A CLUSTER OF GENES CONTROLLING THREE ENZYMES IN HISTIDINE BIOSYNTHESIS IN *SACCHAROMYCES CEREVISIAE*[1,2]

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THE difference in the distribution of genes on the chromosomes of eucaryotes as compared with the gene distribution in procaryotes is often cited as a major evolutionary divergence. Recent studies on a large number of auxotrophic mutants of *Salmonella typhimurium* (DEMEREC 1964) indicate that in this procaryote the majority of gene loci that represent phenotypically related groups are arranged in clusters of two or more on the bacterial chromosome. For example, all ten genes specifying the histidine biosynthetic enzymes are clustered in a small region of the Salmonella chromosome (AMES and HARTMAN 1963). The genes within some of these clusters do not function autonomously; rather, they appear to constitute a polarized unit of integrated expression, an operon (JACOB and MONOD 1961). In the eucaryotes such clustering is generally not observed. By contrast with the close linkage of the histidine genes in Salmonella, the histidine loci of yeast are in general distributed throughout the genome (HAWTHORNE and MORTIMER 1960) as are the histidine loci of *Neurospora crassa* (WEBER and CASE 1960). However, more detailed investigations in Neurospora (AHMED, CASE and GILES 1964) have revealed that the *hist-3* region controls three steps in histidine biosynthesis and appears to be partially analogous to the more extensive histidine operon in Salmonella.

In the yeast, *Saccharomyces cerevisiae*, the gene-enzyme relationships for histidine biosynthesis have been established (FINK 1964). These studies indicated that the structural genes for most of the histidine enzymes, which catalyze the identical biosynthetic reactions as the Salmonella enzymes, are widely dispersed on different chromosomes. However, the *hi-4* mutants appeared to be functionally diverse: some lacked the second enzyme in the pathway, PR-AMP pyrophosphohydrolase; others lacked the third enzyme, PR-AMP 1,6 cyclohydrolase; and a third group lacked the last enzyme, histidinol dehydrogenase. The purpose of the present report is to show that this cluster of *hi-4* alleles has many of the properties of bacterial operons: biochemical heterogeneity, polarized complementation, asymmetric localization of entirely noncomplementing mutants, and suppressibility of polar mutations.

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MATERIALS AND METHODS

Yeast strains: Heterothallic strains of *Saccharomyces cerevisiae* were obtained from Dr. R. K. Mortimer. Histidine-requiring mutants were obtained by treatment with ethyl methanesulfonate (EMS). To ten ml of a 36 hr culture (approximately 2×10^8 cells/ml) at pH 8, 0.3 ml of EMS was added. After 40 minutes the cells were diluted and plated. Mutants were isolated by replica-plating (Lederberg and Lederberg 1952).

Media: The complete medium was: yeast extract peptone (YEP): Difco yeast extract, 1%; Difco Bacto-Peptone, 2%; dextrose, 2%; agar, 2%. Wickerham's (1946) synthetic medium was the minimal medium. It was supplemented (per liter) where necessary with: DL-histidine, 20 mg; uracil, 20 mg; adenine, 20 mg; and DL-tryptophan, 20 mg. Cells were presporulated on a medium (GNA) which contained: dextrose, 10%; Difco yeast extract, 0.3%; Difco nutrient broth, 0.8%. The sporulation medium (RA) contained: raffinose, 0.022%; potassium acetate, 0.97%; agar, 2%.

Genetic methods: For testing the complementation response haploid mutants of the same mating type were striped in six parallel lines on a YEP plate. After a day's growth at 30°C, this plate was cross-replicated by a plate striped with strains of opposite mating type to a third YEP plate for an additional day's growth. This last plate was replicated to minimal medium and growth was scored at the intersection areas where diploids had formed. Tests for allelism with the standard histidine tester strains (von Borstel 1963) were scored after one day, while allelic tests were scored after 1, 2, 4, and 7 days. Heteroallelic diploids which had not grown perceptibly in 7 days were scored as noncomplementing.

Suppression was determined by tetrad analysis. Hybrids were precultured for two days in GNA and sporulated on RA. Asci were dissected by the method of Johnston and Mortimer (1959). The resulting spore isolates were scored by replica-plating on the appropriate media.

The method for allelic mapping is based on the frequencies of X-ray-induced reversion of heteroallelic diploids (Manney and Mortimer 1964). The method of Manney (1964) was carefully followed except for several minor adjustments which had to be made for this study because of the lower dose-output of the X-ray machine. Instead of irradiating the cells on the agar surface of a petri plate, cells were allowed to settle in a monolayer in 10 ml beakers and were irradiated at 2 and 4 kr from a G.E. Maxitron 250 with a 1 mm aluminum filter. The dose rate at the position of the cells was 47.6 r/sec.

Accumulation studies: The methods designed by Dr. Bruce Ames for the detection of imidazole intermediates and used for the yeast histidine mutants have been described (Fink 1964).

Preparation of cells for enzyme assay: After two days growth at 30°C in YEP the yeast cells were collected by centrifugation, suspended in a small volume of 0.01 M Tris-HCl, pH 7.4, and disrupted in a pressure cell similar to that described by Eaton (1962). Cell debris was removed by 10 minute centrifugation at 10,000 $\times$ *g*. Protein was determined by the method of Lowry (Lowry, Rosebrough, Farr and Randall 1951). Extracts were passed through a column of Sephadex G-25 in lieu of dialysis.

Assay of PR-AMP pyrophosphohydrolase: The enzyme carries out the conversion of PR-ATP $\rightarrow$PR-AMP (Figure 1). The assay used has been described by Ahmed, Case and Giles (1964).

Assay of PR-AMP 1,6 cyclohydrolase: This enzyme carries out the reaction, PR-AMP$\rightarrow$ BBMII. The assay used has been described by Smith and Ames (1965).

Assay of histidinol dehydrogenase: This enzyme catalyzes the reaction, L-histidinol + 2 NAD^+ $\rightarrow$2 NADH + H^+ + L-histidine. The assays were carried out on partially purified extracts as follows: to 0.25 ml of 0.2 M triethanolamine-HCl buffer, pH 8.5, were added 0.05 ml of the extract, 0.05 ml of 8 mM NAD^+, and 0.02 ml of 100 mM L-histidinol·2 HCl. The blank contained H_2O in place of the histidinol. The change in optical density at 340 mμ was recorded.

RESULTS

Complementation: One hundred and forty *hi-4* mutants were derived in haploid strains of *S. cerevisiae*. Fifty-eight of these mutants were crossed by wild

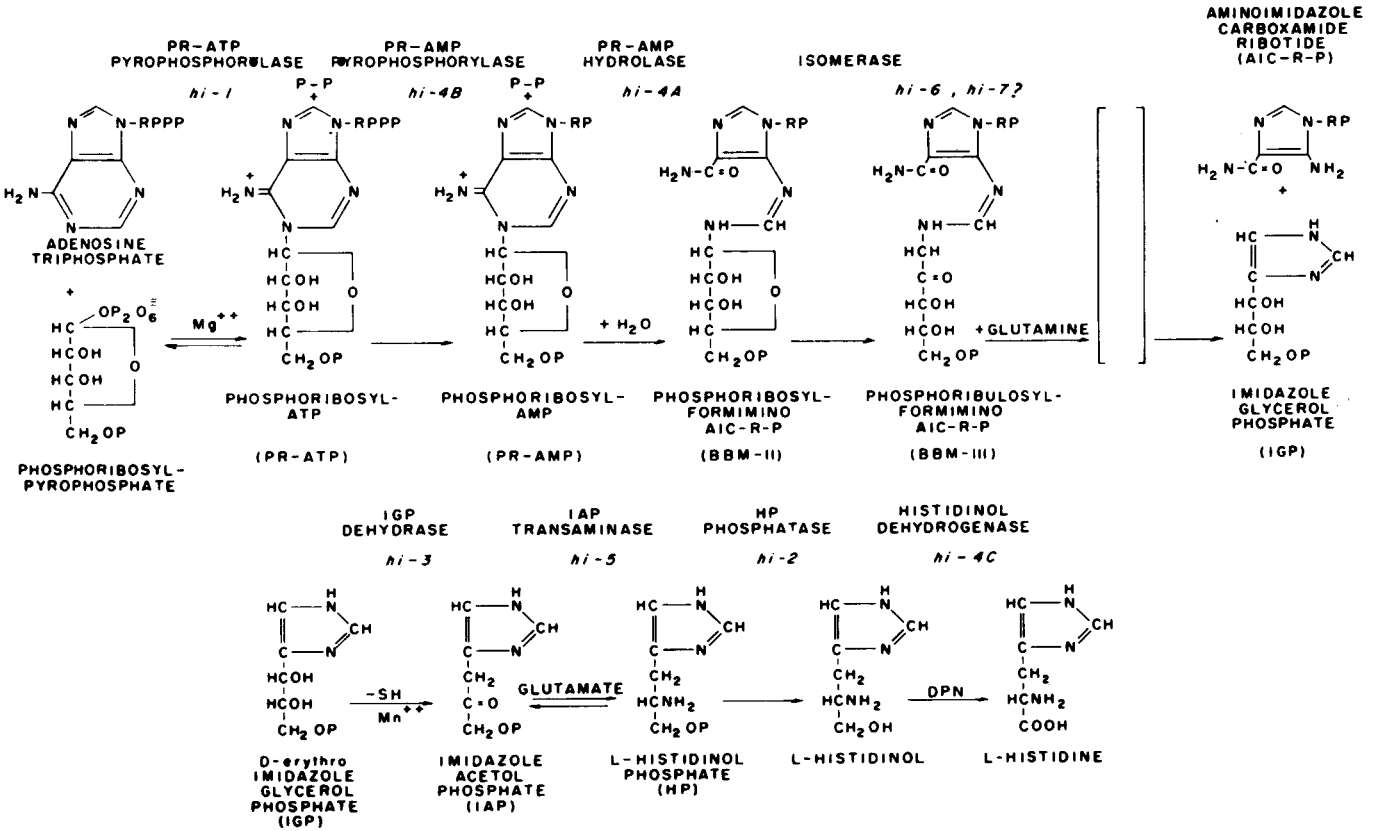

FIGURE 1.—The pathway of histidine biosynthesis based on the studies of SMITH and AMES (1965). The gene-enzyme relationships presented by FINK (1964) are indicated above the chemical structures.

type, and derived in the opposite mating type. Complementation responses were tabulated for all 3,364 pairwise combinations of these 58 mutants. There were no disagreements between the originals and the repeat tests using progeny isolates. The complementation map constructed from these tests is shown in Figure 2. Standard mutant testers exhibiting characteristic complementation responses were then used to assign positions on the complementation map to the remaining 82 mutants which had not been derived in both mating types.

There are three large groups of complementing mutants designated *hi-4A* (481,877), *hi-4B* (331,594), and *hi-4C* (470,721). There is complementation within both A and C. The order of the *hi-4A* mutants relative to the B and C mutants can not be determined on the basis of complementation data alone, since a mutant class overlapping both A and B or both A and C is absent. The order presented in Figure 2 is based upon the genetic analysis. The complementation response within a given group differed noticeably from that between groups. Growth of the complementing diploid was always rapid (observable after 1 day) when the mutant pairs were from different groups, whereas it was often restricted (observable only after 3 or 4 days) when the mutant pairs were from the same group. This latter phenomenon is characteristic of interallelic complementation (GILES 1963). In addition to these mutants with restricted defects there are multiply defective, overlapping ABC types such as 4, 39, 260 as well as BC types such as 147, 702, and 715.

Accumulation: The accumulations characteristic of the *hi-4* mutants were studied by paper chromatography. The results of the chromatography of extracts of 40 *hi-4* mutants appear in Table 1. A comparison of the accumulations of the

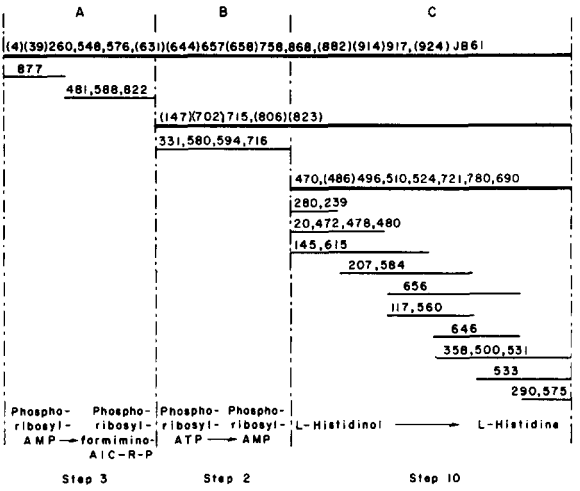

FIGURE 2.—Complementation map of the *hi-4* region in yeast. Each number represents a specific mutant of independent origin. The damage to the polypeptide chain caused by the mutation is represented by the extent of the solid line across the map. Overlapping lines mean that the mutations involved fail to complement, which is presumed to mean that their mutational defects overlap. The mutants enclosed by parentheses are suppressible by S_d.

TABLE 1

The accumulations characteristic of hi-4 *mutants grouped with respect to their location on the complementation map. The ± indicates a slightly weaker accumulation of histidinol*

Mutant	Histidinol	Mutant	Histidinol
ABC 39	—	B 594	—
ABC 4	—	BC 147	—
ABC 631	—	BC 701	—
ABC 644	—	BC 806	—
ABC 917	—	C 721	±
ABC 924	—	C 470	+
ABC 260	—	C 486	±
ABC 758	—	C 510	+
ABC 868	—	C 496	+
A 481	—	C 280	±
A 588	—	C 239	±
A 926	—	C 20	+
A 876	—	C 472	+
A 880	—	C 97	+
A 891	—	C 145	+
A 926	—	C 207	+
A 935	—	C 117	+
A 877	—	C 560	±
B 331	—	C 533	+
B 580	—	C 290	+

hi-4 mutants relative to their positions on the complementation map reveals an unusual situation. Mutants in the A and B groups do not accumulate any imidazoles, whereas all of the C mutants tested accumulate histidinol. Multiply defective types such as ABC or BC mutants do not accumulate any imidazoles. These data suggested that the *hi-4* region might be responsible for more than a single step in histidine biosynthesis. The C mutants appeared to be defective for the last step in the pathway, the conversion of L-histidinol to L-histidine, while the A and B mutants appeared to lack enzymes early in the pathway prior to the formation of imidazole glycerol phosphate.

Enzyme assays: The possibility of functional heterogeneity was verified directly by assaying extracts of the yeast mutants. The results of these assays appear in Table 2. It is clear from these data that the *hi-4A* mutants lack the cyclohydrase which converts PR-AMP into BBM II and that the B mutants are defective for the pyrophosphohydrolase which catalyzes the conversion of PR-ATP to PR-AMP. These two reactions are steps prior to the formation of the imidazole ring (Figure 1). The C mutants are unable to carry out the tenth step, the conversion of L-histidinol to L-histidine, catalyzed by the enzyme histidinol dehydrogenase. Mutants restricted to one complementation group do not affect the enzymatic activity of an adjacent group. The mutants whose defects cross intergroup regions on the complementation map lack or have reduced levels of all three enzymatic activities associated with the region. On the basis of the striking correlation between the biochemical and complementation studies it appears that A, B, and C

TABLE 2

PR-AMP 1, 6 cyclohydrolase, PR-ATP pyrophosphohydrolase, and histidinol dehydrogenase in wild-type and hi-4 *mutant strains*

Mutant	Units of enzyme per mg protein		
	cyclohydrolase	pyrophosphohydrolase	dehydrogenase
ABC 39	0.00	0.10	0.6
ABC 260	0.00	0.17	0.0
ABC 4	0.00	0.00	0.0
BC 147	0.06	0.19	1.2
BC 806	0.00	0.16	0.0
A 481	0.02	1.10	18.0
A 877	0.06	2.30	22.6
B 331	0.94	0.12	21.6
B 594	0.90	0.14	25.3
C 280	0.70	2.26	0.0
C 20	0.85	2.38	0.0
C 290	0.75	2.40	0.0
wild type	0.90	2.50	18.8

represent discrete cistrons organized into a higher functional unit, the *hi-4* region.

Mapping of the hi-4 mutants: The method for allelic mapping in yeast depends upon the sensitivity of heteroallelic diploids to induction of mitotic reversion by X rays (MANNEY and MORTIMER 1964). The increase in revertants due to the radiation is assumed to be proportional to the distance between the two alleles. The order of mutational sites for the *hi-4* region was determined by two-point crosses using this method. Figure 3 is a map of 26 *hi-4* sites of mutation. The most salient features of this genetic map become apparent upon comparison with the complementation map (Figure 4). Each group of mutants, A, B, and C, is restricted to a discrete region of the genetic map. Although, as mentioned before, the A alleles cannot be ordered on the complementation map, the genetic data place them to the left of the B and C alleles. The genetic map is colinear with the complementation map in the regions where this is testable. Thus, the order 280, 560, 575 predicted from the complementation map is identical to the order of these mutational sites on the genetic map.

The most conspicuous feature of this genetic map is the asymmetrical distribution of the noncomplementing mutants. All 11 of the noncomplementing mutants mapped by this procedure are localized within the A region, which comprises about one-third of the entire genetic map of the *hi-4* region. This localization affords the noncomplementing mutants special prominence in view of their biochemical pleiotropy. The other class of pleiotropic mutants, BC, represented by mutants 702, 823, 147, and 806, is not found in either the A or the C region. Their restriction to the B region suggests that once a mutational defect of this type occurs, its effect can proceed only to the right (into the C region) and not to the left (into the A region) of that defect. Otherwise, there would be mutants mapping in B and giving the AB phenotype in the complementation test. No mutants of this type have been found.

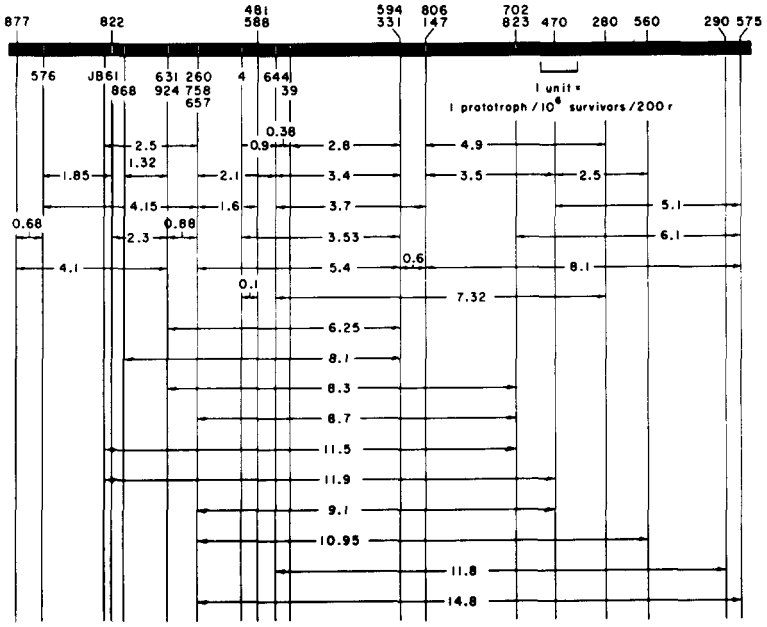

FIGURE 3.—The genetic map of the *hi-4* region based on the X-ray induced reversion frequencies in heteroallelic diploids. The noncomplementing alleles are drawn below the line.

The localization of the noncomplementing mutants to a discrete portion of the genetic map is such a striking feature of the *hi-4* region that it seemed advisable to establish this fact by an independent means. The method which was devised involved the use of double mutants in conjunction with the X-ray mitotic recombination technique. The double mutants were generated by starting with a B mutant which had been selected for growth on histidinol and selecting for a second mutation (of the ABC or C type) which would prohibit growth on histidinol. (For this purpose *hi-4B*-331 was treated with EMS and plated at the appropriate dilution on YEP. These plates were replicated to minimal + histidinol and colonies failing to grow were isolated from the YEP master plates. Eleven mutants were isolated which failed to grow on histidinol and also showed altered complementation patterns.

The successful use of double mutants for mapping depends upon the rarity of double crossover events. If a mutation lies between the sites of the double mutant it will take a double crossover to produce a prototroph; whereas, if it is outside, a single crossover will suffice. Consequently, a vastly reduced frequency of prototrophs is indicative of localization within the region bounded by the sites of the double mutant. Figure 5 shows a series of histidine-requiring diploids, each square representing a different *hi-4* mutant crossed by the same double mutant. The plates were irradiated with 6 kr. The double mutant used in the experiment shown here contained, in addition to the original B site mutation, a newly-induced ABC site mutation (as determined by complementation, biochemical, and genetic analysis) so that the double mutant strand was ABC B . The responses ob-

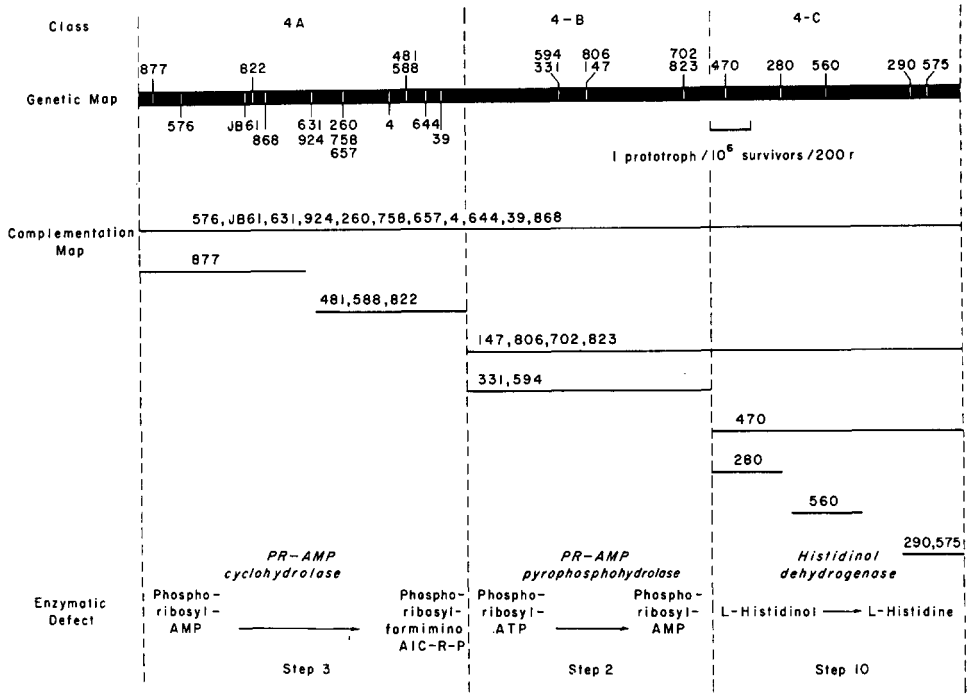

FIGURE 4.—A comparison of the genetic and complementation maps for the *hi-4* region. The enzymatic defects for mutations in each region are indicated at the bottom of the figure.

tained from a plate like the one shown can be represented as a simple array of +'s and −'s depending upon the production of prototrophs. Thus, in the left-hand column of the bottom plate in Figure 5, mutants 868, 548, 260, and 917 appear to lie within the sites of the double mutation while 560 and 486 lie outside of this region. A correlation of these responses with the positions of the mutants on the complementation map shows that all of the noncomplementing alleles (marked by an asterisk) produce low frequencies of recombinants when crossed with this double mutant, whereas all of the strains having a mutation in the C region give high frequencies. This analysis has been extended to show that of the 140 mutants isolated all 22 noncomplementing mutants, as well as all 9 *hi-4A* and all 6 *hi-4B* mutants, are located within the region bounded by this double mutant. All the noncomplementing mutants recombine with high frequencies with at least five other independently isolated double mutants of the composition $\frac{B}{C}$. Several of these BC double mutants which recombine with the noncomplementing mutants and all B mutants (except 594) give low numbers of recombinants with all other C mutants.

These data delineate two mutually exclusive locations in the genetic material: one occupied by the 22 noncomplementing mutants and one occupied by the C mutants. This strongly supports the unusual distribution of the noncomplementing mutants on the recombination map determined by two point crosses.

Suppression: HAWTHORNE and MORTIMER (1963) investigated three suppres-

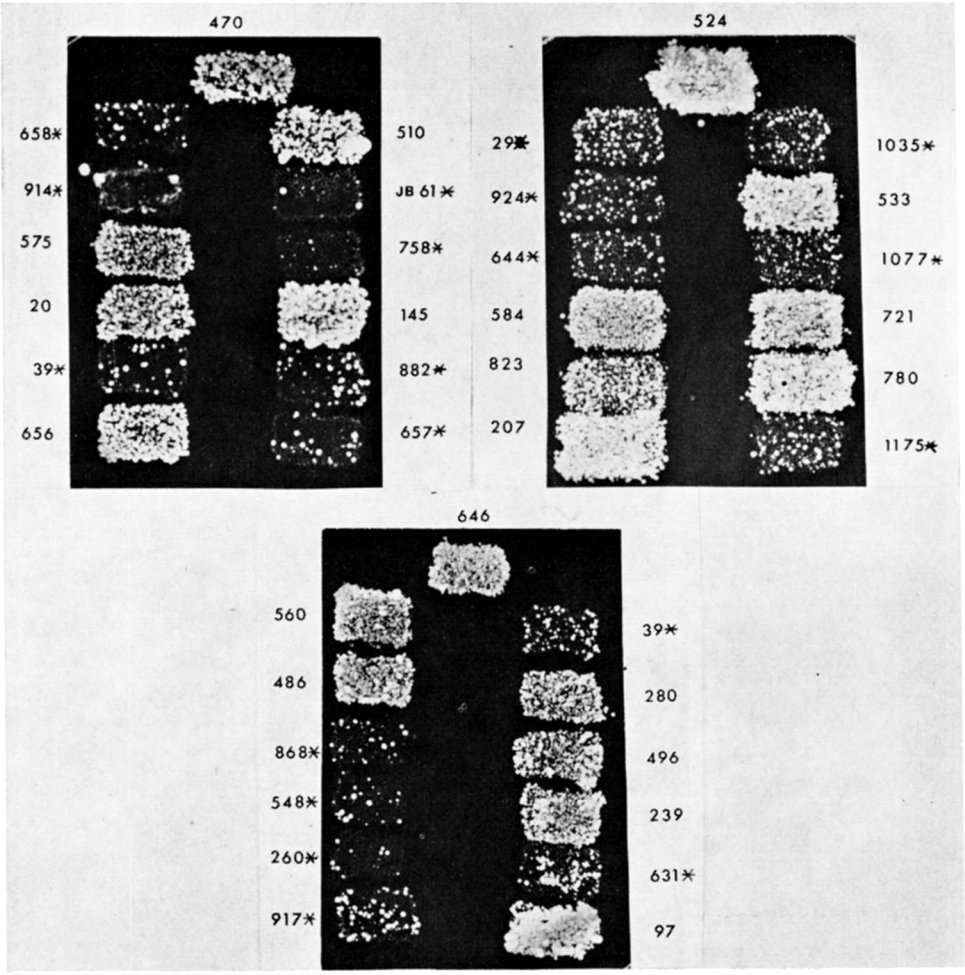

FIGURE 5.—The relative frequency of prototrophs produced by a variety of *hi-4* alleles when crossed by a $\frac{\text{ABC}}{\text{B}}$ double mutant. The white areas of confluent growth are due to *hi*+ recombinants. The infrequent white colonies in the otherwise blank areas are presumably double recombinants. Alleles marked by an asterisk are noncomplementing.

sors in yeast designated S_a, S_b, and S_c. These resembled the "amber" suppressors in *E. coli* by virtue of the fact that they suppressed mutations at phenotypically diverse loci. In addition, a fourth suppressor, S_d, was found to suppress noncomplementing mutants at the tryptophan synthetase locus in yeast (MANNEY 1964).

The *hi-4* mutants were tested for suppression by S_d by analysis of hybrids heterozygous for S_d and the allele being tested and homozygous for *ad-2-1* (adenine requiring and suppressible by S_d). The homozygous *ad-2* allele allows detection of the suppressor-bearing spores (MANNEY 1964). If a *hi-4* allele is suppressible it should give rise to an excess of histidine independent phenotypes

TABLE 3

Segregation analysis of hi-4 *mutants* × *suppressor* (S_d)

Mutant class	Number of tetrads 4:0	3:1	2:2	Spores $S_d^+hi^+:S_d^+hi^-$	Mutant class	Number of tetrads 4:0	3:1	2:2	Spores $S_d^+hi^+:S_d^+hi^-$
ABC					B				
4	0	2	4	7:0	331	0	0	6	6:4
39	0	3	6	8:0	594	0	0	6	4:5
260	0	0	9	7:9	C				
548	0	0	4	2:1	486	0	2	6	14:0
576	0	0	8	8:8	470	0	0	5	2:3
631	4	4	1	13:0	496	0	0	6	4:3
644	3	8	1	8:0	510	0	0	6	6:6
658	1	1	1	2:0	780	0	0	5	4:5
882	2	4	3	10:0	721	0	0	5	2:4
914	1	2	2	5:0	C				
917	0	0	7	7:7	20	0	0	6	4:6
JB61	0	0	4	3:5	97	0	0	4	2:2
924	1	3	3	5:0	117	0	0	5	1:3
A					145	0	0	6	3:4
481	0	0	9	7:9	207	0	0	3	1:2
BC					239	0	0	4	4:7
147	5(?)	0	4	12:0	290*	0	1	5	5:7
715	0	0	7	8:6	326	0	0	5	2:2
806	3	5	4	8:0	329	0	0	5	2:6
823	1	3	2	4:0	448	0	0	4	4:4
702	1	4	0	6:0	475	0	0	5	7:6
					478	0	0	6	3:3

* The wild-type spore from mutant 290 was *ad*$^+$. It was backcrossed to a strain which carried the *ad-2-1* allele. The backcross gave no histidine-requiring progeny. Hence, this was probably a "false tetrad" arising from the chance association of nonsister spores (JOHNSTON and MORTIMER 1959). The (?) after 147 refers to the fact that in the growth test the spores carrying the suppressor showed extremely restricted growth on minimal medium (were only slightly suppressed).

in tetrad analysis. Furthermore, such a suppressible allele should not give rise to spores which carry the suppressor and which require histidine. The data for 39 *hi-4* mutants appear in Table 3. The data are tabulated in the following ways: (1) segregation ratios among four-spored asci (2) $hi^+:hi^-$ phenotypes among suppressor-bearing spores. From these data it is concluded that 13 of the 39 alleles are suppressible by S_d.

The relationship between complementation pattern and suppression is shown in Figure 2. The suppressible alleles are enclosed by parentheses. Only the entirely noncomplementing ABC mutants, the BC mutants, and a noncomplementing C mutant are suppressible. The latter two categories are similar insofar as these types show a polarized loss of activity. These suppressible mutational defects are such that they may complement to the left but not to the right.

DISCUSSION

These studies have revealed a cluster of histidine mutants in yeast which exhibit remarkable phenotypic discontinuities. There are three groups of mutants

as determined by combined genetic, biochemical and complementation analyses. These groups appear to correspond to three different structural genes which specify the enzymes which catalyse the third, the second, and the final steps in histidine biosynthesis. The organization of this locus into a larger functional unit is shown by the noncomplementing ABC and partially noncomplementing BC mutants, which are defective for all three biochemical reactions. These multiply defective types show a polarized loss of function on the complementation map (Figure 2). Once a polar mutation has occurred its effect proceeds to the right but not to the left of the mutational site. Furthermore, the noncomplementing mutants are not distributed randomly throughout the *hi-4* region, but are restricted to one end of that region. This localization of the noncomplementing alleles is distinctly different from the situation arising when a single gene specifies a single enzyme. In diverse studies where complementation analysis, fine-structure analysis, and enzymology have been combined—*E. coli* alkaline phosphatase (GAREN and SIDDIQI 1962), *S. cerevisiae* tryptophan synthetase (MANNEY 1964), *Schizosaccharomyces pombe* adenylosuccinase (MEGNET and GILES 1964)—the noncomplementing mutants are distributed at random within the locus.

Polarization of the complementation map and the asymmetrical localization of noncomplementing alleles are characteristic of operon systems (LOPER, GRABNAR, STAHL, HARTMAN and HARTMAN 1964). These two features seem to be attributable to the effects of an amber mutation occurring within an operon. It was originally proposed that the amber mutants in the RII cistron of T4 were unable to synthesize a complete protein (BENZER and CHAMPE 1962). There is now direct evidence that the molecular consequence of amber mutations is the production of polypeptide fragments (SARABHAI, STRETTON, BRENNER and BOLLE 1964). Incomplete polypeptide chains are produced because the amber mutation gives rise to a nonsense codon (BRENNER, STRETTON and KAPLAN 1965; WEIGERT and GAREN 1965) which prohibits the sequential translation of messenger-RNA. In an operon an amber mutation can have pleiotropic effects. These effects appear to be related to the fact that there is a single messenger for all of the structural cistrons in an operon (MARTIN 1963) and that this messenger is translated sequentially from the operator end (GOLDBERGER and BERBERICH 1965). Many amber mutations appear to abolish the activity of all structural cistrons distal (with respect to the operator) to the site of the mutation (NEWTON, BECKWITH, ZIPSER and BRENNER, in press). Thus, an amber mutation which occurs in the structural cistron nearest to the operator can prevent translation of subsequent cistrons and will appear totally noncomplementing. However, an amber mutation located in the second cistron will be functional for the first cistron and defective for subsequent cistrons. As a result of this, the amber mutations not in the first cistron will be classified as polarity types. Consequently, in an operon the entirely noncomplementing types are restricted on the genetic map. All must map proximal to or within the first structural cistron. This is one of the most significant distinguishing features of an operon. An important corollary to asymmetric distribution of the noncomplementing mutants is the polarization of the

complementation map. Any mutant which loses the function of more than one cistron loses additional functions only distal to the point of mutation, and this loss progresses to the end of the operon.

The operon model satisfactorily explains many of the observations described above for the *hi-4* region. The mutants confined to single regions on the complementation map are attributed to missense mutations producing amino acid substitutions and consequently an altered enzyme for that cistron. The polarized mutants could arise from amber mutations or from nonsense codons arising from frame shifts (although reversion studies gave no evidence for frame shifts among the *hi-4* mutants). The polarity of translation in the *hi-4* region would therefore be from A → C (Figure 2). The suppressor in yeast, S_d, has many of the properties of the amber suppressors in *E. coli* where it has been shown in one case that suppression of chain termination is caused by an altered transfer-RNA (Capecchi and Gussin 1965). Thus, the suppression of a mutation by an amber suppressor indicates that the site of action of the mutation is during protein synthesis. By analogy with the situation in bacteria, mutations which cross inter-cistronic boundaries on the complementation map of *hi-4* should be suppressible. This prediction is substantiated by the data in Table 3 which show that only those mutations identified as polarity mutations on the complementation map are suppressible by S_d.

On this model, *hi-4A*, *B*, and *C* represent three cistrons producing three polypeptides. The BC mutants, therefore, should have the ability to catalyze the A reaction. As can be seen in Table 2, their ability to carry out this reaction is vastly reduced. Furthermore, attempts to separate the A, B, and C activities by gel-filtration and ultracentrifugation have so far been unsuccessful. This suggests that these three polypeptides are associated in a heteropolymeric complex similar to that described for the A and B components of bacterial tryptophan synthetase (Crawford and Yanofsky 1958). Although the individual components of the tryptophan synthetase molecule are responsible for separate reactions, these reactions do not take place at normal rates without the aggregation of the two components. This scheme suggests that the *hi-4A*, *hi-4B*, and *hi-4C* cistrons produce the A, B, and C polypeptides which catalyze all three reactions when associated and none of the reactions when disaggregated. Since the BC mutation is a polar mutation, it allows the production only of A chains which alone have no A activity. However, in a heterozygous diploid, BC/A, the strand carrying the missense A mutation will provide normal B and C chains. These can complex with the A chain made by the strand carrying the BC mutation, yielding a functional complex. This model explains the growth of A/BC diploids and the absence of A activity in the BC haploids. Further work on the physical separation of these polypeptides should reveal whether or not this model is correct.

Clustering of related genes has been described in yeast for the three cistrons specifying the galactose enzymes (Douglas and Hawthorne 1964). However, there is no evidence for polarity mutations in that cluster so that it can not be determined as yet whether these cistrons act as a single unit of genetic transcription or as independent units. The situation described for the *hi-4* region of yeast is remarkably similar to that described for another Ascomycete, *Neurospora*

crassa (AHMED, CASE and GILES 1964). In Neurospora, a cluster of cistrons controls the same three steps: the second, the third, and the last reactions in histidine biosynthesis. This complex shows the properties of polarization and localization of noncomplementing mutants identical to those elaborated above for yeast. In both yeast and Neurospora the order of polarity is reaction 3, reaction 2, reaction 10; whereas, in Salmonella the order is reaction 10, reaction 3, reaction 2. Moreover, histidinol dehydrogenase has a molecular weight (estimated by sucrose gradient centrifugation) of 101,000 in yeast (FINK 1965), 108,000 in Neurospora (AHMED 1964), and 75,000 in Salmonella (LOPER and ADAMS 1965). These observations reflect the high degree of homology in genetic structure between the yeast and Neurospora histidine systems and a clear difference from Salmonella.

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SUMMARY

The biochemistry, genetics and complementation of mutations in the *hi-4* region of yeast have been studied. The *hi-4A* cistron specifies the information for the third step in histidine biosynthesis, the *hi-4B* cistron for the second, and the *hi-4C* for the last. Enzyme assays show that mutants limited to one cistron on the complementation map lack only one enzymatic activity. In addition to these mutants exhibiting localized defects, there are ABC and BC mutants which have lost all three enzymatic activities. These multiply-defective mutants behave as partially complementing or completely noncomplementing types and are characterized by a striking polarization of the mutational defect from the left to the right on the complementation map of the *hi-4* region. The genetic distribution of the *hi-4* alleles was examined by fine-structure analysis both by two-factor and by three-factor crosses. The noncomplementing alleles are not distributed at random throughout the region; rather, all 22 tested are clustered in the A region. Asymmetric localization of the noncomplementing alleles, biochemical heterogeneity, and polarization of the complementation map are characteristics of operons but not of single cistrons. Consistent with the operon interpretation of the *hi-4* region is the observation that only the noncomplementing alleles or alleles having polarized defects within *hi-4* are suppressible by the amber-like suppressor, S_d.

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