Topical Reversion at the *HIS1* Locus of *Saccharomyces cerevisiae* • A Tale of Three Mutants

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ABSTRACT

Mutants of the *HIS1* locus of the yeast *Saccharomyces cerevisiae* are suitable reporters for spontaneous reversion events because most reversions are topical, that is, within the locus itself. Thirteen mutations of *his1-1* now have been identified with respect to base sequence. Revertants of three mutants and their spontaneous reversion rates are presented: (1) a chain termination mutation (*his1-208*, née *his1-1*) that does not revert by mutations of tRNA loci and reverts only by intracodonic suppression; (2) a missense mutation (*his1-798*, née *his1-7*) that can revert by intragenic suppression by base substitutions of any sort, including a back mutation as well as one three-base deletion; and (3) a −1 frameshift mutation (*his1-434*, née *his1-19*) that only reverts topically by +1 back mutation, +1 intragenic suppression, or a −2 deletion. Often the +1 insertion is accompanied by base substitution events at one or both ends of a run of A's. Missense suppressors of *his1-798* are either feeders or nonfeeders, and at four different locations within the locus, a single base substitution encoding an amino acid alteration will suffice to turn the nonfeeder phenotype into a feeder phenotype. Late-appearing revertants of *his1-798* were found to be slowly growing leaky mutants rather than a manifestation of adaptive mutagenesis. Spontaneous revertants of *his1-208* and *his1-434* produced no late-arising colonies.

IN the yeast *Saccharomyces cerevisiae* it is often difficult to study topical reversions, that is, mutations that arise in the same genetic locus as the mutant. The difficulty arises because the topical frameshifts, nonsense, and missense mutations often are swamped out by a plethora of extragenic suppressors, so that an analysis of each revertant becomes an overwhelming exercise. With one interesting exception,[1] *his1* revertants have been reported as arising only at the locus itself (Korch and Snow 1973). No *his1* mutants were found that responded to chain termination suppressors. This puzzling finding was investigated exhaustively with the genetic techniques available in 1973. Our interest in topical reversion led us to study spontaneous reversions of *his1* mutations in the Korch and Snow collection.

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[1] Snow (1980) reported one type of extragenic suppressor of *his1* mutants that depends on interaction of two nonhomologous proteins. This finding presaged the useful two-hybrid system of Fields and Song (1989; Song *et al.* 1991) for demonstrating protein interactions between nonhomologous proteins. Now that the Fields-Song two-hybrid method is available, the protein interaction found by Snow (1980) should be investigated further.

This paper is dedicated to Jan Drake on the Occasion of *his1st-65th* Birthday. It is also a reminder for him to bite the bullet gracefully.

We have analyzed, in depth, reversions of a nonsense, a missense, and a frameshift mutation at *HIS1*. We have confirmed the observation of Korch and Snow (1973) that these reversions of *his1* mutants are indeed topical. The revertants are detected among cells grown in glucose in multiple cultures, where revertants appear from the background only when the limiting metabolite (in these cases, histidine) is exhausted. We have used the P_0 of the Poisson distribution for calculation of the spontaneous mutation rate (*cf.* von Borstel 1978; von Borstel *et al.* 1971), which avoids most types of selection because mutational events rather than mutant frequencies are measured.

MATERIALS AND METHODS

Strains and mutants: According to the customary nomenclature for mutations of *S. cerevisiae*, the first mutant allele of *HIS1* discovered was called *his1-1*, and as mutants were discovered, the enumeration grew until Korch and Snow (1973) mapped *his1-315*. Unfortunately, two of the more used numbers, namely, *his1-1S* and *his1-7S*, were duplicated for the original mutant alleles that Korch and Snow (1973) designated *his1-1F* for the *his1-1* isolated by C. Raut (unpublished results) and *his1-7F* for the *his1-7* of R. K. Mortimer (unpublished results) used by Fogel and Hurst (1967). See Korch and Snow (1973) for a full historical account of the naming of the mutant alleles of *HIS1*. Here we mention the "F" (for Fogel) in Table 1, and it is removed from this designation

TABLE 1

Sequence alterations in *his1* mutants and the source of the mutations

Korch and Snow (1973) name of mutant alleles	Renamed by base position and mutation[a]	Source[b]	Change
his1-49	*his1-159-196(del 37)*	spont.	deletion
his1-1F = his1-39	*his1-208 (A → T)*	UV	lys → stop(TAA)
his1-54	*his1-221 (G → A)*	NA	gly → asp
his1-1S	*his1-364 (A → T)*	UV	ile → phe
his1-62	*his1-374 (G → A)*		ser → asn
his1-19	*his1-434 (delAA → G)*	EMS	frameshift
his1-42^{ts}	*his1-499 (G → A)*	EMS	ala → thr
his1-30	*his1-652 (G → A)*	EMS	gly → met
his1-51	*his1-653 (G → A)*	NA	gly → asp
his1-48, his1-65	*his1-683 (G → A)*	EMS, NA	cys → tyr
his1-7F = his1-3	*his1-798 (G → A)*	UV	met → ile
his1-11	*his1-818 (G → A)*		gly → asp
his1-315	*his1-887 (G → A)*	UV	arg → his

[a] The mutations are renamed for their base sequence using a slight variation of the system being devised for the human genome (Glickman 1997).

[b] spont., spontaneous; UV, ultraviolet radiation; NA, nitrous acid; EMS, ethyl methanesulfonate.

henceforth. The names *his1-1S* and *his1-7S* are retained for the two mutants found by Korch and Snow (1973).

In Table 1 we rename the mutations in a locus to take into account their position in the base sequence as well as the type of mutation that took place. With slight departures, such as keeping the three-letter name for the phenotype itself, this is, in accordance with rules being established for the human genome (Glickman 1997). The human genome nomenclature is being changed now to take in at a glance the most information possible about each mutant base pair. We have altered the rule by enclosing the nature of the mutation in parentheses to permit it to be a removable segment, whereas the proposal for the human genome is to affix the change into the name itself.

For the reversion experiments, the mutations were in different genetic backgrounds (Table 2). The strains containing *his1-1* and *his1-19* originally came from the collection made by Korch and Snow (1973) and were constructed into genetic backgrounds with other mutant loci from our own collection by Savage (1979; see also Manivasakim 1993).

Media: The complex medium of general use is YEPD (1% yeast extract, 2% Difco peptone, and 2% dextrose). The components of synthetic complete media (*cf.* von Borstel *et al.* 1971) are filter-sterilized, except for the glucose and agar, which are autoclaved separately. The components of media are mixed together while the solutions are still hot. We have found that autoclaving the Yeast Nitrogen Base and the amino acids and bases together alters mutation rates.

Measurement of spontaneous mutation rates: The growth-limiting concentration of histidine is 0.2 mg/L for *his1-798* and *his1-208* and 0.4 mg/L for *his1-434*. An average of 2500 cells is inoculated into each compartment of multiple-well assays, and cells go through at least eight or nine doublings in the medium before growth ceases. All experiments are carried out at 26°, and the routine spontaneous mutation rate is determined at 14 days. The incubation temperature must be monitored carefully, because there is a doubling in the spontaneous reversion rate, for most strains, with each 5° increase in temperature from 10 to 30° (R. C. von Borstel and C. M. Steinberg, unpublished data).

The P_0 component of the Poisson distribution is used to calculate the spontaneous mutation rate. Thereby, the spontaneous reversion rate (M) is

$$M = e^{(N_0/N)} - m_b/2C,$$

where N is the total number of compartments, N_0 is the number of compartments not containing revertants, m_b is the average number of mutants in the inoculum, and C is the average number of cells per compartment after growth has stopped (von Borstel *et al.* 1971; von Borstel 1978).

If a large number of compartments is used, experiments are highly repeatable, as shown in Table 3 for reversion of the missense mutant, *his1-798*.

The *his1-798* reversion data are the result of seven replicate tests carried out over an 8-month period. The mean of the seven tests is 6.3443×10^{-8} reversions/cell/generation, the standard deviation is 0.2968, and the coefficient of variation is 0.0468. The preferred measurement of error is the coefficient of variation, which gives an accurate statement of the

TABLE 2

Genotypes of strains

Strain	Genotype	New names for *HIS1* mutations
XV185-14C	*MAT***a** *ade2-1 arg4-17 his1-7 hom3-10 lys1-1 trp5-48*	*(his1-798)*
HP109-1C	*MAT***a** *ade2-1 hom3-10 his1-1*	*(his1-208)*
HP17-3D	*MAT***a** *ade2-1 his1-19*	*(his1-434)*

TABLE 3

Demonstration of repeatability of the spontaneous reversion rate assay: Results of repeated compartmentalization tests for base substitutions in *his1-7* (*his1-798*) of strain XV185-14C

Experiment number	Cells/compartment ($\times 10^{-6}$)	Number of compartments	Number of compartments with mutants	Background mutants/compartment ($\times 10^{8}$)	Mutation rate ($\times 10^{8}$)
	C	N	$N - N_0$	m_b	M[a]
880921	191	498	105	0.032	6.16
881016	228	497	126	0.030	6.34
890401-1	299	482	146	0.031	6.01
890401-2	245	500	137	0.031	6.51
890401-3	280	500	148	0.020	6.24
890505	280	479	141	0	6.23
890514	231	488	134	0.021	6.92

[a] M = reversions/cell/generation.

precision of the P_0 method. Calculation of the standard error or standard deviation requires the multiplication of the variability of the numerator and the denominator, and this leads to a perceived exaggeration of the error limits.

For very low reversion rates, as observed with *his1-434*, we grow the mutants to ~1–5 × 10^7 cells/ml in YEPD medium and then place 1 ml each on 100–160 individual plates of histidine-dropout medium. After cessation of growth, the histidine-independent revertants arise from the background. Because there is still some histidine present in the inoculum that is plated, complete depletion of histidine (and thus cessation of growth) occurs ~6–8 cell generations later (*ca.* 1–2 × 10^9 cells). Thereby, fewer than 2% of the mutants could have arisen in the original culture medium.

For examination of late-appearing mutants, particularly those arising after nearly 30 days, the mutants were examined for growth rate by growing them in a histidine-dropout medium, using synthetic complete medium for the control. The cells were counted with a hemocytometer every 2 hr for at least three doublings of the control in synthetic complete medium.

Extraction of genomic DNA from yeast for PCR: A single yeast colony was transferred into 100 ml of fresh lysing solution (1 m Sorbitol, 20 mm EDTA, 10 μl/ml β-mercaptoethanol, 2 mg/ml Zymolyase 20T) and incubated for 5 min at 37°. One hundred microliters of PCI (70% phenol, 29% chloroform, 1% isoamylalcohol) was added and incubated at 60° with vigorous shaking for 3 min. The aqueous phase was transferred to a fresh 1.5 ml microfuge tube for a standard powdered glass DNA extraction. The final DNA recovery from the powdered glass was in 20 μl of TE (10 mm Tris-HCl, 1 mm ETDA, pH 8.0).

Preparation of sequencing template by PCR and purification on agarose gels: In earlier studies (Lee *et al.* 1988, 1992), shuttle vectors containing the mutant *HIS1* coding sequences were recovered from *S. cerevisiae*, amplified in *Escherichia coli*, and subsequently sequenced using the dideoxy chain-termination method developed by Sanger *et al.* (1977) (^{35}S-αATP). Direct sequencing of yeast colony PCR products for the HIS1 locus was first done by Ritzel *et al.* (1989). Amplication of sequencing template by PCR was done according to the following protocol: the outside primers H1O (5′-GCTGCCAAGTGA GTCACCTCTACC-3′) and H1X (5′-GCATGAAGACGGTAG TAAAGC-3′) were used to amplify the *his1* allele template for sequencing. The PCR reaction mixture included 3 μl of 10× PCR buffer (500 mm KCl, 100 mm Tris-HCl, 50 mm $MgCl_2$), 2 μl of the DNA extract, 1 ng of each primer and 2 μl of Taq polymerase solution, made up to 30 μl with sterile Milli-Q water. Twenty microliters of paraffin oil was added to each sample prior to PCR to reduce evaporation. The primers were made by the Microbiology Services Unit in the Department of Biological Sciences, University of Alberta (Edmonton, Canada).

The above reaction was cycled at 1 cycle (95° for 5 min), 30 cycles (95° for 30 s, 60° for 30 s, 73° for 90 s), 1 cycle (73° for 5 min) followed by a hold at 6°. The PCR was done in a Stratagene Robocycler 96 with thin-wall 200-μl tubes (Rose Scientific, Edmonton, Canada). Twenty microliters of 30% glycerol:0.25% bromophenol blue loading dye was added to each sample. From each sample, 110 μl was loaded on a 1% agarose:0.5× TBE (45 mm Tris-borate, 1 mm EDTA) minigel:0.5 μg/ml of ethidium bromide in a minigel box (Tyler Research Instruments, Edmonton, Canada) and electrophoresed at 60 V. The DNA bands were visualized on a UV transilluminator and cut out of the gel with minimal UV exposure. The DNA was isolated from the gel slices following the GeneClean II protocol with TBE modifier. DNA was resuspended in 20–50 μl of water depending on the size of the band. Confirmation of successful DNA extraction was made by electrophoresing a sample of the purified DNA on a 1% agarose:0.5× TBE gel.

Sequencing of the *his1* mutants using ThermoSequenase with Redivue ^{33}P terminators: The primers H1A (5′-ATGGATT TGGTGAACCATCTAACC-3′), H1B (5′-GTCGACGTAGACT TAGCAATCG-3′), H1C (5′-GTTAGTTCCATGATTGAGAG-3′), and $H1E_2$ (5′-GCTCTGGGAATTGGTGATGC-3′) were used for sequencing the coding strand. The primers H1X (5′-GCAT GAAGACGGTAGTAAAGC-3′) and H1R (5′-TCTGTTCTATC TTATACACGACAA-3′) were used for sequencing the noncoding strand. Dideoxy sequencing (Sanger *et al.* 1977) was done following the ThermoSequenase protocol from Amersham Life Science (Oakville, CA), with annealing temperatures 5° below the T_m for each primer and 50 cycles. Sequencing products were separated by denaturing polyacrylamide gel electrophoresis on 6% 57 acrylamide:3 bis-acrylamide with 8 m urea and 0.5× TBE. Urea was removed from the gels by soaking in 20% ethanol for 5 min followed by vacuum drying at 80° for 1 hr. Fuji RX autoradiography film (Fisher Scientific, Toronto, Canada) was exposed to the dried gel for 12–36 hr.

Sequence analyses were done manually by comparison with

TABLE 4

Revertants of the *his-208* (A → T) (*his1-1*) termination codon 5′-TAA-3′

Number of spontaneous revertants	Mutant codon	Base change	Amino acid insertion
2	GAA	T → G	glu
5	CAA	T → C	gln
1	AAA	T → A	lys
1	TCA	A → C	ser
3	TTA	A → T	leu
3	TAC	A → C	tyr
1	TAT	A → T	tyr

the wild-type *HIS1* sequence. Mutations were confirmed by analysis of the second DNA strand.

RESULTS

Revertants of *his1-208 (A→T) (née his1-1)*: The mutant *his1-208* is caused by a mutation that creates an ochre chain termination codon. The reversion rate for this allele is 1.7×10^{-9} reversions/cell/generation. The sequence changes in the revertants of *his1-208* provide clear evidence that this ochre chain termination codon UAA does not revert by mutations of the anticodon of the tRNA (Table 4). Each histidine-independent revertant of *his1-208* was a single base missense or back mutation within the three-base coding region. All seven possible single base mutations that replace the chain-termination codon with an amino acid codon will support growth. Any mutation to the amber UAG or opal UGA codons would not be expected to grow. None of the revertants were leaky; that is, late-arising revertants have not been observed among spontaneous mutants of *his1-208*. All base substitutions for the UAA codon function as well as any other.

There are a number of ways of explaining the lack of chain-termination suppression in *HIS1*, the most obvious of them being that tRNA suppression of chain termination is usually an inefficient process, and perhaps the codon has to be translated efficiently in order for the enzyme to be active. The efficiency has to be extremely low, because late-arising revertants of *his1-798* can be as slow as 10% of the growth rate of the normal cells, and perhaps less. It would be interesting to test other opal, amber, as well as other ochre mutations at different locations within the locus. However, none was available from the Korch and Snow (1973) collection.

Revertants of *his1-798 (G→A) (née his1-7)*: This mutation is a transversion lying near the 3′-end of the locus. It reverts most frequently by intragenic missense suppression. All reversions occurred on the upstream side of the primary mutation, excepting back mutation at the mutant base. The revertants are classified into three groups: nonfeeders, feeders, and undefined (untested) (Table 5). Two revertants in Table 5 are of particular interest because they are rare: *HIS1-798 (→ 798)*, which is a back mutation (a restoration to the wild-type genotype), and *HIS1-798 (→ Δ199-201)*, a deletion of an entire codon.

For mutagen testing assays, *his1-798* could be useful because it would permit measurement of mutagen-induced transition or transversion types at numerous places within the locus. As would be expected when missense suppression makes many mutation sites available throughout the gene, the spontaneous reversion rate is high, being in the range of 10^{-7} mutations/cell/generation.

Feeders and nonfeeders are about equal in frequency. The feeders are an interesting example of excretion of a metabolite enabling nearby cells to grow into colonies. The metabolite that is excreted was found to be histidine, as determined by analysis of the supernatant of liquid cultures. *HIS1* encodes the first enzyme of the histidine pathway, phosphoribosyl adenosine triphosphate:pyrophosphate phosphoribosyltransferase (E.C.2.4.2.c) (Fink 1964). Feeding of nearby cells suggests that an overproduction of histidine is controlled by the first step of the pathway. This indicates that the capability for feeding is because of a loss of feedback inhibition by histidine. The feeders are located in three main groups in the locus. It is interesting that one base change can elicit overexpression, whereas the same base mutated in a different way, or a reversion in the same codon, may result in a nonfeeder. These are at amino acids 69, 123, 156, and 266, corresponding, respectively, to base pairs at 205 and 206, 367, 472 and 473, and 796 and 798. There is no unique contiguous part of the gene that is mutated in the cross-feeding of revertants of *his1-798* that might encode a feedback inhibition site. This differs from the suggestion put forth by Korch and Snow (1973) that the alleles permitting excretion of histidine were located in a particular portion of the locus. Later work (Lax and Fogel 1978; Lax *et al.* 1978; Fogel *et al.* 1978) showed that there was more than one location, but still these authors advanced the suggestion that regulation of excretion was under the tight control of structural elements within the gene. Enlightenment may be provided by a comparison of the genetic data with a structure derived by X-ray crystallography.

The spontaneous reversion rate for allele *his1-798*, which is discussed as a model system for spontaneous mutation rate measurement (see materials and methods), is 6.3×10^{-8} reversions/cell/generation. Revertants that are feeders could confound mutation rate measurements based on revertant counts, but this problem is avoided when mutational events are counted using the P_0 fraction of the Poisson distribution. Late-arising revertants of *his1-798* are shown in Table 6.

Instead of reaching a plateau by the 14th day at 26°, a few spontaneous revertants continued to appear until

TABLE 5

Revertants of *his1-798 (G → A) (his1-7)*

Mutant base number	Base change	Amino acid alteration	Mutant base number	Base change	Amino acid alteration
	Nonfeeders			Feeders	
15	AAC → AAG	asn^{5} → lys	191	ACT → AAT	hr^{64} → asn
23	ACC → ATC	thr^{8} → ile	205	GGT → AGT	gly^{69} → met
115	TCT → GCT	ser^{39} → ala	205	GGT → TGT	gly^{69} → cys
206	GGT → GTT	gly^{69} → val	206	GGT → GCT	gly^{69} → ala
226	ACT → GCT	thr^{76} → ala	206	GGT → GAT(2)[a]	gly^{69} → asp
227	ACT → AGT	thr^{76} → ser	251	TCT → TAT	ser^{84} → tyr
367	GTT → ATT(2)[a]	val^{123} → ile	367	GTT → TTT	val^{123} → phe
455	GTC → GGC	val^{152} → gly	473	GCA → GTA	ala^{158} → val
472	GCA → ACA	ala^{158} → thr	476	TCA → TTA(2)[a]	ser^{159} → leu
478	TGT → CGT(2)[a]	cys^{160} → arg	492	ATT → ATG	ile^{164} → met
487	GGA → AGA	gly^{163} → arg	612	AGC → AGG	ser^{204} → arg
656	GTC → GCC	val^{219} → ala	758	TCC → TAC(2)[a]	ser^{253} → tyr
664	GCT → TCT(2)[a]	ala^{222} → ser	766	GAC → TAC	asp^{256} → tyr
731	ACG → ATG	thr^{244} → met	792	AGT → AGG	ser^{264} → arg
733	CCT → TCT	pro^{245} → ser	796	ATG → TTG	met^{266} → leu
798[b]	ATA → ATG	ile^{266} → met			
	Undefined[c]				
18	CAT → CAA	his^{6} → gln			
76	GTT → CTT	val^{26} → leu			
154	GTA → TTA	val^{52} → leu			
199–201	ΔGGT	Δ gly^{67}			
605	CCA → GCA	pro^{202} → arg			
664	GCT → ACT	ala^{222} → thr			
744	AGA → AGC	arg^{248} → ser			

[a] The number in parentheses indicates the number of mutants found to have the same mutation.

[b] Back mutation (restoration to wild-type genotype).

[c] The undefined mutants were taken from liquid cultures and were not tested for feeding or nonfeeding capability.

this experiment was terminated at 30 days. Most of the revertants tested that arose after the 14th day grew more slowly than those which arose previously. A few mutants, namely, revertants 3, 13, and 15, grew at the same rate in the presence or absence of histidine. Revertants 3 and 13 are petite mutants and grew slowly with respect to the controls. The only one of these three revertants that was not a petite mutant was revertant 15. Late-arising mutants that grow rapidly have been observed with reversions of the *trp5-48* mutant (data not shown). These revertant colonies are invariably slow-growing mutants, where secondary mutations make the cells grow rapidly. When patches of slow-growing cells are streaked on the plate, papillations appear, constituting rapidly growing cells. The colony from revertant 15, shown in Table 6, was not isolated early enough to obtain a subset of slow-growing revertants. On the other hand, revertant 15, appearing on day 30, may be the first case of adaptive mutagenesis that we have observed. When the DNA sequence is obtained, this revertant will be examined for two or more alterations within the base sequence of *HIS1*.

The mutants described by Cairns and his colleagues (Cairns *et al.* 1988; Cairns and Foster 1991; Foster and Cairns 1992) as being caused by "directed mutagenesis" arose late and were ascribed to mutations taking place in stationary phase in *E. coli*. Ryan (1955) noted that DNA synthesis was taking place during "stationary phase," and this could account for some of the mutations, although he too believed that reversions were arising during stationary phase (Ryan 1959). This Ryan-Cairns deviation from the expectation that mutations take place during genomic DNA replication in *E. coli* has been observed in *S. cerevisiae* as well (von Borstel 1978; Hall 1992; Steele and Jinks-Robertson 1992). The current most popular explanation for the Ryan-Cairns deviation in *E. coli* is that recombinational events are taking place during stationary phase, which, for the most part, lead to frameshift mutations [Harris *et al.* (1994), Foster and Trimarchi (1995); but see Foster (1998); Rosenberg *et al.* (1998)].

All late-arising spontaneous reversions we have studied can be explained as slowly growing mutants due to leakiness of revertants or as fast-growing mutants that

TABLE 6

Nature of late-arising revertants of *his1-798*: Dates of appearance and generation time

Revertant number	Date of appearance (from beginning of experiment)	Generation time (% of control)
1	Early (1–2)	100
2	Early (1–2)	100
3	19	100
4	19	60
5	19	15
6	19	25
7	20	25
8	22	80
9	22	50
10	22	65
11	22	65
12	22	40
13	25	100
14	25	60
15	30	100
16	30	30
17	30	75

Early mutants are the controls.

are due to a secondary spontaneous revertant arising within a pool of slowly growing cells (*cf.* Hall 1990). And the last colonies to arise comprise cells that grow more slowly than all previously arising colonies.

Revertants of *his1-434 (AA→G) (née his1-19)*: This mutation is a deletion of a base along with a transition at the 5′ end of the run of adenines where the deletion occurred. The mutation has an ochre chain termination codon as the next codon in the sequence. The maximum allowable distance over which a reversion can take place is the addition of one base within six bases upstream. Upstream from that position any +1 addition or −2 deletion turns the codon prior to these six bases into an opal chain termination codon. The revertants that have been found to date are shown in Table 7. The two slowly growing mutants arose by day 5, so no late-arising mutants accrued in this experiment. Ten of the revertants were additions of one base pair, and two were deletions of two base pairs. The remaining seven also had base substitutions; five of these were at one or both ends of the run of adenines, and two were outside of the run of adenines.

The reversion rate for *his1-434* is very low (9.4×10^{-12} reversions/cell/generation). To maximize the opportunity for observing late-arising mutants that grow rapidly, a reversion experiment was carried out and observed for 30 days at 26°. The growth rates of each revertant were measured and compared in synthetic medium with and without histidine (Table 7). It is important to note that two revertants shown in Table 6 grew more slowly in medium without histidine, but like the other revertants, they arose within the time frame we call early (within 5 days at 26°), and thus must have occurred during log phase growth on the plate.

In a run of identical bases, the dynamics that produce a −1 reversion also can produce a +1 mutation (Streisinger *et al.* 1966; Fowler *et al.* 1974; Streisinger and Owen 1985; Bebenek *et al.* 1992). The high frequency of base substitution-associated frameshifts seen here was not observed in either of two comprehensive analyses of hypermutability in homonucleotide runs in yeast (Tran *et al.* 1996, 1997). Therefore, it is somewhat astonishing to observe that, in our observations, about 40% of all revertants also had base substitutions, most of which were at the end of the run of identical bases (Table 7). Something like this had been observed by Bebenek *et al.* (1992) *in vitro* when they provided imbalanced frequencies of different deoxyribonucleosides in the substrate for replication of a segment of DNA containing a +1 mutation.

The reasons for the frameshift along with a base change could relate to mismatch repair. For example, we speculate that a misincorporated base leaving the replication fork may bind to a homolog of the *E. coli* MutS protein. This binding may then hold the primer on the template in a slipped position, thus allowing a base to be added or deleted with a higher probability than would have occurred without the misincorporation. Another possibility is that there is a steric hindrance to mismatch repair in some cases of very close mismatches, as suggested by Manivasakam *et al.* (1996). Yet another possibility (S. Rosenberg, personal communication) is that a depletion of mismatch repair components may occur locally, allowing multiple mismatches to persist.

CONCLUSIONS

1. The data presented here are consistent with the notion put forward by Korch and Snow (1973) that *HIS1* mutations are reverted within the locus itself. Nevertheless, we have not excluded all possible *trans*-acting suppressors.
2. An ochre nonsense codon that was not suppressible by any external suppressor was reverted. All possible single base revertants encoding amino acids within the codon were identified.
3. Cells containing reversions of *his1-798* that encoded different amino acids for the same codon could exhibit different feedback inhibition phenotypes.
4. The −1 frameshift mutation in a run of A's reverted by the conventional purine addition or double base subtraction most of the time, but a surprising number of the reversions were associated with base substitutions at one or both ends of the run of A's.
5. Some of the reversions are late-arising mutations. It is not a certainty that these late-arising reversions occurred during stationary phase because most of them were slow-growing revertants.

TABLE 7

Frameshift revertants of the frameshift mutant *his1-434(AA → G) (his1-19)*

Codon and peptide[a] sequences (5′→3′)					Base changes	Nature of mutation (occurrences)	Growth rate[b] (%)
GTT val	GAA glu	AAA lys	ATG met	ACC thr	Wild type	—	
GTT val	GAA glu	AA**G** lys	TGA STOP	CCA	−AA+G	*his1-434* (*delAA → G*) (*his1-19*)	100
GTT val	GAA glu	AA**A** lys	GTG val	ACC thr	+A	revertants (9)	100
GTT val	G**G**A gly	AAA lys	GTG val	ACC thr	+G	revertant (1)	100
GTT val	**C**A**A** gln	AAA lys	GTG val	ACC thr	G → C;+A	revertant (1)	100
GTT val	**A**A**A** lys	AAA lys	GTG val	ACC thr	G → A;+A	revertants (2)	100
GTT val	**C**A**A** gln	AAA lys	**T**TG leu	ACC thr	G → C;G → T;+A	revertants (2)	100
G**C**T ala	GAA glu	AA**A** lys	G**A**G glu	ACC thr	T → C;T → A;+A	revertant (1)	100
GGA gly	AAA lys	GTG val	ACC thr		−TT	revertant (1)	100
GTT val	GAA glu	ATG met	ACC thr		−AG	revertant (1)	∼10
GTT val	GAA glu	AA**A** lys	ACC thr		G → A;−TG	revertant (1)	∼33

[a] The base number of the first base of the valine codon is 426.
[b] Percent of growth on −histidine medium compared with +histidine.

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