MUTATOR ACTIVITY OF PETITE STRAINS OF *SACCHAROMYCES CEREVISIAE*[1]

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ABSTRACT

Petite strains in Saccharomyces exhibit enhanced spontaneous mutation rates of nuclear genes regardless of whether they are cytoplasmically or nuclearly inherited, or whether or not the cytoplasmic petite strains have mitochondrial DNA. In petite strains, the mutation rate for the nonsense allele *lys1-1* is enhanced by a factor of 3-6 and for the missense allele *his1-7* by a factor of 2 as compared with their grande counterparts. The reversion of a third allele, the putative frameshift mutation, *hom3-10*, is not enhanced in a petite background. The results indicate that the spontaneous mutation rate of an organism can be altered by *indirect* intracellular influences.

IT is now generally assumed that spontaneous mutations arise during replication, repair, and recombination of DNA from errors made by the enzymes of DNA metabolism. The level of spontaneous mutations also depends upon indirect influences within the cell. Indirect influences on mutation rates were demonstrated by VON BORSTEL, CAIN and STEINBERG (1971). They showed that cytoplasmic petite strains of yeast (e.g., respiratory-deficient) had mutator activity where their grande counterparts did not.

In order to obtain more insight into the phenomenon of petite mutator activity, we initiated this series of experiments to determine the types of petites that can influence the spontaneous mutation rate, and whether the type of molecular lesions affected are base substitutions, frameshift mutations, or both.

MATERIALS AND METHODS

Strains: The basic strains used in these experiments are listed in Table 1. The strains X1687-12B and X1687-101B were provided by R. K. MORTIMER, and the strains XV185-6A, XV182-6C, and XV182-6D were derived from crosses of the first two strains with strains provided by G. E. MAGNI and S. FOGEL. All cytoplasmic petite strains used in the experiments were from the strains listed in Table 1 and were either of spontaneous origin or were induced with ethidium bromide.

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TABLE 1

Genotype of haploid strains

Strain	Genotype							
X1687–12B	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*	*trp5–48*			
XV185–6A	*ade2–1*	*arg4–17*		*lys1–1*	*trp5–48*	*hom3–10*	*his1–7*	
X1687–101B	*ade2–1*	*arg4–17*	*his5–2*	*lys1–1*		*leu1–12*		
XV182–6C	*ade2–1*	*arg4–4*	*his5–2*	*lys1–1*	*trp5–48*	*met1–1*	*thr1*	
XV182–6D	*ade2–1*	*arg4–4*	*his5–2*	*lys1–1*	*trp5–48*	*met1–1*	*thr1*	*pet1*

The alleles *ade2-1*, *arg4-4*, *arg4-17*, *his5-2*, *lys1-1*, and *trp5-48* are ochre-suppressible mutants. The allele *his1-7* is a missense mutant believed to be revertible by internal missense suppression, and the allele *hom3-10* is believed to be a frameshift mutant revertible by internal frameshift suppression (see VON BORSTEL *et al.* 1973, for the genetic arguments for these assignments).

The symbol [*rho*⁻] designates the cytoplasmically inherited petite character and *pet* designates the nuclearly inherited petite character. Despite this difference of inheritance, the petite phenotypes are similar. Cytochromes ($a + a_3$) and b, as well as a number of respiratory enzymes, are absent and petites are therefore unable to grow on nonfermentable substrates (for review, see SHERMAN 1965). ERY^S and ERY^r designate, respectively, the cytoplasmically inherited determinants for sensitivity or resistance to erythromycin. ERY^r strains show almost no inhibition of growth on glycerol in the presence of 8 mg/ml erthromycin. Independent isolates or erythromycin-resistant strains are designated ERY^{r-1}, ERY^{r-2}, etc.

Media: Many of the media are the same as those described by VON BORSTEL, CAIN and STEINBERG (1971) and VON BORSTEL *et al.* (1973), including the basic synthetic complete medium which we call Mortimer Complete (MC). MC is a mixture of ten amino acids and bases, vitamins, salts and glucose. Occasional modifications of the formulae found in the above two references are listed in footnotes to the tables. Three other media were used in these experiments. A glycerol-complete medium containing erythromycin (YEPG-ERY) was used for the isolation of mutants resistant to erythromycin. YEPG-ERY is one percent Difco yeast extract, two percent Bactopeptone, four percent glycerol, and two percent agar suplemented with 8 mg/ml of erythromycin glucoheptonate. A synthetic complete medium containing glycerol (MCGD) was used to distinguish prototropic diploid colonies that were either grande, [*rho*+] or petite, [*rho*⁻]. MCGD is MC with three percent glycerol and 0.1 percent glucose replacing the two percent glucose commonly used in MC. A synthetic omission medium containing glycerol and erythromycin (MCGD-ERY) was used for the determination of sensitivity of strains to erythromycin. MCGD-ERY is MCGD with 4 mg/ml of erythromycin glucoheptonate added.

Isolation of erythromycin-resistant mutants: Spontaneous mutants resistant to erythromycin were isolated by plating cultures of the sensitive strains X1687-12B and X1687-101B on YEPG and YEPG-ERY.

Determination of erythromycin-resistance in crosses: The [*rho*⁻] strains derived from a resistant strain were crossed with a [*rho*+ ERY^S] strain and the zygotes were then placed on MCGD-ERY. If a cross between [*rho*+ ERY^S] × [*rho*⁻] gave rise to some [ERY^r] diploid clones, then the [*rho*⁻] cells were assumed to be [*rho*⁻ ERY^r].

Determination of suppressiveness of petites: The percentage of petite zygotic colonies was determined on MCGD using the tetrazolium overlay method of OGUR, ST. JOHN and NOGAI (1957). For calculating the degree of suppressiveness, the formula of EPHRUSSI and GRANDCHAMP (1965) was used.

Induction of petities with ethidium bromide: Ethidium bromide is an effective inducer of cytoplasmic petites (SLONIMSKI, PERRODIN and CROFT 1968). This phenanthrene dye was added at 10 μg/ml to logarithmically growing cells of the strains X1687-12B [ERY^r] and X1687-101B [ERY^r] according to the schedule of GOLDRING *et al.* (1970). Samples were taken as a function

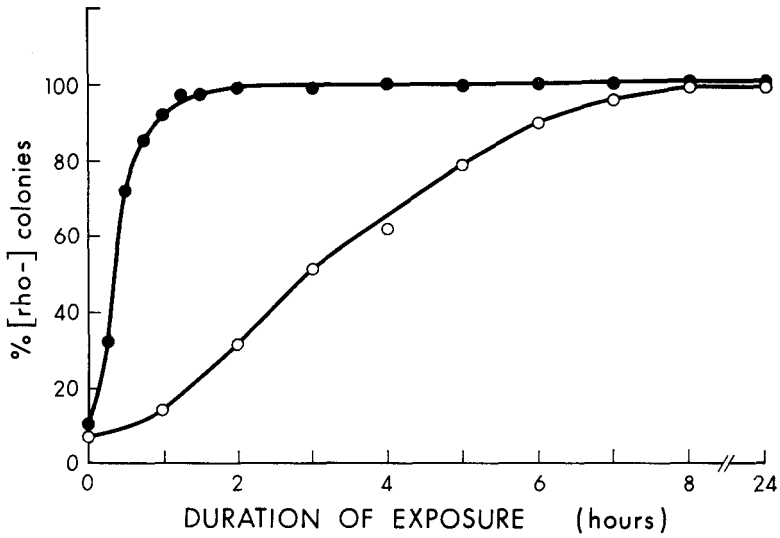

FIGURE 1.—Kinetics of petite induction by ethidium bromide. Ethidium bromide (10 μg/ml) was added to logarithmically growing cells of strains X1687–12B and X1687–101B in MC. Samples were taken at different time intervals, diluted with saline-phosphate (0.15 M NaCl, 0.02 M K_2HPO_4, pH7.5) and plated on yeast-peptone agar + 1% glucose.

● Strain X1687-12B

○ Strain X1687-101B

of time by diluting the plating on glucose-containing medium. The petite colonies were identified by the tetrazolium agar overlay method of OGUR, ST. JOHN and NOGAI (1957).

In Figure 1 it can be seen that the two strains responded very differently with respect to petite induction by ethidium bromide. The petite strains induced by ethidium bromide for use in our studies were taken from cultures treated for 24 hours.

Measurement of spontaneous mutation rates: The method for estimating spontaneous mutation rates has been described by VON BORSTEL, CAIN and STEINBERG (1971). It is a 1000-compartment fluctation test where growth is limited by adding only a small amount of a necessary substrate. Revertants continue to grow. Mutation rates are determined from the proportion of compartments without mutants. For studying reversion of *lys1-1*, 1.0 μg/ml was usually used for limiting the growth; for *his1-7*, 0.3 μg/ml was used; and for *hom3-10*, methionine was used at a concentration of 1.0 μg/ml to limit the growth.

Distinguishing lys1–1 *site mutants from suppressors*: The mutations at the *lys1–1* site itself can be distinguished by two different methods: the revertants can be picked and replica-plated, or 5 μg/ml of adenine in the medium causes the locus mutants to be red and the suppressors to be white (SCHULLER and VON BORSTEL 1974). The former method was used unless otherwise designated in the tables. The latter method is rapid and has approximately 94% reliability.

Detection of mitochondrial DNA: The fluorescent DNA staining procedure of WILLIAMSON and FENNELL (1975), using 4′ .6-diamidino-2-phenylindole (DAPI), was employed for the detection of mitochondrial DNA in whole cells of petite strains. DAPI has been shown to be a selective stain for DNA, mitochondrial DNA molecules, when present, appearing as fluorescent particles in the cytoplasm. Strains diagnosed on this basis as lacking mitochondrial DNA were further examined using DAPI/CsCl gradients (WILLIAMSON and FENNELL 1975). In gradients of this type, mitochondrial DNA forms a fluorescent band separated from that of nuclear DNA and this method, like the cell-staining procedure, is capable of detecting mitochondrial DNA in amounts as low as 50×10^6 daltons per yeast cell.

RESULTS

The spontaneous mutation rates for different cytoplasmic petites derived from the X1687–101B strain of haploid yeast are given in Table 2. In every test it is clear that the reversion rate for the *lysl-1* locus itself is higher in the clones that are cytoplasmic petites. A representative example of the results of fluctuation tests of a grande and a cytoplasmic petite clone are shown in Figure 2 to illustrate the cumulative appearance of spontaneous revertants over the course of an experiment. Mutation rates in the tables are based on the early part of the plateau (see VON BORSTEL, CAIN and STEINBERG 1971). Kinetic data are taken during every fluctuation test and, in general, the revertant accumulation curves resemble those shown in Figure 2. Because petite clones tend to reach a plateau later than do the grande (petite cells have a slower growth rate), the twelfth day was selected for the data presented in the tables.

Three replicates of four clones of another strain, X1687–12B, are listed in Table 3, and these exhibit slightly different mutation rates from those of X1687–101B. One of the clones is the grande strain, and the other three are cytoplasmic petite clones exhibiting different degrees of suppressiveness. A suppressiveness of 0 (a neutral petite) usually indicates the absence of mitochondrial DNA (GOLDRING *et al.* 1970; NAGLEY and LINNANE 1970). It can be seen in Table 3 that a clone having a suppressiveness of 0 retains the capacity to alter the spontaneous mutation rate.

TABLE 2

Spontaneous reversion rates at an ochre site (lys1–1) *in grande and cytoplasmic petite clones of strain X1687–101B*

No.	Strain	Cells/ml ($\times 10^{-6}$)	Mutation rate ($\times 10^{8}$)			Number of revertants	
			Total	Locus	Sup*	Locus	Sup*
22.04	X1687–101B	0.98	4.02	0.35	3.67	7	70
22.06	X1687–101B [*rho⁻-1*]†	1.43	5.49	1.34	4.15	38	113
22.07	X1687–101B [*rho⁻-2*]†	1.38	6.30	1.79	4.51	49	117
22.08	X1687–101B [*rho⁻-1*]‡	1.56	5.40	1.75	3.65	53	107
22.09	X1687–101B [*rho⁻-2*]‡	1.45	6.42	1.93	4.49	54	121
25.01	X1687–101B	1.69	3.77	0.35	3.42	12	110
25.02	X1687–101B (*ERY^{r_2}*)	2.25	3.04	0.40	2.64	18	113
25.03	X1687–101B (*ERY$^{r_{2-1}}$*) [*rho⁻*]†	2.89	3.33	1.13	2.20	64	119
25.04	X1687–101B (*ERY$^{S_{2-6}}$*) [*rho⁻*]†[1]	2.16	4.84	1.34	3.50	57	142
25.05	X1687–101B [*rho⁻*]†[2]	2.44	3.64	0.86	2.78	42	129
25.06	X1687–101B [*rho⁻-1*]†	2.24	4.45	1.35	3.10	59	130
25.07	X1687–101B [*rho⁻-2*]†	2.17	3.92	1.18	2.74	50	112
25.08	X1687–101B [*rho⁻-3*]†	2.46	4.16	1.14	3.02	55	140

* Suppressors.
† Spontaneously arisen.
‡ Induced by ethidium bromide.
[1] Lost the resistance factor to erythromycin.
[2] The petite strain isolated by R. C. VON BORSTEL *et al.*, 1971.

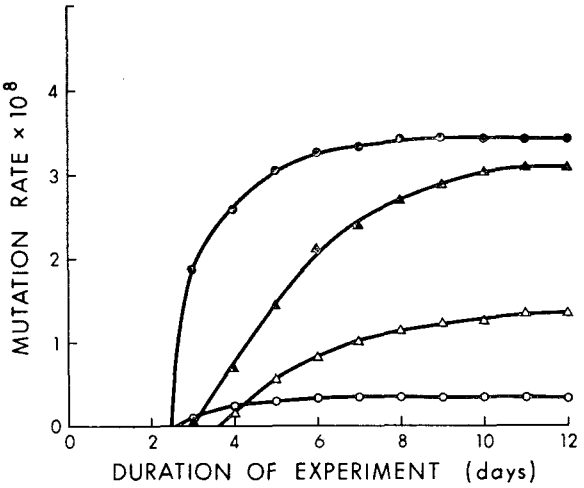

FIGURE 2.—Kinetics of mutant appearance for haploid strains (X1687-101B).
● Suppressors, grande strain (Expt. No. 25.01)
○ Locus revertants, grande strain (Expt. No. 25.01)
▲ Suppressors, petite strain (Expt. No. 25.06)
△ Locus revertants, petite strain (Expt. No. 25.06)

TABLE 3

Spontaneous reversion rates at an ochre site (lys1–1) *in grande and cytoplasmic petite clones of strain X1687–12B*

No.	Strain	Percent suppressiveness	Cells/ml ($\times 10^{-6}$)	Mutation rate ($\times 10^{8}$)			Number of revertants	
				Total	Locus	Sup*	Locus	Sup*
27.01	X1687-12B (*ERY*^{r})[1]	—	1.78	3.15	0.31	2.84	11	97
27.02	X1687-12B (*ERY*^{r}) [*rho*$^{-}$]†	15	1.79	2.60	0.70	1.90	25	67
27.03	X1687-12B (*ERY*^{S}) [*rho*$^{-}$]‡[2]	86	1.99	2.41	0.86	1.55	34	61
27.04	X1687-12B (*ERY*^{S}) [*rho*$^{-}$]‡[1,2]	0	1.79	2.76	1.01	1.75	36	62
35.01	X1687-12B (*ERY*^{r})	—	1.78	3.01	0.31	2.70	11	91
35.02	X1687-12B (*ERY*^{r}) [*rho*$^{-}$]†	15	1.92	1.64	0.47	1.17	18	44
35.03	X1687-12B (*ERY*^{S}) [*rho*$^{-}$]‡[2]	86	1.90	1.83	0.82	1.01	31	39
35.04	X1687-12B (*ERY*^{S}) [*rho*$^{-}$]‡[2]	0	2.40	1.65	0.50	1.15	24	54
35.05	X1687-12B (*ERY*^{r})	—	1.70	2.99	0.24	2.75	8	89
35.06	X1687-12B (*ERY*^{r}) [*rho*$^{-}$]†[3]	15	1.93	2.15	0.55	1.60	21	60
35.07	X1687-12B (*ERY*^{S}) [*rho*$^{-}$]‡[2,3]	86	1.95	1.93	0.86	1.07	33	41
35.08	X1687-12B (*ERY*^{S}) [*rho*$^{-}$]‡[2,3]	0	1.92	1.99	0.61	1.38	23	52

* Suppressors.
† See Table 2.
‡ See Table 2.
[1] Locus data from these experiments were also summarized in Table 4 of VON BORSTEL *et al.* (1973).
[2] These strains lost the resistance factor to erythromycin during treatment with Eth Br.
[3] Medium contained 5 μg/ml adenine in order to recognize *lys1* locus revertants as red colonies (see SCHULLER and VON BORSTEL 1974).

TABLE 4

Spontaneous reversion rates at an ochre site (lys1–1) *in a neutral cytoplasmic petite of strain X1687–12B for which it had been determined that mitochondrial DNA was lacking*

No.	Strain	Percent suppressiveness	Cells/ml ($\times 10^{-6}$)	Mutation rate ($\times 10^{8}$)			Number of revertants	
				Total	Locus	Sup*	Locus	Sup*
51.09	X1687–12B [*rho*$^-$][1]	0	1.93	2.37	0.85	1.52	33	57
51.10	X1687–12B [*rho*$^-$][1]	0	2.05	2.81	0.93	1.88	38	74
51.11	X1687–12B [*rho*$^-$][1]	0	1.96	3.12	1.02	2.10	40	79

This is an experiment done in triplicate with the same [*rho*$^-$] strain. It was the 15% suppressive strain shown in Table 3 and had undergone another mutation to become a neutral petite.

* Suppressors.

[1] Medium contained 5 μg/ml adenine in order to recognize *lys1* locus revertants as red colonies (see SCHULLER and VON BORSTEL 1974).

Some months after its original isolation, we examined the petite strain initially showing 15% suppressiveness for its mitochondrial DNA content. It had none, so it was reexamined for suppressiveness and it had indeed converted spontaneously into a neutral petite. The strain was then tested again for its mutation rate (Table 4). It can be seen that the spontaneous reversion rate for the *lys1–1* is still enhanced.

From Tables 2, 3 and 4, three characteristics of cytoplasmic petites are recognizable: Cytoplasmic petites always cause enhanced spontaneous reversion rates for the *lys1–1* mutant (particularly noticeable in Table 2); they cause their effects no matter the degree of suppressiveness (Tables 3 and 4) even if mitochondrial DNA is absent, and they exhibit a strain-to-strain variation in the degree to which the effects are produced (Tables 2 and 3).

The strain XV185–6A shown in Table 5 was constructed so that the three mutant sites with different properties would be together in the same genetic background. The data are from two replicates of the same experiment. These mutant sites respond differently to the presence of defective mitochondria. Again, the *lys1–1* mutant site exhibits an enhanced spontaneous mutation rate. The *his1–7* mutant site, believed to be a missense mutation, is approximately doubled in its reversion rate. The *hom3–10* mutant site, believed to be a frameshift mutation, has the same spontaneous reversion rates in both strains. Thus, depending upon the mutation type being tested, cytoplasmic petites have either enhanced mutation rates or show no change whatsoever.

The data shown in Table 6 were obtained to determine whether the petite phenotype *per se* was responsible for the observed enhancement of spontaneous mutation rate. Spontaneous reversion of the *lys1–1* site was measured in a strain carrying a mutation in the *pet1* gene. The sister spores differ only with respect to the presence of *pet1*. Again, replicate experiments show that the petite strain exhibits an enhanced spontaneous mutation rate for the *lys1–1* locus itself.

TABLE 5

Spontaneous reversion rates at an ochre site (lys1-1), *at a missense site* (his1-7) *and at a putative frameshift site* (hom3-10) *in the same strain of yeast*

No.	Strain	Cells/ml ($\times 10^{-6}$)	Mutation rate ($\times 10^{8}$)			Number of revertants		Reversion site
			Total	Locus	Sup*	Locus	Sup*	
36.04	XV185-6A	2.30	1.99	0.42	1.57	19	70	*lys1-1*
36.05	XV185-6A [*rho*$^-$]†	2.16	2.17	0.87	1.30	37	55	
36.08	XV185-6A[2]	2.47	—	7.42	—	310	—	*his1-7*
36.09	XV185-6A [*rho*$^-$]†[2]	2.52	—	12.53	—	472	—	
37.05	XV185-6A[3]	1.56	—	0.45	—	132	—	*hom3-10*
37.06	XV185-6A [*rho*$^-$]†[3]	1.73	—	0.48	—	155	—	
36.06	XV185-6A[1]	2.41	2.06	0.23	1.83	11	85	*lys1-1*
36.07	XV185-6A [*rho*$^-$]†[1]	2.39	1.99	0.82	1.17	29	54	
37.01	XV185-6A[1,2]	2.49	—	7.93	—	328	—	*his1-7*
37.02	XV185-6A [*rho*$^-$]†[1,2]	2.51	—	15.37	—	543	—	
37.03	XV185-6A[1,3]	1.84	—	0.59	—	198	—	*hom3-10*
37.04	XV185-6A [*rho*$^-$]†[1,3]	1.73	—	0.55	—	173	—	

* Suppressors.
† See Table 2.
[1] Medium contained 5 μg/ml in order to recognize *lys1* locus revertants as red colonies (see SCHULLER and VON BORSTEL 1974).
[2] Medium contained 0.3 μg/ml histidine and 60 μg/ml lysine.
[3] Medium contained 1.0 μg/ml methionine and 60 μg/ml lysine.

TABLE 6

Spontaneous reversion rates at an ochre site (lys1-1) *in a nuclear petite strain*

No.	Strain	Presence or absence of *pet 1*	Cells/ml ($\times 10^{-6}$)	Mutation rates ($\times 10^{8}$)			Number of revertants	
				Total	Locus	Sup*	Locus	Sup*
27.08	XV182-6C[1]	*PET*+	2.08	2.00	0.24	1.76	10	71
27.06	XV182-6D[1]	*pet1*	2.40	2.81	0.99	1.82	46	83
27.05	XV182-6C[2]	*PET*+	1.98	2.36	0.15	2.21	6	84
27.07	XV182-6D[2]	*pet1*	2.43	2.02	0.58	1.44	28	68

* Suppressors.
[1] Locus data from these experiments was summarized in Table 4 of VON BORSTEL *et al.* (1973).
[2] Medium contained 5 μg/ml adenine in order to recognize *lys1* locus revertants as red colonies (see SCHULLER and VON BORSTEL 1974).

DISCUSSION

We have demonstrated that all petite strains have enhanced spontaneous mutation rates regardless of whether the petite character is cytoplasmically or nuclearly inherited or whether they are suppressive or nonsuppressive petites. Furthermore, it is possible to conclude that modifiers of petite mutator capability exist because the petite mutator activity can differ between strains.

Since the petite character effects are on reversion of the nonsense allele *lys1-1* and the missense mutant *his1-7*, but not on reversion of the putative frameshift

mutant *hom3–10* or of forward mutation to suppression, we conclude that the petite character favours a particular base substitution or nucleotide sequence. A genetic argument has been put forth which proposes that reversions of the *lys1–1* mutant site itself are most likely to be transversions of the AT → CG or TA variety (VON BORSTEL *et al.* 1973; GOTTLIEB and VON BORSTEL 1976). This would suggest that the petite character may favour enhancement of one of these two types of molecular lesion, because the effect of the petite character on suppressor mutations is minimal and base substitutions leading to suppression could be, for example, a specific transition.

Because the neutral petite used in our experiments lacks mitochondrial DNA, we can conclude that it is the petite character itself and not an infective product of mitochondrial DNA metabolism encoded by and leaking from the defective mitochondria that causes an enhancement of the spontaneous mutation rate. Mutator activity of strains lacking the mitochondrial DNA template also excludes other possibilities, such as disturbance of nucleotide pool equilibrium by accumulation of a higher proportion of one of the base pairs in the mitochondrial DNA.

Disturbance of the equilibrium of the nucleotide pools in the petite strains does seem to provide a reasonable excuse for, but not an explanation of, the phenomenon. One can imagine that diminution of GTP or ATP or any of the deoxyribonucleotide pools may cause a tripling of the rate of a particular molecular lesion in the DNA through a variety of mechanisms. It is not unreasonable that petites may differ from grande strains in the sizes of their nucleotide pools. Also, it is known that thymine-deficient mutants of *E. coli* can have altered base transition rates by alteration of exogenous thymidine (BERNSTEIN *et al.* 1972).

Oxidative phosphorylation does not operate in petite strains, and this aspect of energy metabolism in itself could cause a sizeable alteration of the nucleotide pools. But other aspects of energy metabolism might be involved, such as the slower cell cycle in petite strains, which might favour the action of one repair system over another, or conservation of energy by petite strains simply by their permitting the mutation rate to rise.

It is evident that almost any imaginable molecular explanation for the petite mutator activity will suffice, and eventually more than one explanation may be needed. Nevertheless, the results are clear enough to demonstrate that intracellular, albeit extranuclear, influences can alter the spontaneous mutation rate, and these cannot be ignored in any account of the origins of spontaneous mutations.

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