*NONSENSE MUTATIONS AFFECTING THE HIS4 ENZYME COMPLEX OF YEAST**

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Abstract.—The *his4* region of yeast contains the information necessary for the catalysis of three steps in the histidine biosynthetic pathway. The three activities specified by this region remain physically associated during gel filtration and ultracentrifugation and after extensive purification. Nonsense mutations in the "operator distal" *his4C* region lower the molecular weight of the two proximal activities. The proximal activities can, therefore, function without the *his4C* portion of the protein(s). These observations are compatible with the following three possibilities. The *his4* region codes for: (1) an aggregate of three proteins forming a multiprotein complex; (2) a single protein with three catalytic activities; and (3) a single protein which is cleaved to form three proteins which remain associated.

Introduction.—It is not known whether operons exist in the chromosomes of higher organisms. Recent studies on yeast[1] and *Neurospora*[2] have revealed operon-like clusters of genes, yet these gene clusters have several features distinguishing them from the typical bacterial operon.[2] The *his4* region of yeast encodes the enzymes which catalyze the second, third, and last steps of histidine biosynthesis.[3] This region displays many of the properties characteristic of bacterial operons, including biochemical pleiotropy and polarity of nonsense mutations. Nonsense mutations localized at one end of the region can destroy the activity of all three enzymes. Our studies indicate that the three *his4* enzymes remain physically associated even after extensive purification. This association could mean either that the *his4* region specifies a single, multifunctional polypeptide or that the region specifies several different polypeptide chains which associate in a multienzyme complex. Clearly, if the region specifies only one polypeptide chain, it cannot be considered an operon.

In this report we describe the biochemical consequences of nonsense mutations located in the middle of the *his4* region. One of these mutations does not markedly affect the activity of two of the three proteins in the *his4* enzyme complex although it lowers the molecular weight of the complex. In view of this finding, any model of the *his4* region must account for the normal functioning of half of the *his4* complex in the complete absence of the other half.

Methods and Materials.—(1) *Yeast strains:* All the histidine-requiring strains described here were haploid-heterothallic strains of *Saccharomyces cerevisiae* and were isolated from the standard wild-type *S288C* or from strains closely related to it after EMS mutagenesis. Strain *SCF1717* was used to obtain "wild-type" *his4* enzymes because it could be derepressed. This strain contains mutation *his1-123* in the gene coding for the first enzyme in the pathway but has a normal *his4* region. Stocks used were:

SCF1717	α *his1-123*
SCF271	α *his4-864 leu2-1*
A235B	a *his4-260 leu2-1*
A1995A	a/a *his4-864/his4-481*

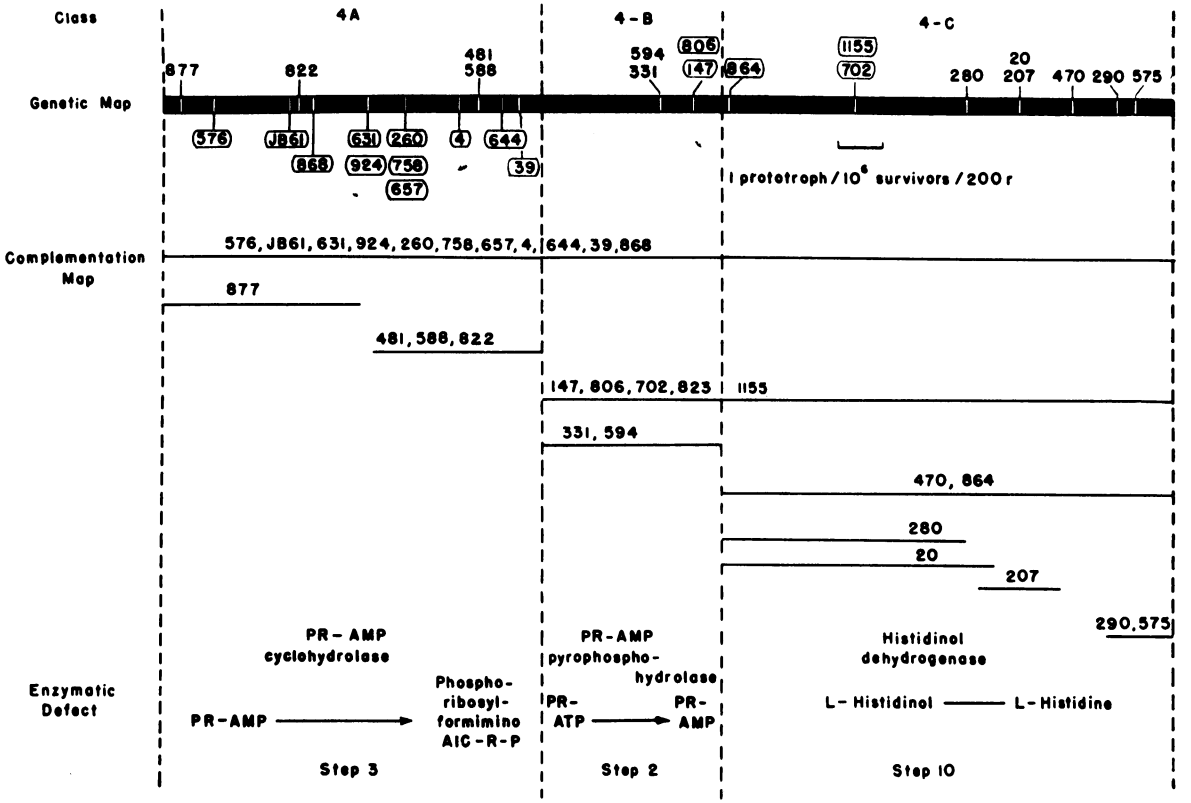
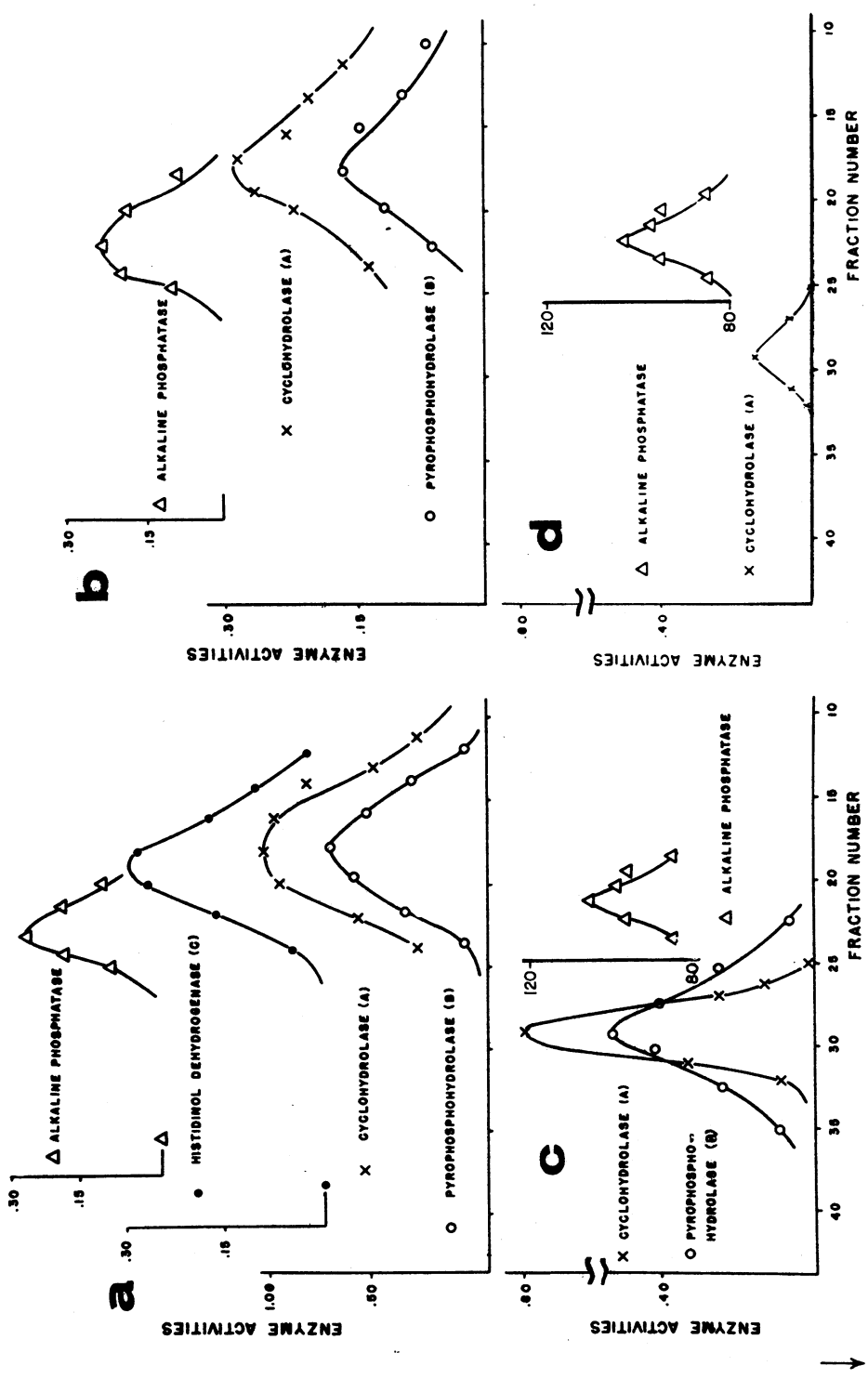


FIG. 1.—The organization of the *his4* region of yeast. At the top of the figure is a genetic map of the region. The numbers circled on the map designate nonsense mutations. Noncomplementing mutants are placed below the line indicating the chromosome. The regions, *A*, *B*, and *C* are based on the complementation map. Allelic complementation occurs within *A* and *C*, but that in *C* is much more detailed than is shown here. Reactions in the pathway of histidine biosynthesis which are affected by mutations in the *A*, *B*, and *C* regions are shown below the appropriate region of the complementation map. Recombination values establishing the position of *his4-864* are: 864–702, 3.01; 864–290, 10.5; 864–806, 2.1; 594–702, 5.1; 806–702, 3.4; 644–864, 4.9; 644–806, 3.4.


a
b
c
d
ENZYME ACTIVITIES
FRACTION NUMBER
ALKALINE PHOSPHATASE
HISTIDINOL DEHYDROGENASE (G)
CYCLOHYDROLASE (A)
PYROPHOSPHONHYDROLASE (B)
PYROPHOSPHO-HYDROLASE (B)

(2) *Growth of strains:* Histidine auxotrophs were derepressed by being grown on histidinol (0.3 mM) or N-acetyl-L-histidine (1 mM). The level of the histidine biosynthetic enzymes in strains grown in this manner is 12-fold higher than wild type (i.e., *S288C*).

(3) *Extraction of enzymes:* Two grams (wet weight) of cells were resuspended in 0.1 *M* Tris-HCl, pH 7.5, and disrupted in a French pressure cell. The cells and debris were removed by centrifugation at 16,000 rpm for 20 min. The protein concentration was approximately 20 mg/ml.

(4) *Enzyme assays:* (*a*) Cyclohydrolase (*his4A*) was measured by following the production of AICAR in the presence of an extract of *Salmonella typhimurium hisT1504his1648* as described earlier.[3] (*b*) Pyrophosphohydrolase (*his4B*) was measured by the same reaction described for cyclohydrolase, except that an extract of *S. typhimurium hisT1504his-E709* was used. (*c*) Histidinol dehydrogenase (*his4C*) was measured by following the reduction of NAD at 340 mμ. The reaction mix contained: 40 μmoles Tris brought to pH 9.0 with HCl, 0.030 μmole NAD, extract (usually 0.03 mg protein), 4 μmoles L-histidinol and water to bring the volume to 0.8 ml.

(5) *Genetic techniques:* The fine-structure mapping was carried out by the X-ray mitotic technique.[4] The techniques used for the mating of strains and the tetrad analysis by ascus dissection were those described by Hawthorne and Mortimer.[5] Suppressible alleles were identified by tetrad analysis and the appropriate backcrosses.

Results.—The genetic and complementation maps of *his4*, together with the corresponding biochemical functions of each region, appear in Figure 1. Strains carrying missense mutations in any one of the three regions (*A*, *B*, or *C*) lack only the enzymic activity associated with that region. Nonsense mutations in *A*, like *his4-260* and *his4-644*, are completely polar, lacking the three enzymic activities associated with the region and complementing none of the other *his4* mutants. Other nonsense mutations like *his4-147* and *his4-806* show a polarized loss of activity from the left to the right.

The nature and location of site 864: Mutant *his4-864* is a *his4C* mutant. Derepressed extracts of *his4-864* contain no detectable histidinol dehydrogenase activity but have normal amounts of cyclohydrolase (*A*) and pyrophosphohydrolase (*B*). Extensive genetic mapping of *his4-864* places it at the beginning of the *C* region.

An analysis of the suppression of the histidine requirement in *his4-864* reveals that this strain is a nonsense mutant. Suppressors of *his4-864* also suppress *ad2-1* and *leu2-1*. These latter two are well-studied nonsense mutations.[6] According to the scheme of Hawthorne and Mortimer,[6] *his4-864* is a nonsense mutation suppressed by class VI suppressors.

Zone centrifugation studies: In wild type, the three activities encoded by the *his4* region sediment together when centrifuged in a sucrose gradient. With varying protein concentrations, all three enzymic activities remain associated at a position in the gradient corresponding to a molecular weight of approximately 95,000 (Fig. 2). In missense mutants (e.g., *his4-331*, *his4-20*, *his4-481*, *his4-280*),

FIG. 2.—Sucrose gradient ultracentrifugation. A crude extract (0.1 ml) was layered on a 5–20% sucrose gradient buffered with 0.1 *M* Tris-HCl, pH 7.5, and centrifuged for 15 hr at 41,000 rpm in the Spinco SW65 rotor. As a standard, 0.02 mg of *E. coli* alkaline phosphatase (mol wt 80,000) was added to the extract before it was layered on the gradient. Alkaline phosphatase was assayed by the method of Garen and Levinthal.[9] Fractions of six drops each were collected. The top of the gradient is on the left, the bottom on the right. (*a*) Strain *SCF1717* (wild-type *his4* region). (*b*) *his4-280*, a *C* missense mutant. (*c*) *his4-864*, a *C* nonsense mutant. (*d*) *his4-702*, a *C* nonsense mutant.

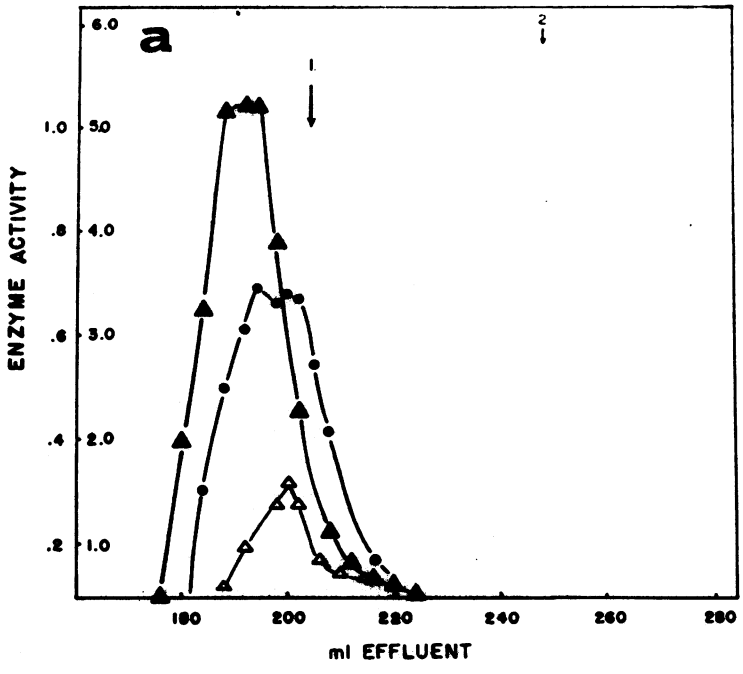

a
1
2
ENZYME ACTIVITY
6.0
5.0
4.0
3.0
2.0
1.0
1.0
.8
.6
.4
.2
160
200
220
240
260
280
ml EFFLUENT

FIG. 3a.

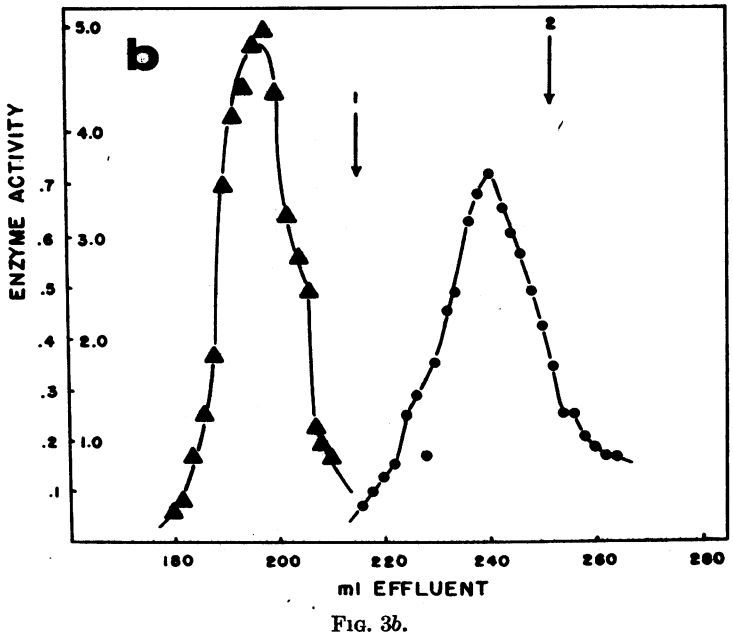

b
1
2
ENZYME ACTIVITY
5.0
4.0
3.0
2.0
1.0
.7
.6
.5
.4
.3
.2
.1
160
200
220
240
260
280
ml EFFLUENT

FIG. 3b.

the proteins corresponding to these activities show the same molecular weight as wild type for the unaffected activities. By contrast, *his4-864*, a *C* nonsense mutant, gives an entirely different sedimentation pattern. In this strain the cyclohydrolase (*A*) and pyrophosphohydrolase (*B*) have a molecular weight of approximately 45,000, roughly half that of wild type. The levels of the *A* and *B* activities in *his4-864* are about the same as wild type, and no special procedures are necessary for their assay. These studies have been confirmed by an analysis of strain *A1995A*. This heterozygote should produce a *his4* complex of normal molecular weight from one chromosome and a low-molecular-weight *A* activity from the other. Centrifugation of an extract of *A1995A* produced a peak of *A* activity of molecular weight 45,000, completely separated from the heavier peak of *C* activity. Thus, the molecular consequence of a nonsense mutation in *his4C* is to lower the molecular weight of the residual *his4* products.

Gel filtration: Chromatography of extracts of wild-type and mutant *his4-864* on Sephadex G-100 also indicates that the residual enzyme activities in this *C* nonsense mutant have a molecular weight about half that of wild type. In wild type, as shown in Figure 3, the cyclohydrolase (*A*) and dehydrogenase (*C*) elute together. The molecular weight for the complex, estimated both on G-100 and G-200, is approximately 165,000. The peak of cyclohydrolase and pyrophosphohydrolase activities in *his4-864* elutes slightly ahead of *Escherichia coli* alkaline phosphatase (Fig. 3) at a position corresponding to a molecular weight of about 85,000. In strain *his4-280*, a *C* missense mutant, the *A* and *B* activities show the same elution profile as wild type. The molecular weight of the *his4* enzymes determined on Sephadex is nearly twice that obtained from ultracentrifugation, perhaps indicative of disaggregation upon dilution in sucrose gradients. However, by both procedures, *A* and *B* activities in *his4-864* have half the molecular weight of wild type.

Antipolar mutants: Several *his4C* nonsense mutants are antipolar and significantly lower the activities of cyclohydrolase and pyrophosphohydrolase. Mutants *his4-702* and *his4-1155* are nonsense mutations mapping in the *his4C* region. Both of these strains are efficiently suppressed by class I subset I suppressors,[7] which suppress the nonsense codeword UAA.[8] Both *his4-702* and *his4-1155* fail to complement *B* mutants and have no detectable pyrophosphohydrolase activity. Cyclohydrolase (*A*) activity is reduced to about 10 per

FIG. 3.—The elution pattern of cyclohydrolase (-·-·-) and dehydrogenase (—Δ—Δ—Δ) from Sephadex G-100. Two ml of a crude extract in 10% sucrose were layered on a 2.5 × 99.5-cm column of Sephadex which had been equilibrated with 0.1 *M* Tris-HCl, pH 7.5, 5 × 10^{-4} *M* L-histidinol, and 5% sucrose. Yeast alcohol dehydrogenase (mol wt 150,000) in the crude extract served as a standard and was assayed by the method of Vallee and Hoch.[10] Purified *E. coli* alkaline phosphatase (0.05 mg) molecular weight 80,000, served as the other marker. Fractions of 2 ml each were collected at a flow rate of 12 ml/hr. (*a*) The pattern of a wild-type extract. (*b*) The pattern of *his4-864*. Vertical scale inside: protein (—▲—▲—▲—) as OD 280. Vertical scale outside: histidinol dehydrogenase activity as Δ OD 340 mμ/ml/min or cyclohydrolase activity as Δ OD 550 mμ/0.05 ml/hr. The fraction of maximum alcohol dehydrogenase activity is represented by an arrow marked *1*. The fraction of maximum alkaline phosphatase activity is represented by an arrow marked *2*. The pyrophosphohydrolase (*his4B*) activity was not assayed here, but in other experiments it was always congruent with cyclohydrolase (*his4A*).

cent that of wild type and has a molecular weight as determined by sucrose gradient centrifugation of 45,000 (Fig. 2) and by Sephadex chromatography of 85,000 (not shown). Thus, some nonsense (ochre) mutations in *C* alter the molecular weight of *A* and drastically lower the levels of both *A* and *B* activities.

Discussion.—The *his4* region of yeast can be interpreted according to the operon model. However, the organization of this region shows details distinct from those commonly shown by bacterial operons. If *his4* is an operon, then *his4 A*, *B*, and *C* regions specify three polypeptides. These proteins must then aggregate to form the final multifunctional product of the *his4* region in order to explain their physical association upon ultracentrifugation and Sephadex chromatography. Extensive purification has failed to separate active components of this aggregate.[11] As Manney[12] has shown, nonsense mutations in yeast as in *E. coli* lead to termination of the polypeptide chain. Nonsense mutations in *his4C* could produce the dual effects seen in Figure 2—termination of the *his4C* chain and disruption of the enzyme aggregate.

What is difficult to resolve with this model, however, is the pleiotropic effects of the mutations in mutants *his4-702*, *his4-1155*, and *his4-864*. The former two mutants show a strong antipolar effect, while the latter does not. This is the exact opposite of the situation found in bacterial operons,[13] where the nonsense mutations closest to the intercistronic barrier (operator proximal) show the greatest antipolar effect. Thus, to interpret *his4* as an operon (with the provision that many of the properties of this region are attributable to the aggregation of the *his4* product) requires the *ad hoc* assumption that the incomplete *C* peptide made by mutants *his4-702*, and *his4-1155* inhibits *A* and *B* activity, while that made by *his4-864* does not.

A second difficulty with the operon model is the complete polarity of *his4A* nonsense mutants. Some complementation between *his4A* nonsense mutants and other *his4* mutants should have been observed at least for the most distal *his4A* mutants such as 644 and 39.

An alternate model for the *his4* region envisions that it encodes a single polypeptide carrying three activities (*A*, *B*, and *C*). This might explain the absolute polarity of *his4A* nonsense mutants and even the behavior of nonsense *his4C* mutants which retain some *hisA* and *hisB* activity. While one might expect that a nonsense mutation in the middle of *his4* should destroy all three activities, this need not necessarily be the case. Some precedent might come from the production of α peptide by mutations in the ω region of the *Z* gene of the *lac* operon.[14] One difficulty with this model would be our inability to detect any difference between the sedimentation and chromatographic properties of the activities in mutants *his4-864* and *his4-702* or *his4-1155*.

Formally, *his4 A*, *B*, and *C* act at one time as a single polypeptide chain and at another as independent polypeptide chains. A mechanism of protein synthesis has been proposed for the production of poliovirus proteins which might be applicable to the present situation.[15] It could be that *his4* produces a single polypeptide chain which is subsequently cleaved into three separate chains by proteolytic enzymes. By this mechanism of protein synthesis a nonsense mutation at the beginning of *his4* would be completely polar. As we obtain more in-

formation on the physical nature of the *his4* protein(s), we should be able to resolve these various possibilities.

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