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K.A.Lyubimova, L.A.Melnikova, A.I.Chepurnoy

ADAPTIVE MUTAGENESIS IN *SACCHAROMYCES CEREVISIAE* AS RESULT OF MIS-REPLICATION OF DNA

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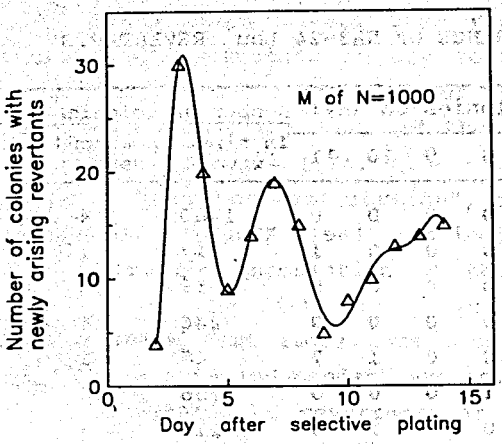

Fig. 1. Appearance curve for Leu^+ revertants in a selective medium with n = $2 \cdot 10^6$ cells in a colony. Abscissae: days after transferring colonies of NA3-24 strain cells on the selective medium without leucine. Ordinates: daily increase in the number of colonies with revertants from the sample of N = 1000 colonies.

based on the following experimental data. Gamma-irradiation of strain NA3-24 cells transferred to the selective medium resulted in increasing number of Leu^+-revertants in the second wave, while the number of revertants in the first wave did not change. Similar results were obtained after adding a small amount of leucine (2 mg/l) to the selective medium. Finally, gamma-irradiation of cells 24 hours before their transfer to the selective medium increases the number of revertants in the first wave, while the number of revertants in the second wave did not change. We can add another important argument to the above-mentioned ones.

Cells of original strain NA3-24 were grown in 20 test tubes filled with a liquid nutrient medium until there were $2 \cdot 10^7$ cells in 1 tube. Then the cells were precipitated in a centrifuge, the remaining medium was washed off, the cells were resuspended in a small amount of water and plated in separate Petri dishes with a leucine-free medium. The experiment was repeated three times, the results being the same. Table 1 shows the results of one of the experiments.

Table 1. TIME-DEPENDENT APPEARANCE OF NA3-24 Leu$^+$ REVERTANTS

Tube	No. of newly arising colonies on day: 2	3	4	5	6	7	8	9	10	11	Number of colonies: In first wave	In second wave
1	860	400	0	0	4	0	0	0	0	0	1260	4
2	13	4	0	0	0	1	0	0	0	1	17	1
3	6	5	0	0	2	1	0	0	0	0	11	3
4	180	66	0	0	1	0	0	0	0	0	246	1
5	34	11	0	0	0	1	0	0	1	7	45	1
6	140	116	0	0	3	1	1	0	0	0	256	5
7	98	15	2	0	0	3	0	0	1	0	115	3
8	60	13	0	0	1	0	0	1	0	8	73	1
9	45	11	0	0	2	0	0	3	0	3	56	2
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	2	0	1	0	0	0	0	3
12	96	104	0	0	1	0	0	0	0	0	200	1
13	550	218	3	0	0	0	0	0	0	0	771	0
14	0	40	8	0	0	1	0	1	2	0	48	1
15	45	0	0	0	2	0	0	3	4	0	45	2
16	14	6	0	0	0	0	1	0	0	1	20	1
17	190	64	0	0	0	0	0	0	0	0	254	0
18	600	178	0	0	3	1	0	0	1	0	778	4
19	15	5	0	0	0	0	0	1	1	0	20	0
Total	2946	1256	13	0	21	9	3	9	10	20	4215	33

	The first wave	The second wave	The third wave front		
				$\bar{x}$ =222	=1.74
				σ^2=117649	=2.31

The data obtained from 19 cultures are given. Cells of strain NA3-24 were grown in test tubes with a liquid nutrient medium from $2 \cdot 10^3$ to $2 \cdot 10^7$ cells. Then cells were washed with water, resuspended and plated on selective plates. Each number given represents the sum of newly arising colonies for each tube on a given day after plating.

These results show that for the first wave the mean number of revertants in one test tube ($\bar{x}_1$ = 222) is much smaller than the dispersion of the mean (σ_1^2 = 117649). For the second wave the mean and the dispersion of the mean have close values ($\bar{x}_2$ = 1.74, σ_2^2 = = 2.31). In the former case we have a "jack-pot distribution", described in experiments by LURIA and DELBRUCK (1943) and indicating that revertants were formed before inoculation of the selective medium. In the other case the distribution is close to the Poisson one, which shows that revertants occurred just in the selective medium as rare independent events.

Appearance of revertants in the second and third waves cannot be explained on the assumption that revertants of the second and third waves were formed before the transfer but showed up late because of small multiplication rate under the conditions of no selective advantages or because of large size of the colony, which made it difficult to detect revertants. It follows from a further set of experiments. Revertants of different morphology from the first, second and third waves (a total of 18 mutants) were individually mixed with cells of the original strain in the ratio of 1 revertant to $1 \cdot 10^4$ cells of the original strain in 1 inoculum. Plated in this way, the colonies were grown on a solid culture medium until the number of cells in a colony reached $3 \cdot 10^6$. Then they were transferred to the selective medium without leucine. The results of all experiments from this set unambiguously showed that there was no delay in appearance of all revertants from the three waves. All checked revertants were detected already in the first wave. The results of detecting 6 revertants from the second wave and 5 revertants from the third wave are given in Tables 2 and 3 respectively.

Thus, revertants from the second and third waves have the same multiplication rate and are as competitive with cells of the original strain as the revertants from the first wave.

Using the number of revertants in the first wave to estimate the number of revertants in the culture at the

Table 2. APPEARANCE OF SECOND-WAVE REVERTANTS IN JOINT CULTIVATION WITH ORIGINAL NA3-24 STRAIN CELLS

t	n	k	Number of revertants on 1 plate in first wave					
			1	2	3	4	5	6
0	0	5	100±10	38±6	136±22	71± 9	104±11	107± 6
0	$1 \cdot 10^4$	5	114± 9	52±6	142±22	76±10	113±17	105± 9
5	$2 \cdot 10^4$	5	105±14	43±6	122±18	73± 8	107±12	105±10

Table 3. APPEARANCE OF THIRD-WAVE REVERTANTS IN JOINT CULTIVATION WITH ORIGINAL NA3-24 STRAIN CELLS

t	n	k	Number of revertants on 1 plate in first wave				
			1	2	3	4	5
0	0	5	150±13	160± 8	103±20	163±9	91± 8
0	$1.3 \cdot 10^4$	5	160±10	154± 6	99± 6	127±8	93±11
5	$2.5 \cdot 10^4$	5	148±24	165± 9	121±16	154±8	125±11
24	$3.4 \cdot 10^6$	5	143±24	141±15	145±19	155±8	137±17

Leu^+ revertants of different morphology from the second and third waves were individually mixed with cells of original strain NA3-24 in the ratio of 1 revertant to $1 \cdot 10^4$ cells of the original strain in 1 inoculum. Plated in this way, the colonies were grown on a solid culture medium at 30°. Then they were transferred to the selective medium without leucine. The data on appearance of these revertants in the first wave are given.

t is the time of cultivation in a nutrient medium (hours),

n is the number of NA3-24 strain cells in 1 colony,

k is the number of plates in the experiment.

moment of transferring the cells to the selective medium, we managed to reveal adaptive mutagenesis in the exponential phase of the culture growth for *Saccharomyces cerevisiae* cultivated in solid nutrient media with different leucine content (Fig. 2). Shortage of leucine in a medium leads to an increase in the reversion rate of the *leu2-1* mutation and does not affect the reversion rate of the *lys1-1* mutation.

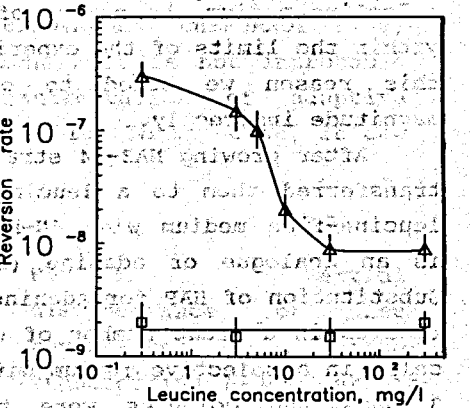

Fig. 2. Rates of spontaneous intralocus reversions of *leu2-1* (△) and *lys1-1* (□) mutation in NA3-24 strain cells cultivated on media with different leucine content. The frequency is expressed in terms of mutants per cell per generation.

The data reported in the paper by CHEPURNOY, MIKHOVA-TSENOVA and BRUNSKOVA (1989) indicate formation of spontaneous *Saccharomyces cerevisiae* mutants during DNA replication. Spontaneous mutants did not occur in the lag period, stationary phase of the culture growth and G_1-phase of the cell cycle where there is no DNA replication. On this strength we ascribed formation of revertants in the selective medium (the second wave of appearance) to residual cell growth caused by a residual leucine pool in cells. The magnitude of this residual growth can be evaluated from the maximum mutation rate observed in our experiments with media containing 0.3 mg/l of leucine ($R \approx 3 \cdot 10^{-7}$ revertants per cell per generation) and from the number of revertants in the

second wave of appearance. Calculation shows that if only 10% of all cells transferred to the selective medium divided, it would justify the number of second-wave revertants observed in our experiment. The scatter in the number of cells in colonies and the experimental error do not allow the 10% increase in the number of cells in a colony to be registered directly. Microscopic investigation of cell suspensions showed that within 14 days of incubation of cells in a selective medium the number of cells in 1 colony fluctuates within the limits of the experimental error equal to 20%. For this reason we tried to evaluate the residual growth magnitude indirectly.

After growing NA3-24 strain cells in a culture medium we transferred them to a leucine-free selective medium and to leucine-free medium with 6N-hydroxy-aminopurine (HAP), which is an analogue of adenine (SHCHERBAKOVA and PAVLOV 1993). Substitution of HAP for adenine in DNA during DNA replication results in a great number of direct mutations. After keeping cells in a selective medium with and without HAP for 1, 2 and 3 days the colonies were transferred to a medium with α-aminoadipate for determination of direct mutations $ADP^+ \rightarrow adp^-$ (CHATTOO *et al.* 1979). Three days keeping of cells in a selective medium without HAP did not lead to formation of adp^--mutants, while keeping in a selective medium with HAP resulted in formation of 12 adp^--mutants ($n = 9 \cdot 10^5$, $N = 1200$). Preliminary experiments showed that the frequency of direct adp^--mutations is below $1 \cdot 10^{-9}$ when cells are cultured in a medium without HAP. If a medium with HAP is used, the mutation frequency increases up to $3 \cdot 10^{-7}$. With the frequency of adp^--mutants per cell per generation equal to $3 \cdot 10^{-7}$, the residual growth in the selective medium is estimated to be 4-8% of the total number of cells transferred to the selective medium. Obviously, this increase cannot be registered directly.

There is another phenomenon associated with formation of spontaneous mutations, which we observed when studying

adaptive mutagenesis. It is partial disappearance of reversions as the selective pressure was reduced. If a culture with revertants is transferred from a medium with low leucine content to a medium with a higher leucine content, a part of revertants disappears (Fig. 3). They disappear in the period between the end of the G_1-phase and the beginning of the S-phase of the next cell cycle. It is "newborn" reversions just formed in the previous phase of DNA replication that disappear (LYUBIMOVA and CHEPURNOY 1992; D.F.STEELE, unpublished observations). It is not improbable that this phenomenon is a manifestation of adaptive mutagenesis or is closely related to it. In any case, it can

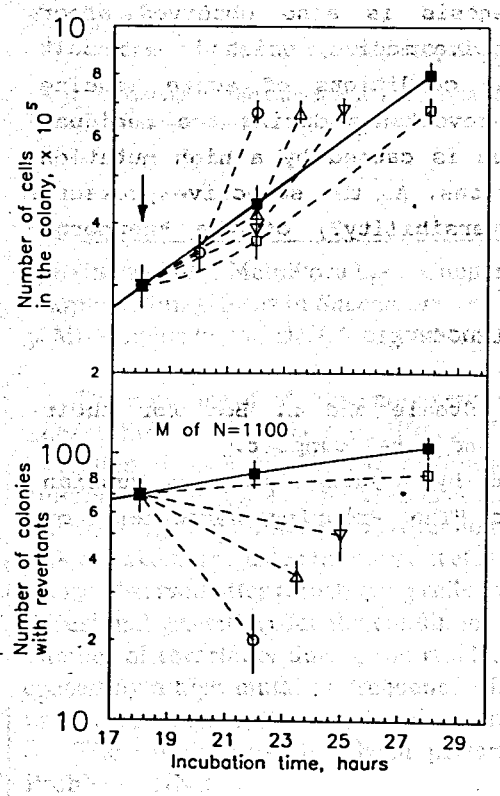

Fig. 3. Partial disappearance of Leu^+ revertants after transferring NA3-24 strain cell colonies from the medium with 3 mg/l (■) of leucine on the medium with 30 (○), 10 (△) and 5 (▽) mg/l of leucine. Transfer from the medium with 3 mg/l of leucine on the medium with the same 3 mg/l (□) of leucine does not lead to disappearance of revertants. The arrow point at the moment of transfer.

be used in further investigations of the nature of spontaneous gene mutation.

CONCLUSIONS

To sum up, we would like to stress that using formation of reversions of *leu2-1* mutation in *Saccharomyces cerevisiae* as an example we managed to demonstrate the presence of adaptive mutagenesis in the exponential phase of culture growth for cells cultivated in media with different leucine content. The mutation frequency of *lys1-1* did not change in this case. Reversions of *leu2-1* mutation are formed in the course of DNA replication. The reversion frequency depends on the medium in which the cells are just at the moment of DNA replication. Adaptive mutagenesis is also observed after transferring cells to a selective medium, which is a result of residual growth under the conditions of acute leucine shortage. A great number of revertants during the residual growth in the selective medium is caused by a high mutation frequency with few cell divisions. As the selective pressure decreases, disappearance (reversibility?) of the "newborn" reversions is observed.

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