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Reversion analysis of [*psi*$^-$] mutations in *Saccharomyces cerevisiae*

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SUMMARY

Mutants of [*psi*], a cytoplasmically inherited factor in the yeast *Saccharomyces cerevisiae* were isolated after treatment with a variety of agents including conventional mutagens and a number of compounds which cause loss of [*psi*] at high frequencies, namely methanol, KCl, dimethyl sulphoxide and guanidine hydrochloride. In [*psi*$^-$] mutants the suppressor *SUQ5* does not suppress ochre mutations such as *ade2.1*.

Reversion analysis of the [*psi*$^-$] mutants revealed three classes: (1) a class of agents producing [*psi*$^-$] mutations which could readily revert to [*psi*$^+$] (methanol, KCl and dimethyl sulphoxide belong to this class), (2) those which could not be shown to revert (GuHCl) and (3) the conventional mutagens which produced both revertible and apparently non-revertible [*psi*$^-$] mutations. We conclude that GuHCl causes a deletion or loss of the [*psi*] factor. Methanol may cause an alteration of 'state' for example, of a promoter, and KCl may be selecting or inducing low copy number variants of [*psi*$^+$] strains. It is possible that DMSO may be involved in regulation of [*psi*].

1. INTRODUCTION

Suppression of ochre mutations can occur in strains of yeast *Saccharomyces cerevisiae* carrying the suppressor mutation *SUQ5*. This suppressor codes for an altered tRNA species capable of recognizing the UAA (ochre) nonsense codon and inserting serine at this position in the chain (Liebman, Stewart & Sherman, 1975). The efficiency of suppression is determined by a cytoplasmically inherited determinant, [*psi*], which segregates in a non-Mendelian fashion in crosses (Cox, 1965). The presence of the [*psi*] factor is required for suppression of certain ochre mutations such as *ade2.1* which is only suppressed by *SUQ5* in a [*psi*$^+$] background. The isogenic *ade2.1 SUQ5* [*psi*$^-$] strain will be unsuppressed and will require adenine for growth. *SUQ5* can suppress certain ochre mutations in [*psi*$^-$] strains but this suppression is weak and sporadic (Cox, 1965).

[*psi*] has proved to be an elusive element. It is subject to the same repair pathways as nuclear genes (Tuite & Cox, 1980) and is probably a DNA plasmid. The psi$^+$ phenotype segregates independently of mitochondrial DNA markers (Young & Cox, 1972) and is found in strains lacking both species of double stranded RNA present in virus-like-particles in killer strains (our unpublished results). Most [*psi*$^-$] strains examined have the endogenous 2 μ plasmid.

Conventional mutagens such as N-methyl-N′-nitro-nitrosoguanidine (NTG), ultraviolet light (UV) and ethyl methanesulphonate (EMS) can induce [*psi*+] to [*psi*−] mutations at a frequency similar to the induction of nuclear mutations. Certain agents have been found to cause a high frequency (up to 100%) loss of [*psi*+] (Tuite *et al.* in preparation). Furthermore, these compounds induce exclusively [*psi*−] mutations, they have not been shown to affect any nuclear genes. The list of these agents includes guanidine hydrochloride (GuHCl) at 0·8–5 mM, dimethylsulphoxide (DMSO) at 2·5–10% (v/v) and a variety of compounds at high concentrations (> 1·8 M) including methanol and chloride ions. This effect is reminiscent of the plasmid 'curing' phenomenon in bacterial systems, and of the curing of mitochondria in yeast by ethidium bromide and acridine dyes.

Young & Cox (1975) have demonstrated that *ade2.1 SUQ5* [*psi*−] strains show an unusually high frequency of reversion to prototrophy ($5{\cdot}1 \times 10^{-5}$) compared with reversion of *ade2.1 SUQ5*+ [*psi*+] or [*psi*−] strains ($1{\cdot}2 \times 10^{-7}$ and $0{\cdot}5 \times 10^{-7}$) respectively). Around 90% of reversions may be attributed to the presence of recessive allosuppressors which may be thought of as nuclear mimics of [*psi*] as they allow suppression by *SUQ5* in [*psi*−] strains though they are incapable of suppression alone. The remaining 10% of the revertants are due to one of the following:

(1) reversion at the *ade* locus,

(2) the appearance of dominant nuclear suppressors which are independent of [*psi*] for their action.

(3) the appearance of a new allele at the *ade2* locus allowing suppression by *SUQ5* in a [*psi*−] background or at the *SUQ5* locus resulting in suppression of *ade2.1* in [*psi*−] strains,

(4) reversion of the cytoplasmic factor [*psi*−] to [*psi*+]. The latter class concerns us here.

A number of [*psi*−] mutants induced with a range of agents were subjected to reversion analysis. The intention was to determine the nature of [*psi*−] mutations induced by each agent. [*psi*−] mutation could occur through alteration of a nucleic acid sequence, by causing an alteration in state of a self replicating system, or by eliminating a plasmid and so curing the cell of [*psi*]. The latter class will be irreversible, as would deletions or major structural alterations of a nucleic acid sequence. [*psi*−] mutants which are revertible could be either point mutations or due to alteration in the 'state' of an autocatalytic system. An example of the latter could be copy-number variants, in which the numbers of a plasmid were maintained below a critical level required for expression of the psi+ phenotype.

2. MATERIALS AND METHODS

(i) *Strains*

The genotype of the parent strain, 243/6a, is: *α ade2.1 his4.580 SUQ5 SUP4.3* [*psi*+] where *ade2.1* is an ochre mutation conferring a requirement for adenine. In adenine requirers a pigment accumulates leading to a red colouration of the

colonies. In 243/6a the *ade2.1* mutation is suppressed by *SUQ5*, a serine inserting ochre suppressor, in the presence of the cytoplasmic factor [*psi*+]. The suppressed strain is white. In [*psi*−] strains *SUQ5* is incapable of suppression of the *ade2.1* mutation so these mutants are red. The amber mutation, *his4.580*, is suppressed by the temperature sensitive amber suppressor *SUP4.3* at 23 °C, but not at 30 °C in [*psi*+] strains (Rasse-Messenguy & Fink, 1973). α is a mating type allele.

The tester strain, which complements [*psi*−] mutations, but no other kind of non-suppressed revertant, was 152/3c of genotype *a ade2.1 his5.2 can1.100 leu1 kar1.1* [*psi*+] (Cox, Tuite & Mundy, 1980), where *a* is a mating type allele, *ade2.1*, *his5.2*, and *can1.100* are ochre mutations, *leu1* confers a requirement for leucine and *kar1.1* causes a block in karyogamy (Conde & Fink, 1976). 209/5d has the genotype *a ade2.1 SUQ5* [*psi*+].

(ii) *Media*

YC is a complete growth medium (Cox & Bevan, 1962) on which both [*rho*+] and [*rho*−] *ade2.1* mutants accumulate a deep red pigment. YNB (Difco yeast nitrogen base without amino acids) is a minimal growth medium solidified with 1·25% purified agar and supplemented with histidine at a final concentration of 10 μg/ml.

(iii) *Mutagenesis*

To obtain (*psi*−] mutants 243/6a was plated on solid YC medium with one of the following agents incorporated (the concentrations used are indicated): Methanol (12·5% v/v), GuHCl (5 mM), DMSO (10% v/v) and KCl (2·5 M). Non-suppressed (red) colonies were purified by streaking on YC medium.

Mutagenesis with the conventional mutagens was as described by Lindegren *et al.* 1965, for EMS and by Cox *et al.* 1980, for NTG. UV mutagenesis was performed by irradiating a 5 ml saline suspension of cells at about 10^7 cells/ml with a dose of 50 J.m^{-2} or 100 J.m^{-2} using a 254 nm lamp. With these mutagens, not all nonsuppressed colonies were due to mutations of [*psi*]. The [*psi*−] mutations were identified by a complementation test. Mutations to [*psi*−] are the only nonsuppressed mutants which are complemented by the tester strain 152/3c. Nonsuppressed red revertants were cross-streaked with the tester strain. Substantial white growth in the region where cells mixed indicated a [*psi*−] mutation (Cox *et al.* 1980).

(iv) *Reversion analysis*

Spontaneous reversion studies were undertaken on log phase cultures of [*psi*−] mutants induced by each of the aforementioned agents. Each isolate was grown overnight at 28 °C on YC, then suspended in 1 ml saline at about 10^7 cells/ml and aliquots plated onto selective YNB plates lacking adenine. Revertant colonies were scored after 4 to 8 days incubation at 28 °C. Reversion due to a [*psi*−] to [*psi*+] event must be distinguished from nuclear revertants having the identical phenotype. All revertants will be white on YC as they are phenotypically ADE+. Replica plating onto YC medium with 5 mM GuHCl incorporated, however caused the [*psi*+] revertant clones to become [*psi*−] and show up red as they were

then unsuppressed. The other revertants remained white as GuHCl has no affect on nuclear genes (Tuite, 1978). A comparison of the two sets of replicas, one on YC and the other on YC with 5 mM GuHCl, revealed the [*psi*$^+$] revertants. The replicas were incubated for 2 days at 28 °C then transferred to the cold (4 °C) before scoring, as this accentuates the red colouration of non-suppressed colonies.

3. RESULTS

(i) *Mutagenesis*

A 5 mM GuHCl treatment produced 100% red colonies which remained red when replica plated onto YC medium. Plating efficiency was 100% at this concentration. Growth on 12·5% methanol allowed 90% survival though the colonies were slower growing. The mutant red colonies which appeared on methanol medium sectored into red and white 'bicycle wheels' when replica plated onto YC. A similar pattern emerges after KCl treatment. 'Stable' red colonies were purified for further analysis.

After 3% EMS and 12·5 μg/ml NTG treatments the survival was 1% and 54% respectively and 3 to 4% of the cells were non-suppressed (red).

(ii) *Reversion of* [*psi*$^-$] *mutants induced by the following treatments*

(*a*) GuHCl (5 mM). Five independently isolated [*psi*$^-$] mutants induced with 5 mM GuHCl were tested for their ability to revert to [*psi*$^+$]. The selection plates were incubated for 8 days for experiment (*a*) and 5 days for experiment (*b*) before the revertants were tested as described (see Table 1). In two independent experiments where different clones were tested, no revertants to [*psi*$^+$] were

Table 1. *Spontaneous reversion of GuHCl induced* [*psi*$^-$] *mutants*

Isolate		No. of revertant colonies	Frequency of reversion to prototrophy	Frequency of reversion to [*psi*$^+$]
243/6a				
ψ^- 29	(*a*)	135	$2{\cdot}7 \times 10^{-4}$	$< 2{\cdot}0 \times 10^{-6}$
	(*b*)	60	$4{\cdot}2 \times 10^{-5}$	$< 7{\cdot}1 \times 10^{-7}$
ψ^- 31	(*a*)	65	$2{\cdot}5 \times 10^{-4}$	$< 3{\cdot}7 \times 10^{-6}$
	(*b*)	222	$2{\cdot}9 \times 10^{-4}$	$< 1{\cdot}3 \times 10^{-6}$
ψ^- 33	(*a*)	2744	$1{\cdot}1 \times 10^{-2}$	$< 4{\cdot}1 \times 10^{-6}$
ψ^- 34	(*a*)	30	$1{\cdot}6 \times 10^{-4}$	$< 5{\cdot}4 \times 10^{-6}$
	(*b*)	230	$6{\cdot}8 \times 10^{-6}$	$< 5{\cdot}3 \times 10^{-7}$
ψ^- 35	(*a*)	4	$3{\cdot}5 \times 10^{-5}$	$< 8{\cdot}7 \times 10^{-6}$
	(*b*)	256	$9{\cdot}4 \times 10^{-5}$	$< 3{\cdot}7 \times 10^{-7}$
	(*c*)	3694	$3{\cdot}5 \times 10^{-4}$	$< 9{\cdot}4 \times 10^{-8}$
209/5d				
ψ^- 1		7452	$1{\cdot}1 \times 10^{-3}$	$< 1{\cdot}4 \times 10^{-7}$
ψ^- 2		231	$2{\cdot}0 \times 10^{-5}$	$< 9{\cdot}0 \times 10^{-8}$
ψ^- 3		221	$3{\cdot}2 \times 10^{-5}$	$< 1{\cdot}5 \times 10^{-7}$
ψ^- 5		340	$1{\cdot}9 \times 10^{-5}$	$< 5{\cdot}0 \times 10^{-8}$
ψ^- 7		357	$2{\cdot}5 \times 10^{-5}$	$< 7{\cdot}0 \times 10^{-8}$

detected. All the revertants were white on both YC medium and on medium with GuHCl incorporated. The frequency of reversion to prototrophy was high, as expected (up to $1{\cdot}1 \times 10^{-2}$). Treatment with a low dose of UV light (5 J.m^{-2}) which gave 100% survival also failed to cause reversion to [*psi*$^+$] (results not shown). One of the mutants, GuHCl ψ^- 35, was tested to see if treatment with a UV dose of 50 J.m^{-2} would induce reversion of [*psi*$^-$] to [*psi*$^+$] as this is a dose shown to cause [*psi*$^-$] mutations in 243/6a. The frequency of reversion to proto-

Table 2. *Spontaneous reversion of* [*psi*$^-$] *mutants induced with 12·5% methanol*

Isolate	No. of revertant colonies	No. of psi$^+$ revertants	Frequency of reversion to prototrophy	Frequency of reversion to [*psi*$^+$]
ψ^- 5	(*a*) 48	3	$6{\cdot}0 \times 10^{-5}$	$3{\cdot}8 \times 10^{-6}$
	(*b*) 435	14	$1{\cdot}0 \times 10^{-4}$	$3{\cdot}2 \times 10^{-6}$
ψ^- 9	38	10	$1{\cdot}0 \times 10^{-4}$	$2{\cdot}7 \times 10^{-5}$
ψ^- 10	159	1	$1{\cdot}7 \times 10^{-4}$	$1{\cdot}1 \times 10^{-6}$
ψ^- 19	95	10	$4{\cdot}5 \times 10^{-5}$	$4{\cdot}8 \times 10^{-6}$
ψ^- 22	109	18	$1{\cdot}0 \times 10^{-4}$	$1{\cdot}8 \times 10^{-5}$
ψ^- 24	(*a*) 58	22	$1{\cdot}0 \times 10^{-4}$	$3{\cdot}8 \times 10^{-5}$
	(*b*) 1116	19	$3{\cdot}8 \times 10^{-4}$	$6{\cdot}4 \times 10^{-6}$
ψ^- 32	65	18	$6{\cdot}5 \times 10^{-5}$	$1{\cdot}8 \times 10^{-5}$
ψ^- 35	80	3	$8{\cdot}0 \times 10^{-5}$	$3{\cdot}0 \times 10^{-6}$
ψ^- 48	51	20	$6{\cdot}5 \times 10^{-5}$	$2{\cdot}5 \times 10^{-5}$
ψ^- 51	35	3	$9{\cdot}1 \times 10^{-8}$	$7{\cdot}8 \times 10^{-6}$

trophy for the untreated control and the UV treatment was $3{\cdot}5 \times 10^{-4}$ and $8{\cdot}5 \times 10^{-4}$ respectively. No [*psi*$^-$] to [*psi*$^+$] revertants were found; the frequency of reversion of [*psi*] was less than $9{\cdot}4 \times 10^{-8}$ for the control and less than $1{\cdot}0 \times 10^{-7}$ for the UV treated sample.

Five independent [*psi*$^-$] isolates of strain 209/5d were tested in the same manner and gave similar results (see Table 1.) The number of cells tested varied between 7×10^6 and 2×10^7. The incubation period of the selection plates was 9 days.

(*b*) Methanol (12·5% v/v). Between $3{\cdot}7 \times 10^5$ and $1{\cdot}0 \times 10^6$ cells were plated onto selective minimal plates which were incubated for 7 days prior to replica plating. The number of revertants tested varied between 35 and 1116. (*a*) and (*b*) represent two independent experiments. Each of the ten independently isolated [*psi*$^-$] mutants induced with methanol yielded [*psi*$^+$] reversions indicated by colonies which were white on YC and red on YC with 5 mM GuHCl (see Table 2). These do not necessarily represent independent reversions; they could be the result of multiplication of a single or a few revertants in the clone.

(*c*) DMSO (10%). In a preliminary experiment to test the DMSO induced [*psi*$^-$] mutants, the selective plates were scored after 4 days incubation at 28 °C and replica plated. The revertants which came up during this time were not [*psi*$^+$] revertants, but it was observed that a number of small colonies appeared during

the incubation period following replica plating. Table 3 illustrates the results obtained when the selective plates were allowed to incubate for 10 days before testing. The [*psi*$^+$] revertants appeared only after prolonged incubation (4–10 days) at 28 °C.

(*d*) KCl (2·5 M). The reversion pattern was similar to that described for the DMSO induced mutants. Eight KCl [*psi*$^-$] mutants were tested, two of these were found to be leaky so that after 3 days incubation on selective medium several

Table 3. *Spontaneous reversion of* [*psi*$^-$] *mutants produced by DMSO treatment*

Isolate	No. of revertant colonies	No. of psi$^+$ revertants	Frequency of reversion to prototrophy	Frequency of reversion to [*psi*$^+$]
ψ^- 1	583	94	$4{\cdot}3 \times 10^{-5}$	$6{\cdot}9 \times 10^{-6}$
ψ^- 10	1349	333	$3{\cdot}8 \times 10^{-5}$	$9{\cdot}5 \times 10^{-6}$
ψ^- 35	1829	567	$7{\cdot}6 \times 10^{-5}$	$2{\cdot}4 \times 10^{-6}$
ψ^- 38	3422	417	$1{\cdot}1 \times 10^{-4}$	$1{\cdot}3 \times 10^{-5}$

Table 4. *Spontaneous reversion of KCl* [*psi*$^-$] *mutants*

Isolate	No. of revertant colonies	No. of psi$^+$ revertants	Frequency of reversion to prototrophy	Frequency of reversion to [*psi*$^+$]
ψ^- 1	394	183	$3{\cdot}1 \times 10^{-3}$	$1{\cdot}5 \times 10^{-3}$
ψ^- 2	2931	66	$6{\cdot}8 \times 10^{-4}$	$1{\cdot}5 \times 10^{-5}$
ψ^- 6	3435	277	$5{\cdot}4 \times 10^{-4}$	$4{\cdot}4 \times 10^{-5}$
ψ^- 12	4141	172	$7{\cdot}6 \times 10^{-4}$	$3{\cdot}2 \times 10^{-5}$
ψ^- 14	5692	339	$1{\cdot}1 \times 10^{-3}$	$6{\cdot}5 \times 10^{-5}$
ψ^- 16	4822	702	$1{\cdot}3 \times 10^{-3}$	$1{\cdot}9 \times 10^{-4}$

ADE$^+$ revertants came up and after a further overnight incubation a lawn appeared. No [*psi*$^+$] revertants had appeared after 4 days among any of the remaining mutants. Many revertants appeared after 6 days incubation and [*psi*$^+$] revertants were among these. The [*psi*$^-$] revertants have a longer lag period when compared with the other classes of revertant which result in a prototrophic phenotype. The plates were replica plated after 6 days incubation at 28 °C.

(*e*) EMS (3 % v/v). Reference to Table 5 shows that three groups of [*psi*$^-$] mutants were found:

1. EMS ψ^- 3 yielded no prototrophs, suggesting the absence of psi-independent suppressors and allosuppressors.
2. EMS ψ^- 4, 5 and 7 showed reversion to prototrophy but no [*psi*$^+$] revertants.
3. EMS ψ^- 2, 6 and 8 showed reversion of [*psi*$^-$] to [*psi*$^+$].

The revertants were allowed 8 days for growth before replica plating.

(*f*) NTG (12·5 μg/ml). The results in Table 5 represent an analysis of revertants which grew within 6 days. The mutants fall into three groups as described for the

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EMS induced [*psi*$^-$] mutants. NTG ψ^- 1, 3, 4 and 6 fall into group (1), NTG ψ^- 2 and 5 into group (2) and NTG ψ^- 7 into group (3). A UV treatment of 50 J.m^{-2} did not produce any revertants of [*psi*$^-$] to [*psi*$^+$] in the isolate NTG ψ^- 5 (results not shown).

Table 5. *Spontaneous reversion of* [*psi*$^-$] *mutants induced with conventional mutagens*

Isolate	No. of revertant colonies	No. of psi$^+$ revertants	Frequency of reversion to prototrophy	Frequency of reversion to [*psi*$^+$]
EMS ψ^- 2	459	178	$3{\cdot}0 \times 10^{-5}$	$1{\cdot}2 \times 10^{-5}$
EMS ψ^- 3	0	0	$< 3{\cdot}5 \times 10^{-8}$	$< 3{\cdot}5 \times 10^{-8}$
EMS ψ^- 4	2780	0	$1{\cdot}2 \times 10^{-3}$	$< 4{\cdot}3 \times 10^{-7}$
EMS ψ^- 5	2357	0	$2{\cdot}2 \times 10^{-4}$	$< 9{\cdot}5 \times 10^{-8}$
EMS ψ^- 6	117	30	$9{\cdot}9 \times 10^{-6}$	$2{\cdot}6 \times 10^{-6}$
EMS ψ^- 7	6	0	$5{\cdot}0 \times 10^{-7}$	$< 7{\cdot}5 \times 10^{-8}$
EMS ψ^- 8	1149	948	$2{\cdot}3 \times 10^{-4}$	$2{\cdot}0 \times 10^{-4}$
NTG ψ^- 1	0	0	$< 8{\cdot}9 \times 10^{-8}$	$< 8{\cdot}9 \times 10^{-8}$
NTG ψ^- 2	28	0	$3{\cdot}8 \times 10^{-5}$	$< 6{\cdot}0 \times 10^{-7}$
NTG ψ^- 3	0	0	$< 5{\cdot}8 \times 10^{-8}$	$< 5{\cdot}8 \times 10^{-8}$
NTG ψ^- 4	0	0	$< 4{\cdot}8 \times 10^{-8}$	$< 4{\cdot}8 \times 10^{-8}$
NTG ψ^- 5	303	0	$2{\cdot}9 \times 10^{-5}$	$< 9{\cdot}8 \times 10^{-8}$
NTG ψ^- 6	0	0	$< 9{\cdot}5 \times 10^{-8}$	$< 9{\cdot}5 \times 10^{-8}$
NTG ψ^- 7	552	38	$4{\cdot}6 \times 10^{-5}$	$3{\cdot}2 \times 10^{-6}$
50 J.m^{-2} ψ^- 1	348	8	$6{\cdot}5 \times 10^{-5}$	$1{\cdot}5 \times 10^{-6}$
50 J.m^{-2} ψ^- 2	1374	14	$1{\cdot}6 \times 10^{-5}$	$2{\cdot}7 \times 10^{-6}$
50 J.m^{-2} ψ^- 4	50	0	$7{\cdot}7 \times 10^{-6}$	$< 5{\cdot}1 \times 10^{-7}$
50 J.m^{-2} ψ^- 5	183	0	$6{\cdot}2 \times 10^{-5}$	$< 3{\cdot}4 \times 10^{-7}$
50 J.m^{-2} ψ^- 9	755	8	$2{\cdot}6 \times 10^{-4}$	$2{\cdot}7 \times 10^{-6}$

(*g*) UV dose of 50 J.m^{-2}. All of the UV induced [*psi*$^-$] mutants showed reversion to prototrophy. No spontaneous [*psi*$^+$] revertants were obtained from the isolates ψ^- 3 or ψ^- 4. The remaining three [*psi*$^-$] mutants showed reversion of [*psi*]. The results shown in Table 5 were obtained after incubation of 6 to 7 days on minimal selective medium.

4. DISCUSSION

The agents causing the conversion of a psi$^+$ to a psi$^-$ phenotype fall into three classes:

(i) agents producing mutations to [*psi*$^-$] which can revert readily, namely methanol, KCl and DMSO,

(ii) those resulting in mutations which could not be shown to revert, namely GuHCl,

(iii) the conventional mutagens which produced both revertible and apparently non-revertible [*psi*$^-$] mutations. These mutagens include NTG, EMS and UV light.

The observation that no revertants of GuHCl induced [*psi*$^-$] mutations could be detected suggests that the mutation caused is a deletion or loss of the factor.

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GuHCl is a protein denaturing agent which is known to affect other cytoplasmically located elements, namely the killer plasmid and mitochondrial DNA. Juliani, Gambarini & Costa (1975*a*) reported that GuHCl induced suppressive petite mutations at a high frequency and that these petites showed altered mitochondrial DNA. They postulate that the target is the mitochondrial membrane, specifically one or more proteins involved in the attachment site of the mitochondrial DNA to the inner membrane so interfering with the normal replication of the DNA molecule (Juliani & Costa, 1975*b*). That they are suppressive petites led the authors to conclude that GuHCl causes deletions in mitochondrial DNA. It is conceivable that membrane attachment sites for the [*psi*] factor are affected in a similar way, preventing [*psi*] replication or causing deletions. A non-revertible 'cured' mutation of a cytoplasmically inherited phenotype is incompatible with its maintenance by means of an autocatalytic regulatory 'state' of a nuclear determinant. 'Mutations' of such systems should always be revertible or, if not, inherited as a chromosomal marker.

Methanol is an unexpected compound in the list of [*psi*] mutagens. It has not been shown to be mutagenic in the Ames test system. The [*psi*$^-$] mutations induced by methanol were shown to revert. Methanol has not previously been reported to cause a change in a nucleic acid sequence in any other system. It is possible, therefore, that methanol is inducing an alteration of 'state' for example by influencing the state of a promoter. 'Leakiness' was observed on some [*psi*$^-$] clones, which could be the result of an intermediate level of expression of a [*psi*$^+$] determinant. On the other hand, methanol has not been exhaustively tested as a mutagen in the very high concentrations used to induce [*psi*$^-$] mutations in (up to 3·9 M). It is not impossible that at these levels it may cause base pair changes. Mundy found five nuclear mutants among a selection of 155 nonsuppressed revertants induced by methanol (Mundy D.Phil. thesis, Tuite, Mundy & Cox, 1980). The preponderance of cytoplasmic reversions in this case may reflect a greater accessibility of the [*psi*$^+$] determinant to the agent.

The reversion patterns of the KCl and DMSO [*psi*$^-$]s are particularly interesting. The earliest revertants that appear on the minimal plates are not [*psi*$^+$] revertants, these appear only after prolonged incubation. Revertibility, of course, implies a point mutation of some kind. However, if such reversion from [*psi*$^-$] point mutations occur, they should, like other revertible mutants be expressible at the time of plating and would appear after the normal interval of incubation. The delayed appearance of the [*psi*$^+$] revertants compared with the other non-suppressed types on these selective plates suggests an 'adaptation' phenomenon. 'Adaptation' could occur as a result of a change of an autocatalytically regulated rate of expression of a determinant from a level too low to have a phenotypic affect to one which allows suppression to occur. Alternatively, it could result from a switch from a low copy number of the [*psi*] determinant to a high copy number necessary for suppression. The signal to increase the copy number may be produced by plating onto a minimal medium lacking adenine.

There is an indication that KCl may select or induce low copy number variants of [*psi*$^+$] strains. Tuite (1978) discovered that many clones recovered after growth of

[*psi*+] strains in 1·8 to 2·5 M KCl solutions maintained replicating instabilities, frequently segregating into stable [*psi*+] suppressed and unstable [*psi*−] clones. We have observed that pure red colonies on media with 2·5 M KCl incorporated sector into suppressed (white) [*psi*+] and unsuppressed [*psi*−] 'bicycle wheels' on transfer to complete medium lacking KCl. Secondly, leakiness was observed with some of the [*psi*−] clones when plated onto selective minimal medium lacking adenine. These observations together suggest that the psi− phenotype might be the consequence of a plasmid copy number maintained at a threshold level for [*psi*+] expression.

DMSO is a membrane active agent (Oliver & Williamson, 1977) which overcomes promoter deficiencies in *E. coli* (Nakanashi *et al.* 1974) and promotes Friend cell differentiation (Friend *et al.* 1971). The DMSO induced [*psi*−] mutants are neither leaky nor very unstable. They differ from the KCl induced [*psi*−] mutants in these respects. DMSO very strongly selects against the growth of [*psi*+] strains so the basis of 'induction' of [*psi*−] mutants by this compound may be the induction or repression of a regulatory 'state'.

Revertible [*psi*−] mutations induced by EMS, NTG and UV irradiation were found, suggesting reversion of a conventional point mutation. Other mutants, namely EMS ψ^- 3, NTG ψ^- 1, 3, 4 and 6 showed no detectable reversion to protrotrophy, hence no observable phenotypic effect of the generally ubiquitous allosuppressors. These revertants, however, show the suppressed phenotype when [*psi*+] is introduced by cytoduction.

The appearance of both revertible and non-revertible [*psi*−] mutations induced by conventional mutagens supports the hypothesis that [*psi*] is DNA and that the [*psi*−] mutations are due to alterations in nucleotide sequences. The mutations induced by KCl, methanol and DMSO may also be base substitutions. However, it is also possible they represent 'change of state' or low copy number variants.

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REFERENCES

Conde, J. & Fink, G. R. (1976). A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. *Proceedings of the National Academy of Science of the United States of America* **73**, 3651–3655.

Cox, B. S. (1965). ψ, a cytoplasmic suppressor of super-suppressor in yeast. *Heredity* **20**, 505–521.

Cox, B. S. & Bevan, E. A. (1962). Aneuploidy in yeast. *New Phytologist* **61**, 342–355.

Cox, B. S., Tuite, M. F. & Mundy, C. J. (1980). Reversion from suppression to non-suppression in SUQ5 [*psi*+] strains of yeast: the classification of mutations. *Genetics* **95**, 589–609.

Friend, C., Scher, W., Holland, J. G. & Sato, T. (1971). Hemoglobin synthesis in murine virus-induced leukemic cells *in vitro*: stimulation of erythroid differentiation by dimethyl sulfoxide. *Proceedings of the National Academy of Sciences of the United States of America* **65**, 378–382.

Juliani, M. H., Gambarini, A. G. & Costa, S. O. P. (1975*a*). Induction of ρ^- mutants in *Saccharomyces cerevisiae* by guanidine hydrochloride. I. Genetic analysis. *Mutation Research* **29**, 67–75.

Juliani, M. H. & Costa, S. O. P. (1975*b*). Induction of ρ^- mutants in *Saccharomyces cerevisiae* by guanidine hydrochloride. II. Conditions that prevent ρ^- induction. *Mutation Research* **30**, 335–342.

Liebman, S. W., Stewart, J. W. & Sherman, F. (1975). Serine substitutions caused by an ochre suppressor in yeast. *Journal of Molecular Biology* **94**, 595–610.

Lindegren, G., Hwang, Y. L., Oshima, Y. & Lindegren, C. C. (1965). Genetical mutants induced by ethyl mthanesulfonate in *Saccharomyces*. *Canadian Journal of Genetics and Cytology* **7**, 491–499.

Mundy, C. R. (1979). The genetics of translation in yeast. D.Phil. thesis, Oxford University, Oxford, U.K.

Nakanishi, S., Adhya, S., Gottesman, M. & Pastan, I. (1974). Effects of dimethylsulfoxide on the *E. coli gal* operon and on bacteriophage lambda *in vivo*. *Cell* **3**, 39–46.

Oliver, S. G. & Williamson, D. H. (1977). Mutants of yeast specifically resistant to petite induction by fluorinated pyrimidines. *Biochemical Genetics* **15**, 775–783.

Rasse-Messenguy, F. & Fink, G. R. (1973). Temperature-sensitive nonsense suppressors in yeast. *Genetics* **75**, 459–464.

Tuite, M. F. (1978). Genetics of nonsense suppressors in yeast. D.Phil. thesis, Oxford University, Oxford, U.K.

Tuite, M. F. & Cox, B. S. (1980). Ultraviolet mutagenesis studies of [*psi*], a cytoplasmic determinant of *Saccharomyces cerevisiae*. *Genetics*. **95**, 611–630.

Tuite, M. F., Mundy, C. J. & Cox, B. S. (1980). A study of some non-mutagenic substances that cause reversion from [*psi*$^+$] to [*psi*$^-$] in *Saccharomyces cerevisiae*. *Genetics*. (In the Press.)

Young, C. S. H. & Cox, B. S. (1972). Extrachromosomal elements in a super-suppression system of yeast. II. Relations with other extrachromosomal elements. *Heredity* **28**, 189–199.

Young, C. S. H. & Cox, B. S. (1975). Extrachromosomal elements in a super-suppression system of yeast. III. Enhanced detection of weak suppressors in certain non-suppressed strains. *Heredity* **34**, 83–92.