALPHA-TEST IN YEAST *SACCHAROMYCES CEREVISIAE*

© A.S. Zhuk [1], E.I. Stepchenkova [2, 3], S.G. Inge-Vechtomov [2, 3]

[1] ITMO University, Saint Petersburg, Russia;

[2] Vavilov Institute of General Genetics Russian Academy of Science, Saint Petersburg brunch, Saint Petersburg, Russia;

[3] St. Petersburg State University, Saint Petersburg, Russia

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❀ **Background.** The alpha-test allows to detect inherited genetic changes of different types, as well as phenotypic expression of primary DNA lesions before the lesions are fixed by repair. Here we investigate ability of the alpha-test to detect base modifications induced by 6-*N*-hydroxylaminopurine (HAP) and determine frequency of inherited and non-inherited genetic changes in yeast strains treated with HAP. **Materials and methods.** The alpha-test is based on mating type regulation and detects cell type switch from α to a in heterothallic yeast *Saccharomyces cerevisiae*. The frequency of mating type switching reflects level of both spontaneous and induced by a mutagen DNA instability. The alpha-test may be performed in two variants: "illegitimate" hybridization and cytoduction. Conducting both complementary tests and analysis of phenotypes of the "illegitimate" hybrids and cytoductants allows to detect the full spectrum of genetic events that lead to mating type switching, such as chromosome III loss and chromosome III arm loss, mutations and temporary lesions, recombination and conversion. **Results.** HAP increases the frequency of illegitimate hybridization by 5-fold, and illegitimate cytoduction by 10-fold. A large proportion of the primary lesions induced by HAP causes temporary mating type switch and the remainder parts are converted into inherited point mutations. **Conclusion.** The alpha-test can detect HAP-induced base modifications and may be used to investigate the ratio between correct and error-prone processing of such primary DNA lesions. Like other genetic toxicology tests the alpha-test has limitations, which are discussed.

❀ **Keywords:** alpha-test; 6-*N*-hydroxylaminopurin; base modifications; primary DNA lesions; genetic toxicology.

ВЫЯВЛЕНИЕ МОДИФИКАЦИЙ ПЕРВИЧНОЙ СТРУКТУРЫ ДНК, ВОЗНИКШИХ ПОД ДЕЙСТВИЕМ АНАЛОГА АЗОТИСТЫХ ОСНОВАНИЙ 6-*N*-ГИДРОКСИЛАМИНОПУРИНА, В АЛЬФА-ТЕСТЕ У ДРОЖЖЕЙ *SACCHAROMYCES CEREVISIAE*

© А.С. Жук [1], Е.И. Степченкова [2, 3], С.Г. Инге-Вечтомов [2, 3]

[1] Федеральное государственное автономное образовательное учреждение высшего образования «Национальный исследовательский университет ИТМО», Санкт-Петербург;

[2] Федеральное государственное бюджетное учреждение науки Институт общей генетики им. Н.И. Вавилова Российской академии наук, Санкт-Петербургский филиал, Санкт-Петербург;

[3] Федеральное государственное бюджетное образовательное учреждение высшего образования «Санкт-Петербургский государственный университет», Санкт-Петербург, Россия

Для цитирования: Жук А.С., Степченкова Е.И., Инге-Вечтомов С.Г. Выявление модификаций первичной структуры ДНК, возникших под действием аналога азотистых оснований 6-*N*-гидроксиламинопурина, в альфа-тесте у дрожжей *Saccharomyces cerevisiae* // Экологическая генетика. – 2020. – Т. 18. – № 3. – С. 357–366. https://doi.org/10.17816/ecogen34581.

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❀ Альфа-тест позволяет выявлять активность различных генотоксических факторов, вызывающих как наследуемые (мутационные и рекомбинационные), так и ненаследуемые (модификационные) изменения генетического материала. Уникальной особенностью альфа-теста является то, что он позволяет изучать фенотипическое проявление первичных повреждений генетического материала еще до их «безошибочного» устранения системами репарации. Альфа-тест основан на использовании системы генетической регуляции клеточного типа и особенностей переключения типа спаривания у гетероталличных дрожжей *Saccharomyces cerevisiae*. В этой работе с использованием мутагенного

аналога оснований 6-*N*-гидроксиламинопурина (ГАП) мы оценили эффективность альфа-теста для выявления таких повреждений генетического материала, как модификации оснований ДНК, а также изучили способность повреждений ДНК, вызванных ГАП, проявляться фенотипически. Альфа-тест проводили в двух взаимодополняющих вариантах: «незаконной» гибридизации и «незаконной» цитодукции, что позволило оценить частоту наследуемых и ненаследуемых изменений, индуцированных ГАП. Мы показали, что обработка клеток ГАП повышает частоту не только точечных мутаций, но и временных повреждений генетического материала, учитываемых в альфа-тесте. Полученные результаты указывают на то, что модификации оснований могут иметь собственное фенотипическое проявление, а альфа-тест обладает достаточной чувствительностью для выявления модификаций оснований ДНК.

❀ **Ключевые слова:** альфа-тест; 6-*N*-гидроксиламинопурин; модификации оснований; первичные повреждения; генетическая токсикология.

INTRODUCTION

The alpha-test is a genetic toxicology method that can detect a wide range of genetic changes, such as gene mutations, loss of an entire chromosome or chromosome arm, gene conversion and recombination, and primary (pre-mutation) DNA lesions [1–3]. The latter is especially important, as the alpha-test allows to study the phenotypic expression of primary lesions, determines their frequency, and estimates the proportion of the primary lesions corrected without error during repair or turned into inherited changes. The alpha-test is based on the features of mating-type regulation in the heterothallic yeast *Saccharomyces cerevisiae*. In the alpha-test, the mutagenic activity of various factors is determined by the mutagen's ability to induce the mating-type switching from α to a in haploid *Saccharomyces* yeast cells. The haploid *S. cerevisiae* cells may have mating type α or a, which is determined by the *MAT* locus located in the right arm, of chromosome III near the centromere. The *MAT* locus may have, two alternative sequences, *MATα* or *MATa* (idiomorphs) in strains of mating types *α* and a, respectively [4]. *MATα* contains two genes, *MATα1* and *MATα2*, which are controlled by a common bidirectional promoter, and encode transcription factors. The *MATα1* product activates the transcription of alpha-specific genes (*αsg*), and the protein encoded by *MATα2* suppresses the expression of a-specific genes (*asg*). If the structural damage or mutations in *MATα* lead to the simultaneous inactivation of both *MATα1* and *MATα2* genes the mating-type switching α to a occurs, because only *asg* are expressed in this case. As a result, cells that initially had the α mating type get the ability to mate with α cells. Genetic information about the mating type in yeast is also stored in the silent *HMRa* and *HMLα* cassettes located in the right and left arms of chromosome III near the telomeres, respectively. In homothallic yeast strains, in contrast to heterothallic strains, during germination of ascospores, a genetically determined the mating type switching occurs regularly due to the replacement of the *MAT* locus sequence with an alternative sequence of one of the cassettes. In heterothallic strains, the spontaneous mating-type switching from α to a occurs with a very low frequency (10^{-7}), which increases when the cells are exposed to DNA-damaging agents, due to an increase in both the frequency of primary lesions and mutations in *MATα* and the frequency of recombination and conversion between *MATα* and *HMRa*. Thus, in the alpha-test, an indicator of the genotoxic activity of a factor under study is the frequency of the mating-type switching from α to a, which corresponds to the frequency of the occurrence of "illegitimate" hybrids of two strains of the same mating type α. The genetic events affecting the *MATα* locus lead to the mating-type switching from α to a, are: temporary lesions and gene mutations, loss of an entire chromosome III or its right arm together with the *MATα* locus, and conversion and recombination, which result ininformation transfer to the *MATα* locus from the silent cassette *HMRa*

In the alpha-test, to assess the frequency of mating-type switching, two strains of the same mating type α with complementary markers are plated on a selective for "illegitimate" hybrids medium on which the parental strains cannot grow. Normally, hybridization occurs only between cells of opposite mating types. When strains of the same mating type α are mixed together, cell hybridization becomes possible only if some cells of the test strain switch their mating type from α to a aftere exposure to a mutagen. The strains used in the alpha-test carry genetic markers that enable not only to select

hybrids but also to identify all genetic events that lead to mating-type switching from α to a.

The alpha-test is performed in two complementary variants: "illegitimate" hybridization and "illegitimate" cytoduction to identify all genetic events leading to an disturbance of the *MATa* locus expression "Illegitimate" hybridization allows to estimate the frequency of loss of the whole chromosome III and its right arm as well as conversion and recombination and the frequency of the comman class of gene mutations and temporary lesions in the *MATα* locus. To determine the frequency of gene mutations and the frequency of temporary lesions at the *MATα* locus separately, it is necessary to perform the alpha-test in the "illegitimate" cytoduction system. To reveal which of the listed genetic events lead to the mating-type switching from α to a, it is necessary to analyze the phenotype of the resulting "illegitimate" hybrids and cytoductants (Fig. 1). After the frequency of each phenotypic classes of hybrids and cytoductants has been determined, it is then possible to quantify the proportion of the primary DNA lesions corrected in error-free maner. However, during their existence in the cell, those primary lessions could influence cellularphenotype (leading to a temporary mating-type switching from

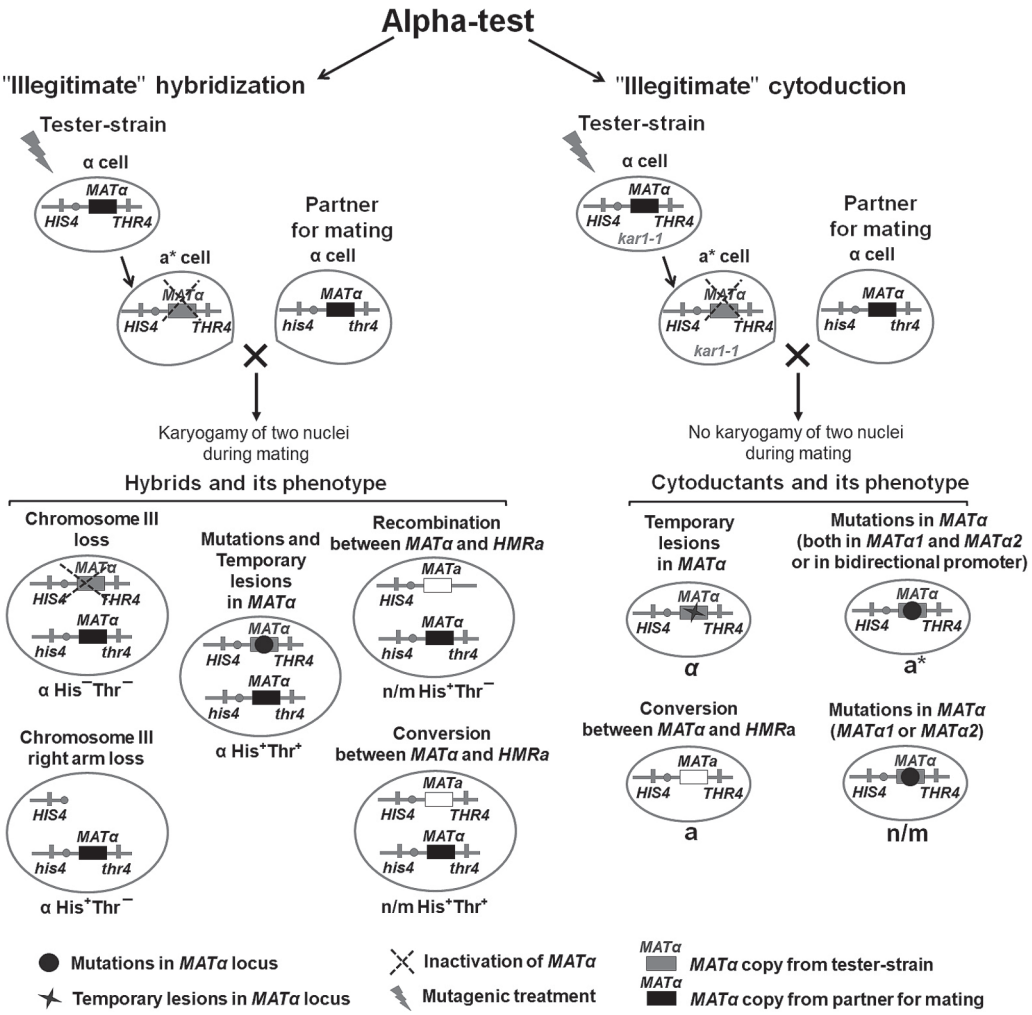
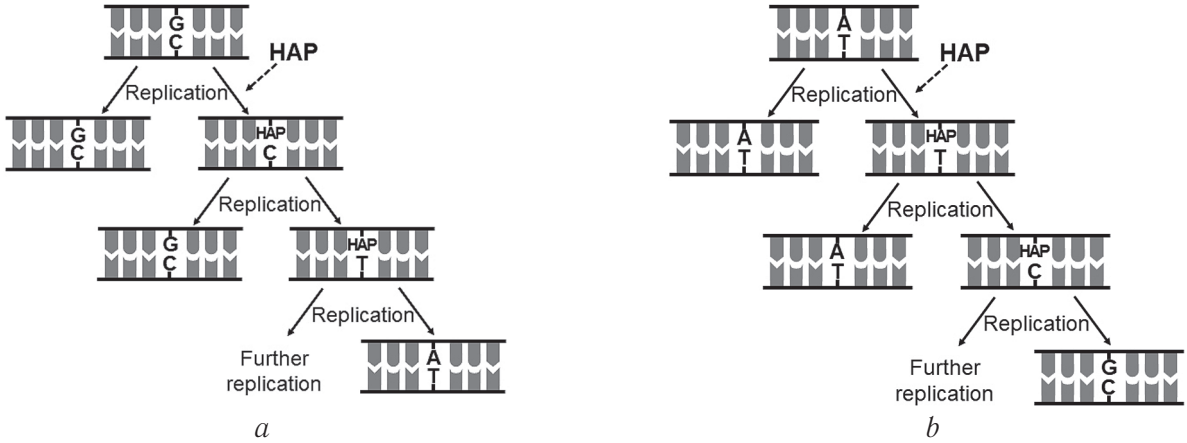


Fig. 1. Genetic events, that can be detected in two variants of the alpha-test in "illegitimate" hybridization and cytoduction.
Note. In "illegitimate" hybridization test the frequency of diploid hybrids formation between two strains of α mating type is estimated. Tester-strain in "illegitimate" cytoduction has *kar1-1* mutation [8] that blocks karyogamy. In addition, for the selection of cytroduktants, the tester strain carries a recessive marker of cycloheximide resistance (*cyh*^{r}) and a cytoplasmic marker [ρ^0] [1]. These genetic markers make it possible to select haploid cells with mixed cytoplasm of both strains and single nucleus of the tester strain, which carried a temporary or inherited change in the genetic material, leading to the mating type switching. "illegitimate" cytoduction results in haploids formation with nucleus of the tester-strain and mitochondria from the mating partner. Since genetic events detected in the alpha-test are recessive, it is preferable to use autodiploid with homozygous *MATα* partner strain to reduce influence its spontaneous mating type switch on the alpha-test results. Phenotype designation: n/m is non-mater, a* is recessive a or a-like faker, which can mate with α cells, but such hybrids have mating type α) [1, 2, 6]



a *b*

Fig. 2. HAP incorporation into DNA during replication and the mechanism of transition induction: (*a*) substitutions GC → AT; (*b*) substitutions AT → GC

α to a). So we can score the proportion of those primary lessions that were fixed in the form of mutations, lead to the loss of chromosome III or its right arm and also induce gene conversion and recombination. The theoretical concept of the alpha-test are described in more detail in previous studies [2, 5–7].

Earlier, several physical and chemical mutagens (ultraviolet radiation, ethylmethanesulfonate, 2-aminofluorene, 1,2,7,8-diepoxyoctane, β-propiolactone, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine, cyclophosphamide, diethylhexylphtrolate, and acrylonitrile diethylstilbestrol) were tested using the alpha-test. The effect of inactivation of DNA polymerase genes involved in translesion DNA synthesis and DNA double-strand break repair genes has been studied [2, 3, 5, 6, 9]. Thus, it was found that the alpha-test is a sensitive test system for detecting mutagens that cause both gene mutations and chromosomal abnormalities and can also be used to study the fundamental mechanisms of mutagenesis and repair.

Despite the proven efficiency of the alpha-test to identify the activity of mutagens with different mechanisms of action, almost nothing is known about the sensitivity of the alpha-test to a whole group of mutagens, which are analogs of DNA nitrogenous bases. In this study, the sensitivity of the alpha-test was analysed using an adenine analog, 6-*N*-hydroxylaminopurin (HAP). HAP in the form of a nucleotide is incorporated into DNA during replication opposite thymine or cytosine, and in the subsequent rounds of replication, the erroneous inclusion of HAP opposite cytosine leads to transition [10, 11] (Fig. 2). It is the ability of HAP to form pairs with both thymine or cytosine that determines its mutagenic properties and the replicative mechanism of mutagenic action. HAP is a universal and strong mutagen for many prokaryotic and eukaryotic organisms, including mammals [10–16]. In yeast, none of the repair systems can recognize and remove HAP from DNA [17]. Therefore, in yeast, in contrast to *Escherichia coli* and humans, HAP cannot induce recombination and single- or double-stranded DNA breaks, which often occur during repair [17, 18]. In yeast, HAP after incorporation into DNA induces only gene mutations such as GC → AT or AT → GC transitions [12, 19–21] (Fig. 2). HAP more often induces GC → AT substitutions, as demonstrated in systems for the induction of reversions for *URA3* and *LYS2* genes in *S. cerevisiae* [19, 20] and DNA sequencing of haploid and diploid yeast strains treated with HAP [12]. With regard to these properties, HAP was chosen as a reference mutagen to assess the efficacy of the alpha-test in relation to analogs of nitrogenous bases. The ratio and frequency of inherited and non-inherited genetic material changes induced by HAP were estimated by using the alpha-test.

MATERIALS AND METHODS

Strains and cultivation conditions

In this study, haploid strains of heterothallic yeast *S. cerevisiae* were used: testerstrain for "illegitimate" hybridization K5-35V-D924 (*MATα ura3Δ leu2Δ met15Δ lys5:: KanMX cyh*^{r}), tester-strain for "illegitimate" cytoduction K5-35V-D924-kar1-1 (*MATα ura3Δ leu2Δ met15Δ lys5::*

KanMX cyhr kar1-1 [rho⁻]), partner for mating as a cytoplasm donor D926 (*MATα//MATα ADE1//ade1Δ leu2Δ//leu2Δ lys2Δ//lys2Δ ura3Δ//ura3Δ his4Δ//his4Δ thr4Δ//thr4Δ CYH^s [rho⁺]*), testers of mating type 78A-P2345 *MATα his5* and 2G-P2345 *MATa his5* [2]. Yeast starins were grown on solid and liquid YEPD complete media and minimal MD medium containing the necessary amino acids, nitrogenous bases, vitamins, and microelements [22]. Agar manufactured by Sigma or Difco was used at a concentration of 20 g/L to prepare solid media. The medium for the selection of "illegitimate" hybrids was prepared based on MD medium supplemented with L-histidine (20 mg/L), L-threonine (150 mg/L), uracil (20 mg/L), and L-leucine (60 mg/L). The medium for the selection of "illegitimate" cytoductants was prepared based on MD medium without glucose with the addition of ethyl alcohol (20 mL/L), the antibiotic cycloheximide (5 mg/L), L-methionine (20 mg/L), L-leucine (60 mg/L), L-lysine (30 mg/L), and uracil. Yeast strains were grown at 30 °C. To treat cells with HAP, independent cultures of the tester-strain in the exponential growth stage of the culture were used. Yeast was grown for 16 h in liquid YEPD medium containing HAP at a concentration of 50 mg/L. Treated cells were then used in the "illegitimate" hybridization and cytoduction tests.

Alpha-test

To determine the frequency of "illegitimate" hybridization, 50 to 100 μL of an overnight cell culture of the tester-strain K5-35V-D924 and the partner strain for mating D926 were mixed together on a selective medium to select hybrids. In "illegitimate" cytoduction, strain K5-35V-D924-kar1-1 was used as a tester. In this case, both strains (K5-35V-D924-kar1-1 and D926) were first plated on complete YEPD medium and incubated together for 48 h and then replica-plated on a selective medium for the selection of cytoductants. Before plating, overnight cultures of both strains were concentrated 10 times to a density of approximately 10^9 cells/mL. In parallel, to assess the survival rate in "illegitimate" hybridization and cytoduction tests, the tester-strain culture was diluted 10^4 or 10^5 times and plated (100 μL) on solid YEPD medium. The number of colonies formed by surviving cells and colonies of "illegitimate" hybrids was calculated on day 3 of incubation, and the number of grown cytoductants was counted 10 days after replicaplating onto a selective medium. In each experiment, 10 to 12 independent cultures were used, and at least three experiments were performed. To analyze the phenotype of "illegitimate" hybrids and cytoductants for further distribution between the phenotypic classes detected in the alpha-test, at least 500 hybrids and cytoductants were picked up for each type of experimental conditions. Then, the selected hybrids and cytoductants were tested for auxotrophy and the mating type. The frequency of each phenotypic class was calculated by multiplying the proportion of the class by the overall frequency of "illegitimate" hybridization or cytoduction in each culture. The statistical significance of differences between the frequency of spontaneous and HAP-induced damage of the genetic material was checked using the Mann–Whitney test at a significance level of 0.05 [23]. On the graphs illustrating the frequency of the different classes of genetic changes, the median and its confidence interval, as determined by the standard method according to GOST R50779.24-2005, were presented. The statistical significance of differences between the compared proportions of the classes of hybrids or cytoductants was assessed using the *Z*-test with Yates's correction for continuity [23]. The confidence interval for the shares was calculated using the Wilson (Score) method [24].

RESULTS

Treatment of cells with HAP increases the overall frequency of "illegitimate" hybridization by about five times. Thus, the frequency of spontaneous "illegitimate" hybridization was 83×10^{-7} (63–140), and after exposure to HAP, the frequency increased to 425×10^{-7} (297–510). To determine the type of genetic changes leading to an increase in the frequency of mating-type switching from α to a, the proportion of each phenotypic class of "illegitimate" hybrids detected in the alpha-test was measured (Fig. 3, *a*). For this purpose, 1322 "illegitimate" hybrids that arose spontaneously and 892 "illegitimate" hybrids induced by HAP were selected. The selected hybrids were tested for histidine and threonine auxotrophy and mating type (see Materials and Methods). The results of this analysis

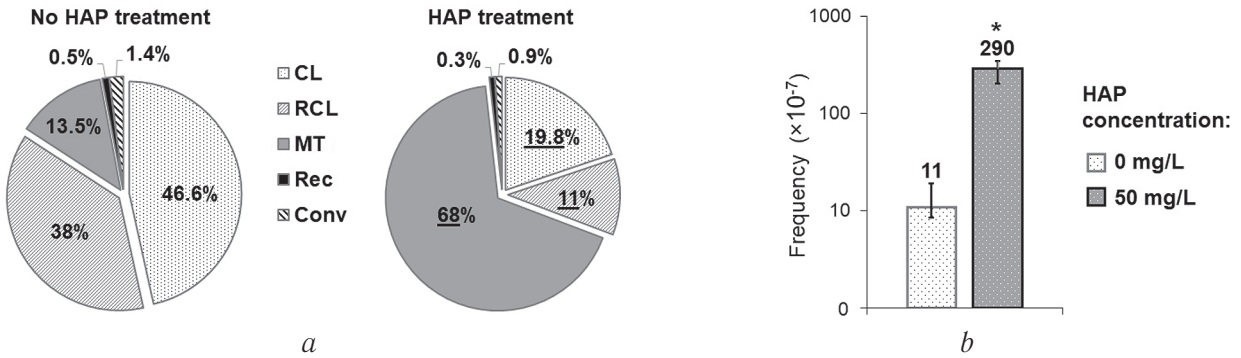


Fig. 3. Ratio of heritable and nonheritable genetics changes induced by HAP (%) (*a*) and frequency of mutations and temporary lesions in *MATα* locus (*b*) detected in "illegitimate" hybridization.
Note: CL – Chromosome III loss; RCL – Right arm of chromosome III loss; MT – Mutations and temporary lesions in *MATα* locus. Rec – Recombination between *MATα* locus and cassette *HMRa*; Conv – Conversion between *MATα* locus and cassette *HMRa*; * – significant difference between the HAP treated and control variants, estimated in Mann–Whitney test (p-value < 0.0001); Underline – difference between proportion of genetic events in the HAP treated and negative control is statistically significant, according to Z-test

enabled to determine the percentage of all phenotypic classes of "illegitimate" hybrids detected in the alpha-test. Thus, HAP treatment induced significant increase (from 13.5% to 68%) in the proportion of the phenotypic class of α His^+ Thr^+ hybrids, which arose as a result of mutations or temporary lesion at the *MATα* locus. This increase in the proportion of the α His^+ Thr^+ class was caused by the decrease in the proportion of all other classes of "illegitimate" hybrids detected in the alpha-test, namely, α His^- Thr^- (loss of chromosome III), α His^+ Thr^- (loss of the right arm of chromosome III), n/m His^+ Thr^- (recombination between *MATα* and *HMRa*), and n/m His^+ Thr^+ (conversion between *MATα* and *HMRa*; Fig. 3, *a*).

At the next stage, the absolute frequency values of the occurrence of "illegitimate" hybrids with the α His^+ Thr^+ phenotype (mutations and temporary lesions) were determined in cultures treated with HAP and cultures not treated with the mutagen. For this purpose, the ratio of the number of the α His^+ Thr^+ "illegitimate" hybrids to the number of living cells in each culture was determined. This indicator, in contrast to the relative frequency measured as a percentage, demonstrated more clearly the effect of HAP on the induction of "illegitimate" hybrids of this class. Based on the data in Fig. 3, *b*, under the action of HAP, the frequency of the occurrence of "illegitimate" hybrids α His^+ Thr^+ increased by more than 20 times. These results were consistent with the literature that HAP in yeast is a strong mutagen, causing transitions [10, 12, 15].

The "illegitimate" hybridization test does not distinguish hybrids resulting from mutations at the *MATα* locus from those resulting from temporary (pre-mutational) genetic material changes, as the hybrids in both cases have the α His^+ Thr^+ phenotype. This problem was solved using the "illegitimate" cytoduction test (see Fig. 4). HAP increased the overall frequency of "illegitimate" cytoduction by an order of magnitude from 10^{-7} to 10^{-6}. To determine which genetic changes caused an increase in the frequency of "illegitimate" cytoduction, 1327 cytoductants arising spontaneously and 732 cytoductants arising after HAP treatment were selected. The selected cytoductants were tested for mating type and auxotrophy. Using these data the percentage of cytoductants of various phenotypic classes that arose under different experimental conditions was determined (Fig. 4, *a*). It was shown that HAP treatment increased the proportion of phenotypic classes of cytoductants arising as a result of the following genetic changes: temporary lesions at the *MATα* locus (cytoductants have mating type α), mutations in *MATα1* and *MATα2* simultaneously or in a bidirectional promoter, *MATα* deletions (cytoductants have the mating type *a**; recessive *a*), and mutations in one of the *MATα1* or *MATα2* genes (cytoductants are sterile – n/m). The latter class could appear only as a result of the initial damage in *MATα1* and *MATα2* simultaneously with the subsequent (after mating) repair of one of the genes. The proportion of cytoductants arising as a result of gene conversion between *MATα* and a silent *HMRa*

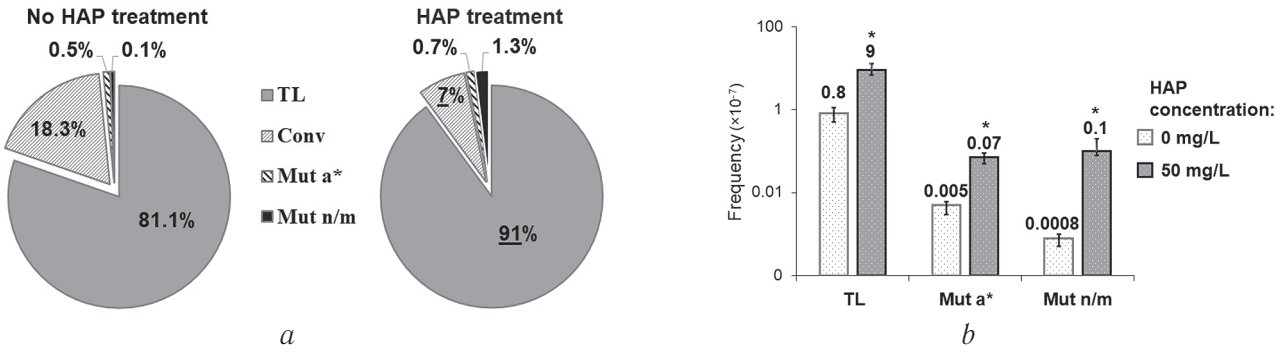


Fig. 4. Ratio of heritable and nonheritable genetic changes induced by HAP in percent (*a*) and frequency of inheritable and non-heritable in *MATα* locus (*b*) detected in "illegitimate" cytoduction.
Note: TL – Temporally lesions in *MATα* locus; Conv – Conversion between *MATα* locus and cassette *HMRa*; Mut a* – Mutations in *MATα* locus (both *MATα1* and *MATα2*, or in bidirectional promoter); Mut n/m – Mutations in *MATα* locus (*MATα1* or *MATα2*); * – significant difference between frequency of genetic events in the HAP treated and control variants, estimated in Mann–Whitney test (p-value < 0.0001); Underline – difference between proportion of genetic events in the HAP treated and control variants is statistically significant, according to Z-test

cassette (cytoductants of mating type *a*), on the contrary, decreased (Fig. 4, *a*).

The frequency of occurrence of "illegitimate" cytoductants of various phenotypic classes was estimated. HAP significantly induced the frequency of cytoductants *a** and n/m by a factor of 14 and 125 (Fig. 4, *b*), respectively, compared to the spontaneous level. Along with the expected result that HAP increased the frequency of gene mutations in one or both *MATα* genes by several orders of magnitude, it was found out that, under the action of HAP, the frequency of tempopary lesions at the *MATα* locus (mating type α cytoductants) increased by 11 times (Fig. 4, *b*). Thus, it can be concluded that primary DNA lesions induced by HAP can manifest itself phenotypically and lead to a temporary mating-type switching from α to a.

DISCUSSION

In this study, the sensitivity of the alpha-test in relation to the purine nitrogenous base analog HAP was evaluated. HAP treatment increased the frequency of inherited and non-inherited genetic material changes detected in the alpha-test. The results are consistent with the literature data that, in yeast, HAP induces only transitions and does not affect recombination [10, 12, 16, 19, 20]. The mutagenic effect of HAP revealed in the alpha-test is generally consistent with the results of other tests, for example, the test for the induction of forward mutations of resistance to canavanine at the same HAP concentration [11, 12, 15]. For the first time, it was demonstrated that HAP-induced primary DNA lesions can affect the cell phenotype and lead to the mating-type switching from α to a. The alpha-test has been proven to be an effective method for detecting such DNA base modifications.

Compared to other methods for genotoxicity testing, the alpha-test has a number of advantages. For example, the alpha-test enables to assess not only the frequency of primary DNA lesions but also trace them further after repair and determine quantitatively the ratio and frequency of inherited genetic material changes, such as gene mutations, conversion, recombination events, and loss of an entire chromosome III or its arm. Despite its high sensitivity and specificity, the alpha-test has some limitations like any genetic toxicology method. One of them is associated with the uneven ratio of classes of genetic events detected in the alpha-test. One of the most frequent genetic events detected in the alpha-test is the loss of an entire chromosome III as well as its right arm containing the *MAT*α locus [25, 26]. Thus, the proportion of hybrids lacking chromosome III, arising spontaneously or under the influence of genotoxic factors varies from 30% to 50% of the total number of "illegitimate" hybrids [2, 5, 6]. The loss of chromosomes or chromosomal fragments as well as recombination are caused by an abnormality of the cytoskeleton or fragmentation of chromosomes as a result of DNA breaks accumulation; therefore, the frequency of these events in cells increases by factors that affect the function of microtubules or lead to single- and double-stranded

DNA breaks. In yeast, unlike *E. coli* and humans, the HAP incorporation into DNA during replication does not further lead to the accumulation of breaks and does not induce chromosomal abnormalities, as yeast does not have enzymes capable to recognize and remove HAP from DNA. Considering these facts and the experience with using the alpha-test to assess the activity of mutagens with a different mechanism of action, it can be concluded that the alpha-test is more sensitive to mutagens that can induce DNA breaks. This study proposes to use a number of modifications of the alpha-test method to improve the sensitivity of the test system to detect factors causing gene mutations, recombination, and conversion, whose relative frequency in the total number of abnormalities detected in the alpha-test is lower than the frequency of chromosome III or its arm loss

To increase the sensitivity of assessing the frequency of the occurrence of relatively rare genetic material abnormalities, using a selective medium for the selection of "illegitimate" hybrids in the "illegitimate" hybridization test, which excludes the growth of hybrids devoid of chromosome III, is suggested. Such a medium should not contain histidine, which is needed for growth of strains, carrying the *his4* mutation, a marker for the chromosome III left arm. In this case, the high frequency of chromosome III loss will not negatively affect the detection of other rarer events. When using a medium without histidine and threonine (*thr4* is a marker of the right arm of chromosome III), the range of events detected in the "illegitimate" hybridization test will be narrowed to the classes "mutations and temporary lesions" (α His^+ Thr^+) and "conversion between *MATα* and *HMRa*" (n/m His^+ Thr^+). The modifications of the proposed testing method can be used in accordance with the objectives of a particular study and the specificity of the mutagenic action of the factors under study.

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❀ Authors and affiliations

Anna S. Zhuk – PhD, Researcher, Laboratory of Genomic Diversity, International Laboratory of Computer Technologies, ITMO University, Saint Petersburg, Russia. SPIN: 2223-5306. E-mail: ania.zhuk@gmail.com.

Elena I. Stepchenkova – PhD, Head of Laboratory of Mutagenesis and Genetic Toxicology, Vavilov Institute of General Genetics Russian Academy of Science, St. Petersburg Brunch, Saint Petersburg, Russia; Assistant, Department of Genetics and Biotechnology, Saint Petersburg State University, Saint Petersburg, Russia. SPIN: 9121-7483. E-mail: stepchenkova@gmail.com.

Sergey G. Inge-Vechtomov – Doctor of Science, Director, Vavilov Institute of General Genetics, Russian Academy of Science, St. Petersburg Brunch, Saint Petersburg, Russia; Professor, Department of Genetics and Biotechnology, Saint Petersburg State University, Saint Petersburg, Russia. SPIN: 3743-7626. E-mail: ingevechtomov@gmail.com.

❀ Информация об авторах

Анна Сергеевна Жук — канд. биол. наук, научный сотрудник, научно-образовательный центр геномного разнообразия международного научного центра компьютерных технологий, Университет ИТМО, Санкт-Петербург. SPIN: 2223-5306. E-mail: ania.zhuk@gmail.com.

Елена Игоревна Степченкова — канд. биол. наук, заведующая лабораторией мутагенеза и генетической токсикологии, Институт общей генетики им. Н.И. Вавилова Российской академии наук, Санкт-Петербургский филиал, Санкт-Петербург; ассистент, кафедра генетики и биотехнологии, Санкт-Петербургский государственный университет, Санкт-Петербург. SPIN: 9121-7483. E-mail: stepchenkova@gmail.com.

Сергей Георгиевич Инге-Вечтомов — д-р биол. наук, директор института, Институт общей генетики им. Н.И. Вавилова Российской академии наук, Санкт-Петербургский филиал, Санкт-Петербург; профессор, кафедра генетики и биотехнологии, Санкт-Петербургский государственный университет, Санкт-Петербург. SPIN: 3743-7626. E-mail: ingevechtomov@gmail.com.