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Genetics

Nonsense suppressors of yeast cause osmotic-sensitive growth

(tRNA mutants/mapping/*Saccharomyces cerevisiae*/efficiency of suppression/antisuppressors)

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ABSTRACT Many nonsense suppressors of *Saccharomyces cerevisiae* cause growth inhibition on hypertonic media. Eight tyrosine-inserting UAA (ochre) suppressors, eight tyrosine-inserting UAG (amber) suppressors, a leucine-inserting UAG suppressor, and a serine-inserting recessive lethal UAG suppressor cause osmotic sensitivity, whereas a serine-inserting UAA suppressor does not cause sensitivity. Although the mechanism is not understood, the growth inhibition of specific suppressors on hypertonic media is correlated with their efficiencies of suppression. This heretofore unknown property of nonsense suppressors is useful for mitotic mapping, selecting tRNA mutants, selecting antisuppressors, and scoring nonsense suppressors.

Numerous suppressors of nonsense mutations in yeast have been isolated and genetically mapped (see ref. 1, for a recent review). Various of these suppressors in the yeast *Saccharomyces cerevisiae* have been demonstrated to insert residues of tyrosine (2–4), serine (5, 6), and leucine (7) at the sites of either UAG (amber) or UAA (ochre) codons. Like nonsense suppression in *Escherichia coli* (see ref. 8, for a review), nonsense suppression in yeast is due to altered tRNAs that have gained the ability to translate chain-terminating codons (9, 10). In this paper† I report that yeast strains carrying certain of these nonsense suppressors are unable to grow on hypertonic media. Although the mechanism responsible for the osmotic sensitivity is unknown, this property enables the scoring of nonsense suppressors in the absence of suppressible markers and can be used in studies that require revertants with reduced or no suppressor activity.

MATERIALS AND METHODS

Yeast Strains and Genetic Nomenclature. The basic strains used in this study are described in Table 1. Only the abbreviated version of the recently described (4) nomenclature for the genotypes of suppressors will be used here. The UAA (ochre) and UAG (amber) suppressors are indicated, respectively, by *o* and *a* following the locus designation (1). For example *SUP2-o* and *SUP2-a* refer to, respectively, UAA and UAG suppressors of the *SUP2* locus. UAA suppressors and UAG suppressors of yeast refer to suppressors that act on, respectively, UAA and UAG mutants. *sup-RL1* refers to the recessive-lethal UAG suppressor (6). The wild-type symbol, *sup+*, designates strains lacking all suppressors. A non-Mendelian genetic determinant ψ^+ increases the efficiency of suppression of the UAA suppressors (5, 12, 13). The ψ factor has no effect on amber suppressors (13). Strain no. 12 is ψ^+. All other ochre suppressor-bearing strains are ψ^-.

Media. The routine nutrient medium consisted of 1% Bacto-yeast extract, 2% Bacto-peptone, 2% dextrose, and when needed for solidification, 2% ionagar (Wilson Diagnostic, Inc.). For cytochrome *c* analysis, only 1% dextrose was used in the growth media. The hypertonic media were prepared by supplementing nutrient medium with potassium chloride, ethylene glycol, dextrose, or glycerol.

Scoring Methods. Growth of various strains on hypertonic media was tested by transferring drops of suspensions of freshly grown cells onto normal and hypertonic nutrient media which were then incubated at 30° for 1–6 days. Cell suspensions were transferred from compartmented dishes by the use of a metal head with inoculating rods spaced at intervals corresponding to the compartments. The nutritional markers and the canavanine-resistant marker (*can1*) were also scored by testing growth of cell suspensions on appropriate media. On a technical note, certain batches of hypertonic media, especially if they are allowed to dry for more than a few days, do not support growth of *any* yeast strain. It is therefore essential to test all such media using a control strain known to be capable of growth on hypertonic media.

Quantitative Growth Measurements. Fresh stationary phase cells were used to inoculate nutrient medium alone and the nutrient medium supplemented with 2.5 M ethylene glycol. The initial cell density of the inoculated cultures was 1 to 2 × 10^6 cells per ml. The cultures were incubated at 30° with vigorous shaking, and growth was followed by measuring turbidity with a model 800-3 Klett-Summerson photoelectric colorimeter using a no. 62 (560–650) light filter.

Cytochrome *c* Analysis. The efficiency of suppression of *cyc1* alleles was scored by estimating the level of cytochrome *c* using low-temperature (−190°) spectroscopic examination of whole cells (14). For representative strains, low-temperature spectrophotometric recordings were also made using a modified Cary model 14 spectrophotometer (15).

RESULTS

Association of Osmotic Sensitivity with an Ochre Suppressor. A detailed analysis of osmotic sensitivity associated with nonsense suppressors of yeast was undertaken following an observation that a strain carrying UAA suppressor, *SUP4-o*, did not grow on nutrient medium that contained 2.5 M ethylene glycol whereas growth was observed for related strains that did not contain the suppressor. The analysis of eight tetrads from a diploid that was heterozygous for *SUP4-o* showed that the inability to grow on the hypertonic medium always segregated with the *SUP4-o* mutation, indicating that either the *SUP4-o* itself or a linked gene prevents growth on this medium. To distinguish between the two possibilities, revertants of suppressor-bearing strain CL180-156B were analyzed. Since the *his5-2, trp5-48,* and *can1-100* are ochre mutations that are suppressed by *SUP4-o,* the strain is canavanine-sensitive and does not require histidine or tryptophan for growth. Revertants of this strain, selected for canavanine resistance, that had become auxotrophic for histidine and tryptophan concomitantly, were judged to have lost suppressor activity. Twelve such revertants that were tested had all gained the ability to grow on the ethylene glycol medium. In a separate experiment, all 30 rev-

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† The results have been briefly reported previously [A. Singh (1976) *Genetics* 83, s72].

Table 1. Basic strains

Strain no.	Strain designation	Genotype	Source
1	CL180-156B	*SUP4-o* α *his5-2 trp5-48 can1-100 cyc1-13 ilv3 met1*	C. W. Lawrence
2	R8	*sup4-o-R1* α *his5-2 trp5-48 can1-100 cyc1-13 ilv3 met1*	This work
3	W87	*sup+* **a** *D ade2-1 lys2-1 trp5-2 leu1-12 ura1-1 + + met4-1* *sup+* α *d ade2-1 lys2-1 trp5-2 leu1-12 + ura3-1 can1-100 +*	(11)
4	952RW87	*SUP2-o* **a** *D ade2-1 lys2-1 trp5-2 leu1-12 ura1-1 + + met4-1* *sup+* α *d ade2-1 lys2-1 trp5-2 leu1-12 + ura3-1 can1-100 +*	(11)
5	22RW87	*SUP3-o* **a** *D ade2-1 lys2-1 trp5-2 leu1-12 ura1-1 + + met4-1* *sup+* α *d ade2-1 lys2-1 trp5-2 leu1-12 + ura3-1 can1-100 +*	(11)
6	164RW87	*SUP4-o* **a** *D ade2-1 lys2-1 trp5-2 leu1-12 ura1-1 + + met4-1* *sup+* α *d ade2-1 lys2-1 trp5-2 leu1-12 + ura3-1 can1-100 +*	(11)
7	186RW87	*SUP5-o* **a** *D ade2-1 lys2-1 trp5-2 leu1-12 ura1-1 + + met4-1* *sup+* α *d ade2-1 lys2-1 trp5-2 leu1-12 + ura3-1 can1-100 +*	(11)
8	64RW87	*SUP6-o* **a** *D ade2-1 lys2-1 trp5-2 leu1-12 ura1-1 + + met4-1* *sup+* α *d ade2-1 lys2-1 trp5-2 leu1-12 + ura3-1 can1-100 +*	(11)
9	30RW87	*SUP7-o* **a** *D ade2-1 lys2-1 trp5-2 leu1-12 ura1-1 + + met4-1* *sup+* α *d ade2-1 lys2-1 trp5-2 leu1-12 + ura3-1 can1-100 +*	(11)
10	16RW87	*SUP8-o* **a** *D ade2-1 lys2-1 trp5-2 leu1-12 ura1-1 + + met4-1* *sup+* α *d ade2-1 lys2-1 trp5-2 leu1-12 + ura3-1 can1-100 +*	(11)
11	136RW87	*SUP11-o* **a** *D ade2-1 lys2-1 trp5-2 leu1-12 ura1-1 + + met4-1* *sup+* α *d ade2-1 lys2-1 trp5-2 leu1-12 + ura3-1 can1-100 +*	(11)
12	AS455-13B	*SUQ5-o* **a** *cyc1-2 ade2-1 lys1-1 his5-2 can1-100*	This work
13	AS467-5A	*SUP6-o* **a** *cyc1-2 arg4-17 met1*	This work
14	D588-6C	*sup+* **a** *cyc1-76 trp1-1 tyr7-1 leu2-1 lys1-1*	(3)
15	B-1782	*SUP6-a* **a** *cyc1-76 trp1-1 tyr7-1 leu2-1 lys1-1*	(3)
16	B-1765	*SUP7-a* **a** *cyc1-76 trp1-1 tyr7-1 leu2-1 lys1-1*	(3)
17	A95	*SUP-a* **a** *cyc1-76 trp1-1 tyr7-1 leu2-1 lys1-1*	This work
18	SL210-3A	*sup+* **a** *cyc1-179 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys1-1*	(4)
19	L-113	*SUP2-a* **a** *cyc1-179 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys1-1*	(4)
20	L-138	*SUP3-a* **a** *cyc1-179 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys1-1*	(4)
21	L-165	*SUP-4-a* **a** *cyc1-179 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys1-1*	(4)
22	L-133	*SUP5-a* **a** *cyc1-179 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys1-1*	(4)
23	L-270	*SUP8-a* **a** *cyc1-179 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys1-1*	(4)
24	L-137	*SUP11-a* **a** *cyc1-179 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys1-1*	(4)
25	L-125	*SUP52-a* **a** *cyc1-179 met8-1 tyr7-1 trp1-1 ade3-26 ilv1-1 leu2-1 his5-2 lys1-1*	(4)
26	MBD16	*SUP-RL1* **a** *cyc1-179 ade3-26 his1 his5-2 leu2-1 lys1-1 trp1-1 tyr7-1 can1* *sup+* α *cyc1-179 + + his5-2 + + trp1-1 tyr7-1 can1*	(6)

The *ade2-1, arg4-17, can1-100, cyc1-2, his5-2, leu2-1, lys1-1, lys2-1 met4-1, trp5-2,* and *trp5-48* markers are ochre-suppressible; *ade3-26, cyc1-76, cyc1-179, ilv1-1, met8-1, trp1-1,* and *tyr7-1* are amber-suppressible; and others are not suppressible by either ochre or amber suppressors. Isogenic sets of strains are grouped together; strain 2 was directly derived from strain 1, strains 4–11 from strain 3, strains 15–17 from strain 14, and strains 19–25 from strain 18.

ertants of CL180-156B selected for growth on the hypertonic medium had lost the suppressor activity. From some reversion experiments, strains were occasionally recovered that still had detectable suppressor activity although the level of suppression, as judged by the suppression of auxotrophic markers, was markedly reduced. These partial revertants had correspondingly an intermediate level of growth on the hypertonic medium. The partial revertants will be discussed later. The reversion analysis thus established that suppression by *SUP4-o* causes growth inhibition on the hypertonic medium.

Growth inhibition caused by *SUP4-o* is not specific to ethylene glycol. The effect is seen when the nutrient medium is made hypertonic by addition of potassium chloride, dextrose, glycerol, or ethylene glycol. Tests using various concentrations of these compounds showed that approximate concentrations that permit clearest distinction between *SUP4-o* and *sup+* strains by the method described in ***Materials and Methods*** are: potassium chloride, 1.5 M; others, 2.5 M. Fig. 1 shows a comparison of growth characteristics of these two strains on normal nutrient medium and on nutrient medium made hypertonic with 2.5 M ethylene glycol. There is no significant difference in growth properties of these two strains on the normal nutrient medium. On the hypertonic medium, however, the suppressor strain does not grow at all, whereas the strain without the sup-

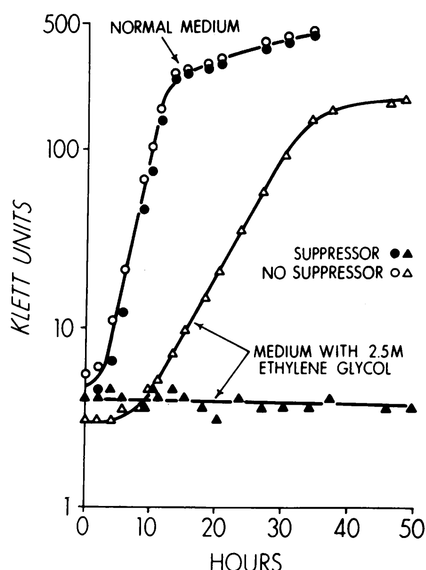

FIG. 1. Growth characteristics of a *SUP4-o* strain and an isogenic strain without the suppressor on normal and on hypertonic media. The two strains are, respectively, strain nos. 1 and 2 in Table 1. The strain without the suppressor was a spontaneous revertant of strain no. 1 and was selected for canavanine resistance and concomitant loss of suppression of the *his* and the *trp* markers.

pressor grows, although the growth rate is poorer than on normal nutrient medium.

To test whether the osmotic sensitivity caused by *SUP4-o* is a dominant or a recessive property, the *SUP4-o* strains were crossed to wild-type, *sup+*, strains that grew well on the hypertonic media. These diploids consistently failed to grow on the ethylene glycol medium, whereas on the other hypertonic media their growth levels could not be distinguished from the growth level of the haploid *sup+* strain. The reason for this specificity for diploid strains is not understood. However, the same phenomenon is observed with other ochre and amber suppressors that are osmotic-sensitive (see below).

Other Ochre Suppressors. Strains carrying other ochre (UAA) suppressors were also tested for their growth response on hypertonic media. These results are summarized in Table 2. Strain no. 3 (Table 1) grew on all hypertonic media. The eight strains (nos. 4–11) derived from it, each carrying a different UAA-specific suppressor, grew on hypertonic media containing potassium chloride, dextrose, or glycerol, but none of these grew on hypertonic medium containing ethylene glycol. The haploid strains containing any one of these suppressors show osmotic-sensitive growth on all four hypertonic media. Two revertants of each of the eight *SUP/sup+* strains were isolated on ethylene glycol, and all of these revertants had lost suppressor activity, as judged by absence of growth on adenineless, lysineless, or tryptophanless media.

Another UAA suppressor, *SUQ5-o*, does not cause growth inhibition on hypertonic media. The *SUQ5-o* strain listed in Table 1 (no. 12) contains the non-Mendelian genetic determinant ψ^+, without which suppression by *SUQ5-o* is barely detectable (5, 12, 13). In addition, tests with several other ψ^+ *SUQ5-o* as well as ψ^- *SUQ5-o* strains have shown that in these strains also *SUQ5-o* is not associated with osmotic-sensitive growth.

Osmotic Sensitivity Caused by Amber Suppressors. To determine if osmotic sensitivity is a characteristic of all tyrosine-inserting suppressors or whether it is restricted to tyrosine-inserting ochre suppressors only, I also tested amber suppressors for their effect on growth on hypertonic media. The tests included all suppressors for which amino acid insertions are known (Table 2). Strain nos. 14–26 (Table 1) were the basic strains used in these tests. Strain no. 14 grew on the hypertonic media whereas the *SUP6-a* and *SUP7-a* strains directly derived from it did not. Strain no. 17, which contains an UAG suppressor mutation, *SUP-a*, at an unidentified locus, was not osmotic-sensitive. The osmotic-sensitive growth response caused by the UAG suppressors, *SUP6-a* and *SUP7-a*, in haploid and diploid strains was similar to that of UAA suppressors described above.

Table 2. Osmotic-sensitive growth response of various nonsense suppressors

Suppressor	Type	Amino acid inserted (ref.)	Growth response
SUP2-o, SUP3-o, SUP4-o, SUP5-o, SUP6-o, SUP7-o, SUP8-o, SUP11-o	UAA	Tyrosine (3)	Sensitive
SUQ5-o	UAA	Serine (6)	Normal
SUP2-a, SUP3-a, SUP4-a, SUP5-a, SUP6-a, SUP7-a, SUP8-a*, SUP11-a*	UAG	Tyrosine (5)	Sensitive
SUP52-a	UAG	Leucine (8)	Sensitive
SUP-RL1	UAG	Serine (7)	Sensitive
SUP-a	UAG	Unknown	Normal

* Amino acid inserted was inferred from genetic data.

The osmotic sensitivity of amber suppressor strains derived from SL210-3A (strain no. 18) could not be tested directly because the parent strain did not grow well on most hypertonic media. It should be noted here that other mutations in *S. cerevisiae* have been identified that cause osmotic sensitivity and that are not related to suppressors (A. Singh and F. Sherman, manuscript in preparation). Studies with amber suppressors were further complicated because many amber suppressor strains grow poorly on glycerol complete medium (1, 16), and strains with poor growth on glycerol medium have correspondingly poor growth on hypertonic media (Singh, unpublished). The results shown in Table 2 were obtained by a combination of pedigree analysis and reversion analysis, and by testing diploids obtained by crossing the haploid strains to *sup+* strains that grew well on hypertonic media. The osmotic sensitivity associated with *SUP52-a* was recessive on all the hypertonic media whereas the growth inhibition caused by other amber suppressors was, as with the ochre suppressors, dominant on the ethylene glycol and recessive on the other hypertonic media.

While testing numerous subclones of various UAA and UAG suppressor strains, clones of some UAG suppressor strains were occasionally encountered that had retained the suppressors, as revealed by their effect on the suppressible auxotrophic markers, but that were not inhibited by hypertonic media. This instability is apparently related to efficiency of suppression. Many highly efficient UAG suppressors are notoriously unstable; suppressors with lower efficiencies are frequently found in cultures of these suppressors (16). Two different alleles of *SUP5-a*, one of the high-efficiency variety and the other of low-efficiency variety, described by Liebman and Sherman (16) were tested for osmotic sensitivity. It was found that the diploids heterozygous for the high-efficiency *SUP5-a* did not grow on the ethylene glycol medium whereas the diploids heterozygous for the low-efficiency *SUP5-a* did.

Osmotic Sensitivity and Efficiency of Suppression. Since high efficiency of suppression by both UAA (12, 13) and UAG

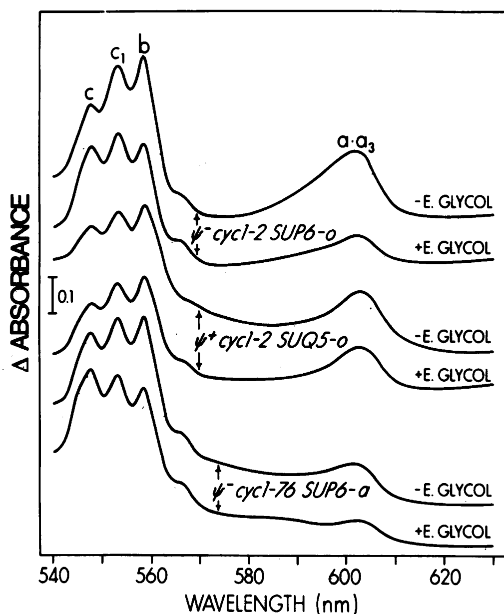

FIG. 2. Low temperature (−190°) spectrophotometric recordings of intact cells, demonstrating effect of ethylene glycol (E. Glycol) on the suppression of nonsense *cyc1* alleles. The concentration of ethylene glycol was 0.5 M. The three strains are, from top, strains nos. 13, 12, and 15 in Table 1. Note that *SUQ5-o* strain contains the ψ factor without which suppression by *SUQ5-o* is barely detectable (5, 12, 13); ψ^+ *SUP6-o* strains are inviable (13); and ψ factor has no effect on amber suppressors (13).

(16) suppressors cause growth retardation, two types of experiments were undertaken to test if hypertonic growth media increase efficiency of suppression. Several partial revertants of *SUP4-o,* which were selected from CL180-156B and which had reduced suppression of the *his5-2* and *trp5-48* markers, were grown on histidineless and tryptophanless media with increasing concentration of ethylene glycol. The growth of the partial revertants on deficient media could not be enhanced by the addition of ethylene glycol.

Spectroscopic examination indicated that under some hypertonic conditions *SUP6-o* and *SUP6-a* acted on the respective ochre (*cyc1-2*) and amber (*cyc1-76*) alleles at higher efficiency. The increases were roughly the same when growth medium contained 0.25, 0.5, 0.75, or 1.0 M ethylene glycol. At higher concentrations there was no detectable increase. Most consistent increases occurred when growth media contained 0.5 M ethylene glycol. It was estimated that, at this concentration, cytochrome *c* increased from roughly 10 to 20% to 25 to 40% of the normal amount in the *SUP6-o cyc1-2* strain and from roughly 30 to 40% to 50 to 60% in the *SUP6-a cyc1-76* strain. Fig. 2 shows low-temperature spectra of three strains grown on normal and on hypertonic media. In the *SUP6* strains, which are osmotic-sensitive, the relative amount of cytochrome *c* increased under hypertonic conditions whereas there was no increase in the *SUQ5-o* strain, which is not osmotic-sensitive. However, as will be discussed later, the significance of these increases as an explanation of osmotic sensitivity is not clear.

DISCUSSION

It is apparent that osmotic sensitivity is not intrinsic to the translation of the nonsense codons, UAA (ochre) and UAG (amber), since both UAA and UAG suppressors exist (Table 2) that do not cause osmotic-sensitive growth. Furthermore, since growth inhibition on hypertonic media is caused by suppressors that include tyrosine-inserting, serine-inserting, and leucine-inserting, this property is not associated with suppression by any one specific type of mutant tRNA.

The reversion analyses show that a lack of sufficient amount of a normal tRNA species is not a cause of osmotic sensitivity. Complete loss of suppressor activity in more than 200 revertants isolated was invariably accompanied by the ability to grow on hypertonic media. Furthermore, revertants of suppressor strains selected for the ability to grow on hypertonic media had either partially or completely lost suppressor activity. Most of the suppressor-inactive revertants contain secondary mutations within the suppressor loci (11, 17). Recombination analysis (11) of two such revertants of *SUP4-o,* which were selected for growth on hypertonic media, showed that both had retained the original suppressor mutation; suppression was inactivated by a second mutation within the gene. Thus, the revertants still contained the respective mutant tRNA although they were no longer osmotic-sensitive.

Experiments to test whether hypertonic media increase the efficiency of suppression to a deleterious level gave ambiguous results. The hypertonic conditions caused no detectable increase in suppression of auxotrophic markers. Under some hypertonic conditions, suppression of nonsense *cyc1* mutants apparently increased. However, the significance of this increase as a cause of osmotic sensitivity is not clear. Increases occurred at low osmolarities at which there is no significant growth inhibition of *SUP* strains in comparison to wild-type strains. Furthermore, at higher osmolarities (>1 M), where *SUP* strains were clearly inhibited, no detectable increase in suppression efficiency was observed. It is also important to note that UAA suppressors *SUP2–SUP8* and *SUP11* in ψ^- background, have an efficiency of suppression similar to that of *SUQ5-o* in ψ^+ background (2, 5). The former suppressors are osmotic-sensitive and the latter is not.

Another cause of osmotic sensitivity of suppressors may be that hypertonic conditions promote misreading by the mutant tRNAs. This phenomenon could be similar to streptomycin-induced misreading in *Escherichia coli* (see ref. 18 for a recent review). It was suggested that streptomycin causes misreading by inducing a distortion of ribosomes (19). The restoration of function by hypertonic conditions in osmotic-remedial mutants of yeast (20) and *Neurospora* (21, 22) is thought to result from conformational changes in otherwise nonfunctional proteins. Robosomes of bacteria are known to be capable of conformational changes (see ref. 23 for a recent review). Therefore, it is not unreasonable to think that hypertonic conditions can induce conformational changes in the tRNA-ribosome complex and thus cause misreading.

Whatever the mechanism of osmotic sensitivity, this property is useful for a variety of studies.

(*i*) Scoring of suppressors. One may follow segregation of suppressors in pedigrees without suppressible markers by growth tests on hypertonic media. This property may also be useful for allelism tests among suppressors.

(*ii*) Mitotic mapping. Hypertonic media may be used to select homozygous (*sup*+ /*sup*+) mitotic segregants from appropriate hybrids heterozygous for a suppressor and for an unmapped marker. Concomitant homozygosis of the new marker would indicate that it is on the same chromosome arm as the suppressor.

(*iii*) Selection of secondary mutations of tRNAs. Methods exist for selecting inactive UAA suppressors that suppress the ochre mutation, *can1-100,* which confers canavanine resistance (see refs. 11 and 17). Secondary mutations of UAA suppressors can now be easily selected on hypertonic media. More significantly,

secondary mutations of UAG suppressors can be selected using this new phenotype.

(*iv*) Selection of antisuppressors. The osmotic sensitivity of suppressors may be used to select for mutations in other genes called antisuppressors (see refs. 1 and 24), which reduce or eliminate suppressor activity.

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