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Non-Mendelian Mutation Allowing Ureidosuccinic Acid Uptake in Yeast

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Mutants of *Saccharomyces cerevisiae* capable of growth on a minimal medium supplemented with ureidosuccinic and glutamic acids have been isolated from a pyrimidineless strain. One of these mutants consistently yielded a non-Mendelian meiotic segregation. Moreover, the mitotic transmission of the mutation was very high. It is suggested that the mutation is nonchromosomal and could be mitochondrial. However, this mutation behaves very differently from other mitochondrial mutations.

The gene *ura-2* of yeasts encodes aspartic transcarbamylase activity. A *ura-2* mutant might be expected to grow on a minimal medium supplemented with ureidosuccinic acid (USA), because this metabolite is the product of the enzyme. However, this is not the case; an additional mutation is required for USA to be used by the mutant. In an earlier study, a recessive mutation showing a normal Mendelian segregation was found and named *ure-1*. This mutation confers on yeast cells the ability to incorporate USA. It also decreases the glutamic acid level and increases the glutamine level in the amino acid pool. The incorporation of USA seems to be related to the depletion of glutamic acid in the pool, because after growth on a high concentration of glutamic acid the mutant is no longer able to incorporate USA. USA and glutamic acid are not taken up by the same permease because there is no competition between them when the initial kinetics of uptake is measured (2, 7; Lacroute, Thesis, 1966).

To understand better the physiological mechanisms implicated in this pool phenomenon, mutants which are able to take up USA even in the presence of large amounts of glutamic acid have been selected. Such mutants are 30 times less frequent than the *ure-1* mutants. These new mutations have been studied genetically, and most of them show Mendelian segregation when crossed with the wild strain. They have been shown to belong to a new locus named *ure-2*, which is nonallelic with *ure-1*. One mutation of this class gives non-Mendelian segregation. This paper presents information obtained on that mutant.

MATERIALS AND METHODS

Organisms. All yeast strains used were *Saccharomyces cerevisiae* derivatives from strain FL100 (haploid, a, wild type; 2) and from an isogenic strain, FL200 (haploid, α, wild type). The isogenic strain FL200 was isolated by sporulation of a diploid resulting from a cross between two different auxotrophic mutants of FL100, one of them having been mutated in the mating type (5).

Genetic analysis. The methods described by Mortimer and Hawthorne (4) were used.

Media and culture methods. Yeast Nitrogen Base (Difco) without amino acids was used as the minimal (nonsupplemented) growth medium. The other media and the culture methods were the same as previously described (3).

Chemicals. The biochemicals were purchased from Calbiochem, Los Angeles Calif., and from E. Merck, A. G. Darmstadt, Germany.

RESULTS AND DISCUSSION

Isolation and characterization of non-Mendelian mutant ure-3. Strain FL100, a, *ura-2-6*, deficient in aspartic transcarbamylase activity, was plated after ultraviolet (UV) mutagenesis on minimal medium supplemented with DL-USA (300 μg/ml) and L-glutamic acid (500 μg/ml). This procedure should select mutants able to utilize USA in the presence of glutamic acid. Thirteen colonies were obtained; subclones of these mutants were tested on different media to verify the phenotype and thus to discard the suppressor mutations or reversions in the gene *ura-2-6*. Seven subclones were able to grow on minimal medium, and two subclones grew poorly on USA with glutamic acid; these strains were not studied further. The other four mutants were crossed

with a strain of opposite mating type (FL201 *ura-2-6*), and the phenotypes of the diploids were analyzed. Table 1 gives the growth requirements of the parent strains, of the mutant subclones, and of the diploids; it also shows whether they excrete uracil in the presence of USA.

Comparisons of the phenotypes of the diploids with the phenotypes of the parent strains show that the ability to grow on USA is dominant in all cases (the *ure-1* mutants obtained previously were all recessive). The new mutations present in subclones 1, 3, and 4 are semidominant (i.e., the heterozygous diploids obtained with these mutants are able to grow on USA but not on USA with glutamic acid), whereas the diploid obtained with subclone 2 is completely dominant and behaves exactly like subclone 2 itself.

The segregation of the ability to use USA was tested in tetrads obtained from the diploids FL100 *ura-2-6* subclone 1 × FL201 *ura-2-6* and FL100 *ura-2-6* subclone 2 × FL201 *ura-2-6*. The sporulation of these two diploids was very poor. Four tetrads were obtained from the diploid with subclone 1, and they show perfect Mendelian segregation; i.e., two spores are able and two spores are unable to use USA in the presence of glutamic acid. This mutation is located in a new gene that has been shown to be distinct from *ure-1* and has been named *ure-2*. This locus will not be described in the present paper.

Five tetrads were obtained from the diploid formed with subclone 2 (diploid named FL464), and the four spores given by each of them were able to grow on USA in the presence of glutamic acid. This result could be explained in two ways: (i) it could result from gene conversion or a mitotic crossing-over in the original diploid, or (ii) the mutation present in subclone 2 is non-Mendelian. To discriminate between these two hypotheses, five independent diploids were made between FL100 *ura-2-6* subclone 2 and FL201 *ura-2-6*; all of them again showed the completely dominant phenotype. Since conversion and mitotic crossing-over are infrequent events, this result makes the first explanation very unlikely. Moreover, the four spores of one tetrad from FL464 were backcrossed with *ura-2-6* mutants which are unable to use USA. The diploids obtained were all able to grow on USA. The tetrads isolated after sporulation were examined for USA utilization and gave, in most cases, four spores able to use USA. For example, the diploids which yield the highest sporulation were obtained in the cross between FL464-1B (a *ura-2-6 ure x*) and FL201 *ura-2-6* (α *ura-2-6* +). From these diploids, 10 tetrads were analyzed: 8 of them gave 4 (*ure*) and 0 (+); 1 gave 3 (*ure*) and 1 (+); and 1 gave 1 (*ure*) and 3 (+). Many other crosses have been examined since, and always a non-Mendelian segregation has been obtained; the majority of tetrads were of the type giving four (*ure*) and zero (+). It is, therefore, clear that this new mutation, which has been named *ure-3*, is non-Mendelian.

Transmissbility of the non-Mendelian mutation. The transmissibility of a non-Mendelian mutation has been defined as the percentage of diploids from a cross between a mutant and a wild strain that exhibit the mutant phenotype. In our case, the transmissibility is very high because,

TABLE 1. *Phenotypes of the different mutant strains and their diploids on different media*[a]

Strain	Genotype	Minimal medium	Uracil	USA	USA + GA	Uracil excretion on USA medium
FL100 *ura-2-6*	*ura-2-6*	−	+	−	−	−
FL201 *ura-2-6*	*ura-2-6*	−	+	−	−	−
FL100 *ura-2-6* subclone 1	*ura-2-6 ure-2*	−	+	±	+	+
FL100 *ura-2-6* subclone 2	*ura-2-6 ure-3*	−	+	±	+	+
FL100 *ura-2-6* subclone 3	*ura-2-6 ure-2*	−	+	±	+	+
FL100 *ura-2-6* subclone 4	*ura-2-6 ure-2*	−	+	±	+	+
FL100 *ura-2-6* × FL201 *ura-2-6*	*ura-2-6* / *ura-2-6*	−	+	−	−	−
FL100 *ura-2-6* subclone 1 × FL201 *ura-2-6* (FL 464)	*ura-2-6 ure-2* / *ura-2-6* +	−	+	±	−	−
FL100 *ura-2-6* subclone 2 × FL201 *ura-2-6*	*ura-2-6 ure-3* / *ura-2-6* +	−	+	±	+	+
FL100 *ura-2-6* subclone 3 × FL201 *ura-2-6*	*ura-2-6 ure-2* / *ura-2-6* +	−	+	±	−	−
FL100 *ura-2-6* subclone 4 × FL201 *ura-2-6*	*ura-2-6 ure-2* / *ura-2-6* +	−	+	±	−	−

[a] USA = ureidosuccinic acid; GA = glutamic acid. The excretion of uracil was measured by the halo produced on USA medium by an indicator pyrimidineless strain spread on the petri dish.

without selection, every zygote that was isolated produced progeny with the mutant phenotype. In fact, all of the zygotes produced some mutants in their progeny. FL464-1A (α *ura-2-6 ure-3* +) and FL100 *arg-32* (a + + arg^-) were crossed, and zygotes were selected by prototrophy. Among 359 prototrophic colonies tested, all were found to excrete uracil in the presence of USA, showing thereby that clones contain a significant portion of mutant diploid cells. Also, we spread cells from each colony on minimal medium and tested the ability of the new colonies formed to excrete uracil in the presence of USA. The results establish that virtually all of the cells in the initial colony had the mutant phenotype.

This result could be explained by a faster rate of growth of the cells inheriting the non-Mendelian mutation, but this was not the case. On the contrary, they grow more slowly than the wild type. If one assumes that there is a genetic determinant in two allelic forms, a normal one and a mutated one, and if there are multiple copies of this genetic determinant in each cell, an asymmetry in the transmissibility can be influenced by two phenomena: (i) there could be an asymmetry in the rate of multiplication of the replicon bearing the mutated gene versus that of the wild-type replicon, or (ii) there could be an asymmetry in the probability of genetic exchange or of recombination between the two replicons. This second possibility was discussed in detail by Coen et al. (1).

Since all of the strains used were isogenic, the asymmetry in transmissibility favoring one of the allelic forms was not probable; nevertheless, there is an asymmetry and a very strong one. There are no means to discriminate between the two possibilities of asymmetry since it is necessary to have at least two different mutations on a replicon to demonstrate genetic recombination and three different mutations to show an asymmetry in genetic recombination. It seems more likely, however, that it is an increase in the rate of multiplication of the mutated replicon which explains its high transmissibility.

A preferential inhibition of the mutated replicon multiplication in the zygotes has been attempted by submitting the parental strain bearing the *ure-3* mutation to UV irradiation prior to the cross with the opposite mating type strain. This technique was used successfully by Sager and Ramanis (6) to inhibit the transmission of chloroplastic genes from a parental chlamydomonas to the zygote.

Table 2 shows that UV irradiation has no appreciable effect on the transmissibility, even with a UV dose that severely impairs zygote formation. Only one diploid, among 23 tested, for the time 3 min, did not receive the *ure* phenotype, so

TABLE 2. *Effect of UV irradiation on the transmissibility of the non-Mendelian mutation*[a]

Irradiation (min)	Mating (hr)	Diploids obtained[b]	Diploids cured[c]
0	6	570 × 100	0/26
1	6	224 × 100	0/31
3	9	5 × 10	1/23
6	9	117	0/28

[a] The cells of strain FL464-1A (*ura 2-6 ure-3*) were irradiated in sterile water after exponential growth in a complete medium. After each time of irradiation, the cells were collected by centrifugation, and the pellet was mixed on a petri dish of complete medium with strain FL100 *arg-32* (a + + arg^-). The required time for mating was long because UV irradiation introduces a delay in the mating. The UV lamp used was a germicidal tube, and the power was 5 erg per cm^2 per sec.

[b] Number of diploids obtained for 2 μliters of mating suspension.

[c] Number of diploids cured of the non-Mendelian mutation/number of diploids tested.

that even if this result was caused by the UV irradiation the effect is very small. This result does not support a mitochondrial localization of the *ure-3* mutation.

The transmissibility has also been measured in crosses involving our *ure-3* strain and nonisogenic strains from other laboratories, especially strain 4592C (M. Grenson) and strain X 1687-12B (R. K. Mortimer); with these two strains the transmissibility was as high as in isogenic crosses. But, in a cross with a spore isolated from Yeast Foam (YF-1B), the zygotes gave wild diploids and *ure-3* diploids in their progeny and the transmissibility was reduced.

Two possibilities may account for the high transmissibility of the *ure-3* mutation. The *ure-3* mutation could by itself give a higher rate of multiplication to the corresponding replicon, but this seems unlikely. The second possibility is that another mutation in the replicon bearing the *ure-3* mutation must occur to induce a higher rate of multiplication or a higher frequency of genetic transmission of the replicon. This second type of mutation would always be selected simultaneously with the *ure-3* mutation, because the *ure-3* mutation, being a recessive mutation, would express its phenotype only when the wild-type replicon had disappeared from the cells. This second possibility is more likely and is in agreement with the scarcity of this type of mutation. It must be possible, if this second hypothesis is right, to obtain recombinants between the two mutations, one recombinant having the wild-type phenotype with a high transmissibility and the other bearing a *ure-3* phenotype with normal transmissibility.

Localization of the mutation. The non-Mendelian mutants currently known in yeast have generally been shown to be mutations in mitochondrial deoxyribonucleic acid. One easy way to ascribe mutations to a change in mitochondrial deoxyribonucleic acid is to demonstrate that the production of cytoplasmic petites (ρ^-) often results in loss of the non-Mendelian mutation (1).

The strains FL 465-1A (α *ure-3*), FL465-5D (a *ure-3*), FL464-1A (α *ure-3 ura-2-6*), and FL464-1B (a *ure-3 ura-2-6*) were treated with ethidium bromide for a short time, or overnight, to induce ρ^- mutants. The ρ^- colonies recovered after the two lengths of treatment were tested for the *ure-3* phenotype.

Twenty-eight ρ^- colonies obtained from FL465-1A were tested, and all were found to be *ure-3*; in the same way, the 81 FL465-5D ρ^- colonies and the 52 FL464-1A ρ^- colonies analyzed were all *ure-3*. Only the FL464-1B colonies showed 2 wild types among 56 ρ^- colonies tested. It is clear that the ρ^- induction is not related to a frequent loss of the *ure-3* genetic determinant. This result was confirmed in the following way. Eight independent ρ^- *ure-3* subclones, isolated from FL465-1A, were crossed with FL100 (a ρ^+ +), and five independent zygotes were isolated from each cross. All of the diploids obtained were found to be ρ^+ *ure-3*; this shows that there is no interference between the high transmission of *ure-3* coming from FL465-1A and the high transmission of ρ^+ coming from FL100. Some mitochondrial mutations are apparently not linked to ρ^+ (P. Slonimski, *personal communication*). Therefore, it is possible that *ure-3* is a mitochondrial mutation belonging to a replicon different from the replicon bearing the ρ^+ information; *ure-3* could also be a mutation of a nonmitochondrial cytoplasmic replicon, but the only deoxyribonucleic acids that have so far been detected in yeast are nuclear and mitochondrial deoxyribonucleic acids.

Throughout the discussion of this work, it has been assumed that *ure-3* is a genic mutation, but (as pointed out by B. Cox) there is still the possibility that it is a case of stable hereditary phenotypic adaptation involving a double antagonistic repressor system according to the Novick hypothesis for stable adaptation. Such a model is very difficult to differentiate from a genic cytoplasmic mutation, and a better understanding of the molecular basis of the *ure-3* phenotype would be needed. The fact that we have obtained *ure-3* mutations only after UV mutagenesis and never spontaneously is not proof of a genic mutation because UV irradiation also affects gene expression.

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