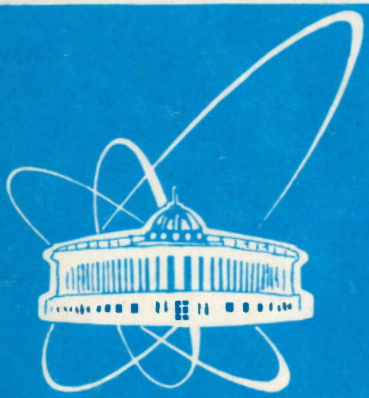

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CHARACTERISTICS OF SPONTANEOUS REVERTANTS IN HAPLOID YEAST

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INTRODUCTION

Earlier it was reported that reversion rate in adenine-auxotrophic (Iljina *et al.*, 1985) and leucine-auxotrophic (Chepurnoy *et al.*, 1989) haploid yeast increases with decreasing concentration of the above metabolites in the medium. In *Ade*$^-$-yeast the appearance rate of *Ade*$^+$-revertants in the logarithmic phase of growth was constant as the adenine concentration varied from 1 to 100 mg·l^{-1}. After transfer to a medium without adenine, or during the stationary phase of growth at low initial concentration of adenine (when the medium is depleted of this metabolite) the reversion rate increased by a factor of 15–150 (for different strains) in the case of intragene suppressors (locus revertants,"L") and by a factor of 2–3 in the case of intergene suppressors (suppressor revertants, "S"). In *Leu*$^-$-yeast the rate of both types of reversion decreased with leucine concentration increasing from 3 to 300 mg·l^{-1} during the logarithmic phase of growth.

Previously we ascribed this difference to different effect produced by the metabolites in question on growth of yeast cultures. However, a desire to know the *Leu*$^+$-reversion rate during residual growth of *Leu*$^-$-yeast on a selective medium (i.e. without leucine) led to a number of experiments, their results being given below.

MATERIALS AND METHODS

Strains

The strains used were the same as in the previous paper (Korogodin *et al.*, 1991): haploid yeast *Saccharomyces cerevisiae*, strain P–192 *(a ade2-192)* from Prof. I.A.Zakharov (PINP) and strain NA3–24 *(a leu2-1 lys1-1)* from A.I.Chepurnoy (JINR). Mutation *ade2-192* seems to be missense and mutations *leu2-1* and *lys1-1* nonsense. Revertants, appeared after transferring the growing cultures to the selective medium,were analyzed.

Media

Full and minimum nutrient media were used (Zakharov *et al.*, 1984). In experiments with strain P-192 1, 10, or 100 mg·l^{-1} of adenine were added to the minimum medium. In experiments with strain NA3-24 3, 30, or 300 mg·l^{-1} of leucine and 30 mg·l^{-1} of lysine were added to the minimum medium. A selective medium was the same minimum one without the respective limiting metabolite(s).

Cultivation

Yeast was grown on nutrient media applied to nuclear micropore filters (Floroff, 1984). After incubation on initial media the number of cells in a colony was found and they were transferred together with the filters to a selective medium. Some experiments had alternate versions: without crushing initial colonies before transferring them to the selective medium and with crushing each colony separately; in the latter case "multiple" and "singular" secondary colonies of revertants grow on smears of initial colonies (Korogodin *et al.*, 1989). Crushing allows one to separate rever-

tants that managed to produce several cells before their transfer to the selective medium and revertant represented by a single cell. Incubation on the selective medium went on until revertant colonies stopped appearing completely, which was not more than 21 days long. During incubation on the selective medium the appearing revertant colonies (as large as $6 \cdot 10^5$ cells/col.) were daily registered and then differentiated into intragene (L) and intergene (S) suppressors.

Differentiation of revertants

The analysis of *Ade*$^+$-revertants was based on the need for adenine and on the colour of revertant colonies. It was found in the experiments that on the media employed L-revertants form white colonies and S-revertants form pink ones (Korogodin *et al.*, 1989). The gene screening check of the fact gave coincidence in 98% (Korogodina *et al.*, 1988a).

The analysis of *Leu*$^+$-revertants was based on the need for metabolites. The revertants were transferred to (1) a minimum medium with lysine without leucine, (2) a minimum medium with leucine without lysine, (3) a minimum medium without lysine and leucine. Revertants growing on medium (1) were considered intragene suppressors (L) and those growing on all three media were considered intergene suppressors (S).

Statistics

In various version of the experiment with strain P–192 we used from 30 to 100 Petri dishes to get about 100 revertant colonies for each version. In experiments with strain NA3–24 about 20 Petri dishes were used for each group. 220 colonies were inoculated in each Petri dish. Registration results for NA3–24 revertants were normalized to 20 Petri dishes, and these data are given below.

Mathematical processing

The data were processed in a PC AT/286 computer. The processing allowed us to trace appearance of revertants in time (before or after transfer to the selective medium) and to know specific growth features of different

revertants on media with or without limiting metabolites.

We considered the reversion process to be related to DNA replication (Korogodin *et al.*, 1991). The formula to find the number of revertants appearing in a population of multiplying cells was

$$M_t = r \cdot n \cdot 2^{t/\tau_o}, \qquad (1)$$

where M_t is the number of revertants by the moment of time t, r is their appearance rate, n is the original number of cells of the initial strain (in this case $n = 1$), τ_o is the cell cycle duration for initial cells.

A derivative of (1) is formula (2) used to determine appearance time t_i of the i-th revertant (from the moment of plating auxotrophic cells onto the nutrient medium):

$$t_i = \frac{ln(i/r) \cdot \tau_o}{ln2} - t_o, \qquad (2)$$

where t_o is the time for one cell to multiply to the number of cells plated.

Conventional division time (CDT) was a quantity often used in processing the data by appearance dynamics of revertants. It is the mean duration of the revertant cell cycle from the moment of appearance till the moment of detection. It was found that one can detect a revertant colony of $6 \cdot 10^5$ cell and more by the naked eye. CDT was calculated from the experimental revertant appearance curve, by formulae (1) and (2) and by the Monte Carlo method.

Different appearance time of revertants on selective media can be caused by (1) different appearance time of revertants at the same rate of their multiplication, (2) appearance of revertants only on the initial medium with different rate of their multiplication, (3) different appearance time of revertants and different rate of their multiplication on the initial and selective media. Formulae (1) and (2) and the Monte Carlo method allow these situations to be simulated and compared with the experimental data.

RESULTS

Appearance dynamics of revertants

At first we shall see what is the effect of crushing colonies before their transfer to the selective medium on appearance of NA3–24 revertants (P–192 data were published earlier, see Korogodin *et al.*, 1989). The experimental results are given in Table 1. We see that crushing does not practically affect appearance of revertants, at least after pre-growing yeast on media with 3 and 30 mg·l^{-1} of leucine. As the leucine content of the medium increases, the yield of revertants decreases by a factor of about 2.

In Fig. 1 there are appearance histograms for P–192 and NA3–24 revertants on selective media. We can see the following distinctions: (1) appearance of revertants in strain P–192 is finished on the 12th day, in NA3–24 on the 18th day; (2) L-revertants make up about 40% of all revertants in P–192 and about 70% in NA3–24; (3) the appearance maximum for revertants of both types is in the first half of incubation time (i.e. the first 6 days) for strain P–192 and in the second half (i.e. the last 9 days) for NA3–24.

Distinctions between the strains become even greater when we compare yield dynamics of multiple and single L-revertants (Fig. 2). The yield of multiple revertant is 5% for strain P–192 and 35% for NA3–24.

The data in Fig. 2 question the reliability of multiplicity or singleness as a criterion for appearance time of revertants. Indeed, in most cases multiple colonies of revertants indicate that initial revertants have already appeared and managed to divide before crushing. Imitation of multiple revertants by several single ones independently appearing on the same smears of primary colonies may work only if the number of initial colonies with single revertants exceeds 20%, which was hardly ever observed in the experiments. As to single colonies, their number is the sum of those appearing on the selective medium (i.e. after crushing) and those appearing on the initial medium (before crushing) but failing to divide. Single revertants may arise from potentially multiple revertants consisting of two–four cells, if the cells merged into a single colony after crushing. Yet, the number of

colonies like that seems to be small. So, the number of single revertants appearing on the initial medium can scarcely be more than two times larger than the number of multiple ones. The data (Fig. 2) about the appearance time of most (over 90%) single P–192 revertants are beyond question for their number is 20 times as large as that of multiple revertants and they are formed during the residual growth of the culture on the selective medium. As to the appearance time of single NA3–24 revertants, which are only twice as numerous as multiple ones, this issue demands special analysis.

Appearance time of single NA3–24 revertant colonies

With P–192 and other earlier described *Ade*$^-$ strains (Korogodin *et al.*, 1989, 1990, 1991), where the number of single L-revertant colonies exceeds that of multiple ones by an order and a half or two orders of magnitude, it is perfectly clear that the great majority of those colonies grow from revertants appeared after crushing, i.e. on the selective medium.

Strain NA3–24, where single L-revertants are about twice as numerous as multiple ones (Fig. 2), is a different case. To examine appearance time of single revertants in this strain we employed four independent approaches. They were to compare curves of accumulated frequencies for multiple and single L-revertants; to determine growth curves for L-revertants on a medium with leucine and on the selective medium; to simulate mathematically the yield of multiple and single L-revertants with their cells multiplicating at different rate. The fourth approach was to analyze multiple revertants appearing on the selective medium at different time.

Let us consider curves of accumulated frequencies for appearance of L-revertants after initial cells growing on media with 3, 30, 300 mg·l^{-1} of leucine during the logarithmic phase of growth (Fig.3). We see that in all three cases the curves for multiple and single revertants are very close and attain 100% at the same time. Similar data were obtained for cultures incubated on leucine media up to their stationary phase of growth. It means that the appearance time of multiple and single revertants on the selective medium is approximately the same ranging from 2 to 20 days of incubation. Taking into account the fact that all multiple revertants came

into being before transfer to the selective medium, one can assume that at least the majority of single revertants were formed at the same time.
The growth rate τ_i of multiple and single revertant cells on the initial medium (with 3 mg·l^{-1} of leucine) was almost the same: $\tau_i \simeq (2.9 \div 6.4)$ h^{-1}. It is smaller than for original auxotrophic cells (about 7.2 h^{-1}). However, bearing in mind that under ordinary conditions revertants are not isolated but grow in close contact with a colony of auxotrophic cells, one can assume that the growth rate of both will be almost the same approaching 7.2 h^{-1}. Finally, on the basis of (1) and (2) with the Monte Carlo method we calculated the number of multiple and single revertants on the assumption that single revertants are also those that failed to divide before our crushing the initial colonies. As shown in Table 2, the calculation results are in good agreement with the experimental data.
Good agreement between the three methods of determining appearance time of single NA3–24 revertants allows a conclusion that the great majority of both multiple and single revertants appear during cultivation of auxotrophic cells in medium with leucine before transfer to the selective medium. It could be expected if reversion in strain NA3–24 would be associated with the S-phase of the cell cycle and the residual growth on the selective medium would be small. The former assumption was specially checked with cells of this strain and turned out to be in good agreement with the experimental results (Chepurnoy, 1989; Korogodin *et al.*, 1991). The results of checking the latter assumption are given in Table 3 and are also in good agreement with it: on the medium with 3 mg ·l^{-1} of leucine, where the yield of L-revertant is the largest, there is no residual growth within the experimental error.
All this is very important for the analysis of cell reversion process in strain NA3–24. The fact that both multiple and single revertants in this strain are formed only on the initial medium, i.e. on a medium with leucine, means that calculation of reversion rates must involve the sum of revertants of both types rather than each group independently, as in the case of strain P–192 and the like. Hence two questions arise: (1) what causes difference in the yield of revertants (especially L-type) in this strain during the

logarithmic phase of growth on media with different leucine concentration (remember that there is no difference like this for cells of *Ade*$^-$ strains)? and (2) why appearance of these revertants on the selective medium is too long, up to 20 days? We shall try to answer both questions.

Dependence of the Leu$^-$ yeast revertant yield on the leucine content of the medium

The yield of NA3–24 L- and S-revertants transferred to the selective medium in the logarithmic and stationary phases of growth as a function of the leucine concentration in the medium is shown in Table 4. The data on single and multiple revertants are united. The rate of registered L- and S-revertants is seen to decrease with increasing leucine concentration, which was first described by Chepurnoy *et al.*, (1989).[1] There arises a question if this increase is caused by the leucine concentration or by anything else.

Noteworthy are the data in Table 4 about cultures transferred to the selective medium in the logarithmic and stationary phases of growth. For all three initial concentrations of leucine in the medium the reversion rates in the stationary phase are smaller than in the logarithmic one. But the most important is that it is accompanied by a reliable decrease in the absolute number of cells in colonies of L-revertants. Note that transition from the logarithmic to the stationary phase at 3 mg·l^{-1} of leucine is followed by practically complete disappearance of leucine from the medium. At 30 mg·l^{-1} its concentration decreases, and at 300 mg·l^{-1}, i.e. with a 10-fold excess over the norm, its concentration varies but slightly. Nevertheless, in all three cases we have a great decrease in the absolute number of revertants appeared.

The NA3–24 data (Table 4) and P–192 data (Korogodin *et al.*, 1991) are compared in Fig. 4. Exactly opposite situations are observed in these strains. Transition to the stationary phase in P–192 is attended by a

[1] Difference in reversion rates given in Table 4 and published by Chepurnoy *et al.*, (1989), Korogodin *et al.*, (1991) results from difference in revertant registration techniques: we registered all revertants that grew in 20 days while in earlier publications only the revertants that grew in the first 6 days were registered.

sharp increase in the number of revertants appeared at initial adenine concentrations of 1 and 10 mg·l^{-1} while at its excess concentration (100 mg·l^{-1}) the number of revertants appeared in both phases is approximately the same. On the other hand, in NA3–24 much fewer revertants appear after the stationary phase than after the logarithmic one at any of the three leucine concentrations (3, 30, 300 mg·l^{-1}).

It is natural to associate these results with different causes. In the first case it is an increase in mutability of the *ade2–192* gene after the medium ran out of adenine. In the second case it is a decrease in revertant appearance with increasing dimensions of initial auxotrophic colonies.

The latter conclusion can be checked by independent processing of our experimental results. Table 4 was used to plot the number of revertants appeared versus the number of cells in initial auxotrophic colonies in Fig.5. By conventions, the number of revertants appeared after the logarithmic phase at 3 mg·l^{-1} of leucine was taken to be 1.00. The part of revertants appeared is seen to decrease monotonously with increasing number of cells in colonies irrespective of whether it is attended by an increase (in different groups) or a decrease (in transition to the stationary phase) in the leucine content of the medium. The minimum appearance of revertants is 10% of the initial number.

Thus, the increase in the yield of revertants with decreasing leucine concentration in the medium observed in the logarithmic phase of growth can be considered to result from an increase in the part of revertants appearing in subsequent incubation on the selective medium rather than from an increase in their appearance rate. It is clearly seen in Fig.6, where appearance of revertants after incubation on media with different leucine concentrations is normalized to the same number 10^6 of initial cells in colonies and the results are expressed in terms of the revertant number distribution over the CDT.

Causes of difference in time of revertant appearance on the selective medium

The causes of difference in time of revertant appearance on the selective medium are best analyzed with strain NA3–24 grown on a medium with 3 mg·l^{-1} of leucine for in this case its revertant formation time is no longer than 54 hours (the end of the logarithmic phase) and the appearance time is as long as 20 days. We use the above leucine concentration because it allows the maximum appearance of revertants (Fig.5).

Let us consider two possible causes of so long revertant appearance. One cause is uniform (or random) distribution of logarithmic-phase revertants in CDT (it will be recalled that CDT is the mean duration of a cell cycle for the given revertant from the moment of its formation to the moment of its appearance). The other cause is nonuniform distribution of revertants over different CDT values. In Fig.7 there are results of simulation of the former situation. The curve in Fig.7A shows accumulation of revertants in a growing yeast population. Fig.7B shows random distribution of revertants in CDT. The appearance curve in Fig.7C corresponds to this distribution. Comparing Fig.7B, C with Fig.6A and Fig.1C, where the experimental results are shown, we see that are not similar at all. Thus, we can reject the simplest assumption that the newly formed revertants are randomly (uniformly) distributed in CDT.

For more detailed study of revertants growing in different time, 128 revertants randomly chosen from among those appearing on the selective medium within 20 days were plated on the medium with 3 mg·l^{-1} of leucine. Appearance time of colonies, their morphology and trend to form secondary colonies (or sectors) were registered for each revertant. Then 27 revertants with different appearance time were randomly chosen from among the above 128 ones and additionally analyzed. We determined the curves of their growth on the medium with 3 mg·l^{-1} of leucine and on the medium without leucine, morphology of colonies on both media, appearance time of colonies after plating individual cells and after preliminary implantation of 1–5 revertant cells in print with about 10^4 initial auxotrophic cells. In the latter case the suspension with revertants and auxotrophic cells in

the ratio $1:10^4$ was inoculated onto a nutrient medium. These prints were made on the selective medium and on a medium with leucine where they were crushed either immediately or 24–48 hours later and then transferred to the selective medium. The results were as follows.

All 128 revertants can be classified by their colony morphology into three groups: (1) large uniform light-creamy colonies with a slightly darker top, (2) medium and small light colonies, (3) colonies of different size and colour from small and brown to large and light. In subsequent replating colonies of the first two groups kept their features while colonies of the third group either reproduced their phenotype or produced heterogeneous colonies again.

The curves in Fig. 8 show distribution of the colonies of the three groups in appearance time. We see that the appearance maximum falls on the 5th–7th day for the first group and on the 13th–15th day for others. Noteworthy is the distribution "tail" for the first group extending to the end of the observation time. The main cause for it may be fast growing secondary colonies entering this group, especially at later time. They are formed in primary small colonies of the 3rd group and imitate the 1st group colony phenotype.

On determining the curves of revertant growth on the full and selective media we found two things (Fig.9). First, growth curves of all revertants have a lag phase from 8 to 30 hours long for the selective medium and do not have it for the medium with leucine; slopes of logarithmic phase sections of the growth curves differ but slightly for both media while the values of τ_j vary from 2.9 to 6.4 h^{-1}. Secondly, neither the lag phase duration nor the multiplication rate of revertant cells correlated with their appearance time. The lag phase in the case of the selective medium might only mean that the revertant cell are less competitive under these conditions, which was also indicated by the brown colour of many colonies (it usually results from accumulation of dead cells in a colony).

Finally, an experiment on analysis of multiple revertants appeared on the selective medium in different time shows the following. To carry out

this experiment, we mixed initial auxotrophic cells with an insignificant number of multiple revertant cells, five of which appeared early (in 3–5 days) after transfer to the selective medium and the other five appeared late (in 17–20 days). The results showed that appearance time of these revertants plated together with auxotrophic cells is longer indeed as compared with revertants, especially those of the second group, plated alone. The difference in revertant appearance time for both groups is as large as 1–2 weeks, and the revertants appeared are greatly different in morphology. Early appeared colonies of five revertants belong to the 1st group in phenotype, late appeared ones belonged to the 3rd group (see Fig.8). All this strongly suggests that different appearance time of these revertants result from genetic distinctions manifesting themselves in their competitiveness rather than from different reversion time of auxotrophic cells.

The fact that there is no strict correlation in appearance time for revertants registered immediately after their appearance and in subsequent experiments can have the following explanation. First, this time greatly depends on the location of revertant cells in colonies of initial auxotrophic cells. Secondly, more highly competitive secondary mutants, especially frequent among revertants of the 3rd group (the most numerous one), inevitably broke up the order of their appearance.

Thus, different appearance time of NA3–24 yeast L-revertants results mainly from genetic heterogeneity of the revertants and higher secondary mutation rate of those which were least competitive from the very beginning and thus had the largest appearance time. However, it does not apply to all auxotrophic strains of yeast. For example, P–192 revertants, especially of L-type, are more competitive than initial auxotrophic cells, have high morphological homogeneity and relatively short appearance time of only 2–4 days since the moment of formation.

DISCUSSION

We would like to discuss the above material from three points of view.

First, it is the effect produced by different concentrations of essential

metabolites on the mutation rate of genes that control their synthesis. While the sharp increase in mutability of the ade2 locus in a medium without adenine is proved (Korogodin *et al.*, 1990), the similar conclusion drawn earlier for leucine (Korogodin *et al.*, 1991) has to be rejected.

A decrease in the yield of leucine revertants with increasing leucine content of the medium results mainly from decreasing appearance of less competitive revertants, which becomes especially pronounced with increasing number of cells in initial auxotrophic colonies. A vivid example is furnished by Fig.6; it is clearly seen that an increase in the size of initial colonies is first of all followed by the disappearance of those distribution curve maxima that correspond to the majority of mutants characterized by low competitiveness and late appearance (compare Fig.6 and Table 4).

Secondly, the latter phenomenon might have the same cause as the extended appearance time of NA3–24 revertants on the selective medium. This cause is great heterogeneity of revertants in competitiveness (Andreadis *et al.*, 1984; Brisco and Kohlhaw, 1990). As the leucine content of the medium and the size of initial colonies increases, the lag of most revertants possibly grows so long that they might fail to appear because of depletion and/or drying of the medium in Petri dishes but start appearing as soon as they are transferred to a fresh selective medium.

Thirdly, and finally, a possible cause of so high heterogeneity of the revertants in this strain needs discussing. It is likely to be the large number of sites in the leu2 gene, their mutations functioning as intragene suppressors (Jones and Fink, 1982; Kohlhaw, 1983; Sherman, 1982). Though partly restoring synthesis of leucine, most of these mutations produce strong pleiotropic effect, which reduce competitiveness of the respective revertants. It gives rise to great morphological heterogeneity of these revertants. Additional mutations in these cells may neutralize the negative effect of primary mutations responsible for reversion and thus contribute to partial or full repair of the initial phenotype. It manifests itself in wide diversity of colonies growing in re-plated small primary colonies of late appearing revertants. It allows an assumption that appearance time (or its derivative CDT) is approximate representation of how sites whose muta-

Table 1. Effect of crushing initial colonies on the yield of NA3–24 revertants (registered 21 days after transfer to the selective medium)

Leucine content, mg·l^{-1}	Yield of revertants (L+S), $\times 10^{-7}$	
	Noncrushed colonies	Crushed colonies
3	8.6 ± 1.1	7.9 ± 1.3
30	4.9 ± 0.8	4.3 ± 0.7

Table 2. Mathematical simulation results (A) compared with the experimental data (B) on the number of multiple and single L-revertants appearing on a medium with 3 and 30 mg ·l^{-1} of leucine in the logarithmic phase of growth. Strain NA3–24.

Medium	3 mg ·l^{-1} of leucine		30 mg ·l^{-1} of leucine	
Index	A	B	A	B
Culture age, h	53.98	54.00	22.02	22.00
Number of mult. revert.	325	325	356	356
Number of single revert.	595	595	491	472

Table 3. Residual growth of NA3–24 yeast on the selective medium after pre-growth on a medium with 3 mg ·l^{-1} of leucine

Culture age, days	Number of cells in colony, $\cdot 10^5$		Residual growth coefficient*
	Initial	Final	
0.8	0.92	0.55	0.60
7.0	2.3	1.9	0.82
7.0	5.5	6.5	1.20
11.0	2.0	2.8	1.40
13.0	1.9	2.5	1.32
15.0	4.0	6.7	1.68
21.0	2.7	2.8	1.02
Mean with error			1.15 ± 0.14

* The residual growth coefficient is the final number of cells in a colony divided into the initial one.

tion produces a suppressing effect (L-suppressors) are distributed over the *leu2* gene.

Also, the same cause (i.e. multiplicity of sites for intragene suppression of leucine synthesis violation) might be responsible for higher appearance rate of NA3–24 L-revertants as compared with adenine-auxotrophic strains from the *ade2* group. For example, in the logarithmic phase of growth the appearance rate of NA3–24 L-revertants is about 6.3×10^{-7} per cell per division, while for strain P–192 it is $6.6 \cdot 10^{-10}$, i.e. by three orders of magnitude smaller. It is of interest that mutation rates of intergene suppressors (S-revertants) are close in both cases, being $1.5 \cdot 10^{-7}$ for NA3–24 and $(0.1–0.6) \cdot 10^{-7}$ for stains from the *ade2–* group (Korogodin *et al.*, 1990). It may be related to different functions of the adenine nucleotide and leucine amino acid in cell life (Kunz, 1982; Satyanarayana *et al.*, 1968).

Table 4. Appearance rates and absolute number of NA3–24 locus (L) and suppressor (S) revertants formed in the logarithmic and stationary phases of growth on media with different leucine concentrations.

Leucine mg·l^{-1}	Growth phase	Number of cells in col.	Revertants (type)	Rate ·10^{-8}	Number of col. of revertants		Appear. coef.**
					Regist.	Expect.*	
3	Logar. (54 h.)	$3.3 \cdot 10^5$	L	63.5	921	921	1.00
			S	15.3	223	223	1.00
	Stat. (96 h.)	$5.5 \cdot 10^5$	L	34.2	822	1537	0.53
			S	8.5	206	371	0.56
30	Logar. (22 h.)	$9.7 \cdot 10^5$	L	19.4	828	2710	0.31
			S	5.1	216	656	0.58
	Stat. (96 h.)	$2.3 \cdot 10^6$	L	7.3	734	6425	0.11
			S	3.4	342	1554	0.22
300	Logar. (22 h.	$1.3 \cdot 10^6$	L	14.1	803	3631	0.22
			S	4.6	261	879	0.30
	Stat. (96 h.)	$2.1 \cdot 10^6$	L	6.7	616	5867	0.10
			S	3.9	355	1419	0.25

* Rates of L- and S- revertants appearance in all groups are supposed to be equal to the rates in the logarithmic phase onto the medium with 3 mg·l^{-1} leucine.

** Ratio of the registered number of revertants to the expected one.

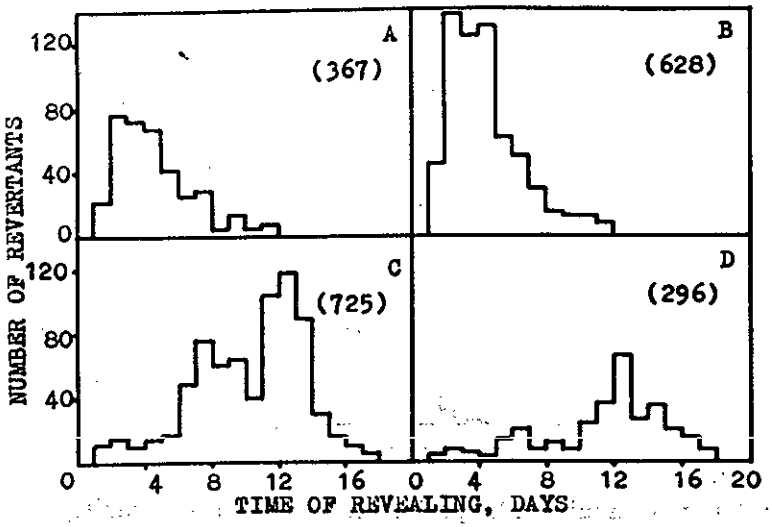

Fig. 1.

Appearance of locus (A, C) and suppressor (B, D) revertants in strains P–192 (A, B) and NA3–24 (C, D) on the selective media after 30-hour incubation on a medium with 10 mg·l^{-1} of adenine (A, B) and after 48-hour incubation on a medium with 3 mg·l^{-1} of leucine (C, D). The cultivation conditions are such that approximately the same total number of revertants (numbers in parentheses) appear in both strains.

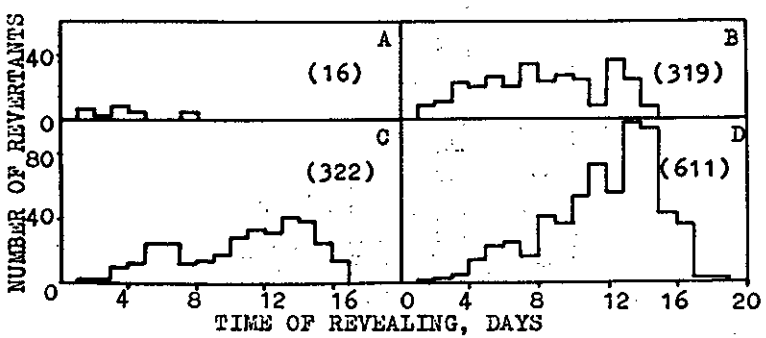

Fig. 2.

Appearance of multiple (A, C) and single (B, D) locus revertants in strains P–192 (A, B) and NA3–24 (C, D) on the selective media under the same cultivation conditions as in Fig.1. In parentheses is the number of revertants.

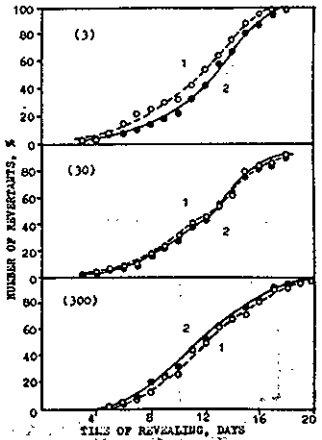

Fig. 3.

Curves of accumulated frequencies for appearance of multiple (1) and single (2) locus revertants in strain NA3–24 after incubation during the logarithmic phase of growth on media with different concentration of leucine (number in parentheses is mg·l^{-1}).

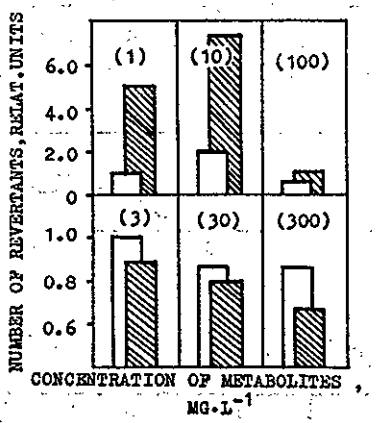

Fig. 4.

The number (relative units) of locus revertants appeared on selective media after incubation during the logarithmic (open bins) and stationary (hatched bins) phases of growth on media with different concentration of adenine or leucine (number in parentheses are mg·l^{-1}). The top row is strain P–192, the bottom row is NA3–24.

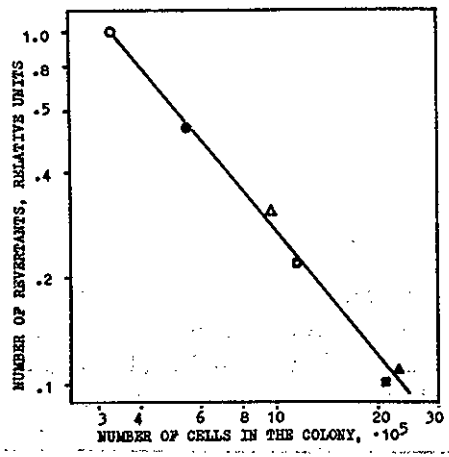

Fig. 5.

Part of NA3–24 locus revertants appeared on the selective medium after pre-incubation during the logarithmic phase of growth (open symbols) and the stationary phase (filled symbols) on media with different concentration of leucine. Circles, triangles and squares are 3, 30, 300 mg·l^{-1} of leucine respectively.

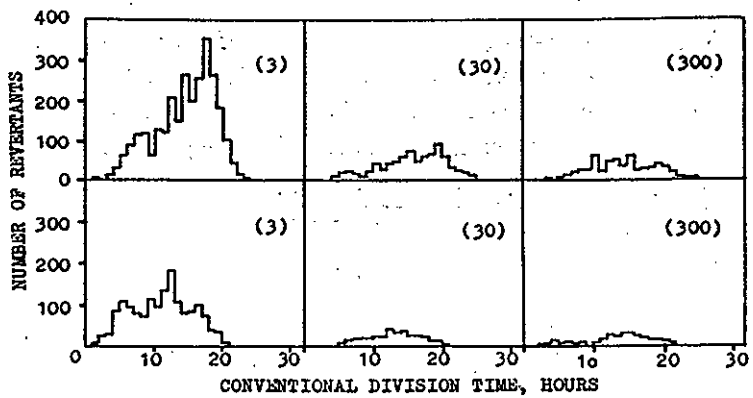

Fig. 6.

CDT distribution of revertants appeared after the logarithmic (top row) and stationary (bottom row) phases of growth on media with different concentration of leucine (number in parentheses is mg·l^{-1}). Normalization to 10^6 cells in an auxotrophic colony.

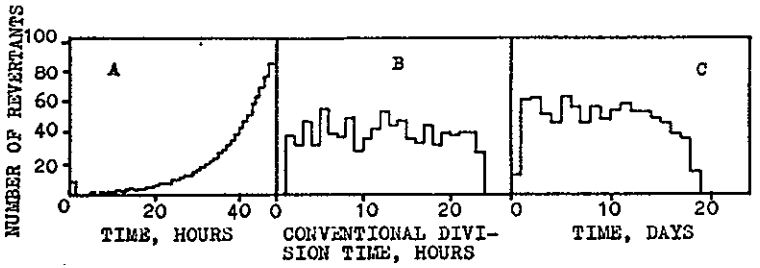

Fig. 7.

Accumulation of revertants in a growing culture (A), CDT values for these revertants (B) and their appearance dynamics (C) for strain NA3-24 after 46-hour growth on a medium with 3 mg·l^{-1} of leucine. The reversion rate is taken to be $6 \cdot 10^{-7}$. Simulation results.

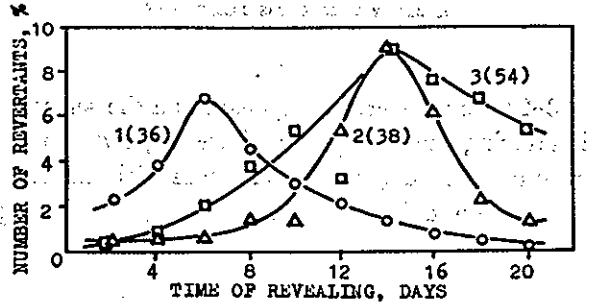

Fig. 8.

Appearance of NA3–24 locus revertants of different groups on the selective medium (see the text), as follows from the analysis of randomly selected 128 revertants appeared in 20 days after 46-hour incubation on a medium with 3 mg·l^{-1} of leucine. Curves 1–3 correspond to revertants of groups 1, 2, 3. In parentheses is the number of revertants of each group.

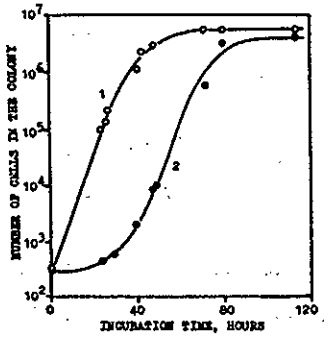

Fig. 9.

Growth curves for one of the NA3-24 revertants on a medium with 3 mg·l^{-1} of leucine (1) and on the leucine-free selective medium (2). The data of a typical experiment.

References

Andreadis,A., Hsu, Y.-P., Hermodson, M., Kohlhaw, G., and Schimmel, P. (1984). Yeast *LEU2*. Repression of mRNA levels by leucine and primary structure of the gene product. *J.Biol. Chem.* **259**, 8059-8062.

Brisco, P.R.G. and Kohlhaw, G.B. (1990). Regulation of yeast *Leu2 J.Biol. Chem.* **265**, 11667-11675.

Floroff, G.N. (1984). Shynthesis of superheavy elements and applications of the methods of nuclear physics in the related areas. *Vestnik Akad. Nauk SSSR* **4**, 35-48.

Chepurnoy, A.I., Mikhova-Tsenova, N., Bruntskova, H. (1989). Spontaneous mutations of *leu2* gene in *Saccharomices cerevisiae*. *Genetika* **25**, 1952-1959.

Ilyina, V.L., Korogodin, V.I. and Fajszi, Cs. (1985). Effect of adenine concentration in the medium on the frequency of reversions in haploid yeast. *Genetika* **21**, 1643-1649.

Jones, E.W. and Fink, G.R. (1982). Regulation of amino acid and nucleotide biosynthesis in yeast. In Strathern J.N., Jones E.W. and Broach J.R.(eds.): *The molecular biology of the yeast Saccharomyces. Metabolism and gene expression*. New York, Cold Spring Harbor Laboratory, 230-239.

Kohlhaw, G.B.(1983). Regulation of leucine biosynthesis in lower eukaryotes. In Herrmann K.M. and Somerville R.L.(eds.): *Amino acids biosynthesis and genetic regulation*. London-Amsterdam-Ontario-Sidney-Tokio, Addison-Wesley publishing company, 285-300.

Korogodin, V.I., Abetyan, N.O., Brunckova, H., Janpoladyan, N.L., Korogodina, V.L., Mikhova-Tsenova, N., Simonyan, N.V., Fajszj, Cs. and Chepurnoy,A.I. (1989). Spontaneous mutagenesis and cell cultivation conditions. In *Ontogenesis, Evolution, Biosphere*. Nauka, Moskow, 59-71.

Korogodin, V.I., Korogodina, V.L. and Fajszi, Cs. (1990). Mutability of genes depends on their functional state - a hypothesis. *Biol. Zent. bl.* **109**, 447-451.

Korogodin, V.I., Korogodina, V.L., Fajszi, Cs., Chepurnoy, A.I., Mikhova-Tzenova, N., Simonyan, N.V. (1991). On the dependence of spontaneous

mutation rates on the functional state of genes. *Yeast* 7, 105-117.

Korogodina, V.L., Koltovaya, N.A., Lyubimova, K.A., Korogodin, V.I. and Fajszi, Cz. (1988). Estimation of the contribution of double mutants to the observed spectrum of revertants, occuring in auxotrophic yeast. *JINR Communication*, P19-88-835, Dubna.

Kunz, B.A. (1982). Genetic effects of deoxyribonucleotide pool imbalances. *Environmental Mutagenesis* 4, 695-725.

Satyanarayana, T., Umbarger, H.E. and Lindegren, G. (1968). Biosynthesis of branched-chain amino acids in yeast: regulation of leucine biosynthesis in prototrophic and auxotrophic strains. *J.Bacteriol.* 96, 2018-2024.

Sherman, F.(1982). Suppression in the Yeast *Saccaharomyces cerevisiae*.In: In Strathern J.N., Jones E.W. and Broach J.R.(eds.): *The molecular biology of the yeast Saccharomyces. Metabolism and gene expression.* New York, Cold Spring Harbor Laboratory, 463-485.

Zakharov, I.A., Kozhin, S.A., Kozhina, S.A. and Fyodorova, I.V. (1984). *Manual and Methods in the Genetics of Yeast* Saccharomycetes. Nauka, Leningrad.

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