ALLELIC COMPLEMENTATION IN THE FIRST GENE FOR HISTIDINE BIOSYNTHESIS IN SACCHAROMYCES CEREVISIAE. II.
COMPLEMENTATION MAPPING OF MUTANTS AND A SUBUNIT MODEL OF THE ENZYME1,2

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ABSTRACT

Sixty-two alleles of the histidine-1 (his1) gene were tested for complementation. The 44 complementing mutants fell into 31 complementation groups which were used to construct a complex complementation map with 18 complementation units. Cluster analysis of the complementation map by either visual inspection or the computer method of Gillie and Petto (1969) shows two very definite clusters.

The molecular weight estimate of the hist enzyme, phosphoribosyl adenine triphosphate: pyrophosphate phosphoribosyltransferase, is $1.8 \times 10^5$ by sucrose density gradient analysis and $2.4 \times 10^5$ by Sephadex gel chromatography. Correlating the length of the his1 gene to the molecular weight of the enzyme indicates that this enzyme is composed of 6 subunits, as is the analogous enzyme in Salmonella typhimurium.

A model of the subunit and tertiary and quaternary structure of the enzyme has been developed from consideration of the genetic and complementation data, the distribution of the various mutant types within the gene, and the biochemical properties of the enzyme encoded by the his1 gene.

GENETICS can quite possibly provide a tool to aid the investigation of the tertiary and quaternary structure of multimeric proteins, since intragenic complementation is known to involve the interactions of the subunits (Schlessinger and Levintal 1963; Fincham and Coddington 1963a,b). Thus, analysis of the pattern of complementation may facilitate analysis of protein conformation.

The most recent and lucid review of complementation is presented by Fincham (1966). Intragenic complementation occurs when some mutants defective in the same biological function are united in a common cytoplasm, forming a diploid or heterokaryon which then is no longer mutant in phenotype.

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Many explanations have been proposed to explain this kind of complementation, which is distinct from complementation between mutants of different genes in the same biosynthetic pathway (i.e., intergenic complementation). The protein-protein interaction hypothesis of Catchside and Overton (1958), Brenner (1959), and Fincham (1959) has withstood the critical test of in vitro production of complementation by mixing the proteins of two allelic mutants with the subsequent formation of a functional protein which is a hybrid of the two different subunits (summarized by Fincham 1966). The properties of the in vivo and in vitro complementation products are very similar, and optimally they are aggregates of an equal number of both mutant subunits (Fincham and Coddington 1963a,b; Schlesinger, Torriani and Levinthal 1963; Suyama and Bonner 1964). Intragenic complementation is known to occur only in proteins with more than one polypeptide subunit, i.e., in multimeric proteins.

As yet there has been no investigation of sufficient scope to satisfactorily test several predictions of the various models of intragenic complementation. In this study these models have been tested and a specific hypothetical model of the tertiary and quaternary structure of an active multimeric protein has been constructed. This has been done by relating the extensive data on intragenic complementation, genetic mapping, and mutant characterization to some of the physical and biochemical properties of the enzyme, phosphoribosyl adenosine triphosphate:pyrophosphate phosphoribosyltransferase (E.C. 2.4.2.2), coded for by the first gene for histidine biosynthesis, histidine-1, in Saccharomyces cerevisiae.

MATERIALS AND METHODS

The media and the his2 mutants of S. cerevisiae used in this study are described by Korch and Snow (1973). In addition, MHA medium was minimal medium supplemented with 1.5 \cdot 10^{-4} M L-histidinol and 5 \cdot 10^{-4} M adenine sulfate.

Genetic procedures: The complementation responses of 62 his1 alleles were determined by one or both of the following methods.

For the first method, templates with 22 haploid strains of the genotype a his1-x leu2 (or tyr1) or a his1-x tyr1 (or leu1) were made on YEPD plates. Each was subsequently replica-plated 24 hours before use to 3 YEPD plates which were incubated at 30°C. At the time these templates were made, small samples of the haploid strains of the genotype a his1-x ade2 or a his1-x ade2 was spread onto a fourth series of YEPD plates with 0.3 ml of sterile water and incubated for 24 hours at 30°C to make lawns of the haploid strains. Then the templates were replicated onto lawn plates of the opposite mating type. These mating plates were incubated for 24 hours and then replica-plated onto minimal (M) plates supplemented with histidine to check for mating (only the diploids being able to grow), and onto minimal plates to check for complementation. Both replicas were incubated at 30°C for 3 days, at which time the M+histidine plates were scored for complementation at this time and after 5 days of incubation. Complementation was scored as strong (++), weak (+), or none (0).

The second method involved estimation of colony size. From the M+histidine replicas made in the first method and stored in the refrigerator, small samples were taken and streaked on M+histidine plates for a viability check, and onto M plates for complementation testing. The samples were streaked over a 1/8 sector of each plate with a loopful of sterile water. The plates were incubated at 30°C and checked for complementation after 1, 2, 4, and 7 days. They were scored semi-quantitatively using a 30X dissecting microscope on the following scale of colony size: no growth (0); very slight growth, probably slight leakiness of the colonies which remained
about this size for 7 days (1); slight growth, weak complementation (2); intermediate growth, intermediate complementation (3); apparent wild-type growth, strong complementation (4). These values were judged in reference to a standard minimal plate with these different colony sizes preserved on it. Ambiguities were resolved, if possible, by repetitions of the crosses.

The rules by which the \textit{his} complementation map was constructed were as follows:

1. Complementing mutants are represented by nonoverlapping lines and noncomplementing mutants are represented by overlapping lines.

2. "A mutant or group of mutants as represented by a line, or a circle, or any continuous combination of lines and circles, such that the number of complementation units covered by a mutant, and the number of branching points and number of units, is the minimum consistent with rule 1." (GILLIE 1966).

3. Where complementation tests for a particular cross were contradictory, the response of the majority of crosses was assumed. If there was not a majority of one type of response, an assumption was made which was the simplest compatible with the data, so that new features were not introduced into the map based on ambiguous results.

4. Leaky alleles (i.e., 28, 32, and 42) were added to the map after all the other alleles had been included. They were allowed to divide complementation units but not to create new structures in the map.

The following definitions of GILLIE (1966) were used:

1. "A complementation unit is the distance used to separate two adjacent points on the map, defined by the ends of lines representing complementing groups."

2. "A complementation group consists of a number of mutants which have the same qualitative complementing properties."

Biochemical procedures: Yeast phosphoribosyltransferase was extracted in 0.1 M Tris-HCl (pH 7.5) buffer with 0.1 M NaCl, 0.4 mM histidine, 0.5 mM EDTA, and 2.8 mM \( \beta \)-mercaptoethanol (VOLL, APPELLA and MARTIN 1967). The enzyme was assayed by the method of VOLL, APPELLA and MARTIN (1967) in the reaction mixture that consisted of 30 \( \mu \)moles of Tris-HCl buffer (pH 8.5), 3 \( \mu \)moles of MgCl\(_2\), 45 \( \mu \)moles of KCl, 3 \( \mu \)moles of ATP, 0.2 \( \mu \)moles of PRPP, 5 \( \mu \)l of inorganic pyrophosphatase (diluted 1:11 with water), and enzyme in a final volume of 0.3 ml. The adenosine triphosphate (ATP) and 5-phosphoribosyl-1-pyrophosphate (PRPP) concentrations used are higher than those used by VOLL, APPELLA and MARTIN (1967) because initial studies had shown these concentrations as being best. Subsequently, it was found that at this concentration of ATP there is 38\% less activity than at half of this concentration (1.5 \( \mu \)moles per 0.3 ml) which had been used by VOLL, APPELLA and MARTIN (1967). A unit of activity is defined as a change in absorbance of 0.01 at 290 nm in 10 minutes, which corresponds to the formation of 0.83 \( \mu \)moles of PR-ATP per 10 minutes. Bovine liver catalase was assayed by a modification of the method of BEERS and SIZER (1952) suggested by the Worthington Biochemical Co.; yeast alcohol dehydrogenase was assayed by the method of VALLEE and HOCH (1955); rabbit muscle aldolase was assayed as suggested by the Worthington Biochemical Co.; and \textit{E. coli} alkaline phosphatase was assayed by the method of GAREN and LEVINTHAL (1960). Ferritin and chicken ovalbumin were detected in Sephadex G-200 column eluates by their absorbance at either 280 or 230 nm. Protein concentration was determined by the method of LOWRY \textit{et al.} (1951) using ovalbumin as a standard against a blank of the extraction buffer.

Phosphoribosyltransferase was extracted from \textit{S. cerevisiae}, strain RS 15/3, of the genotype \textit{a his4B-331 holl}, which was donated by DR. GERALD FINK. Allele \textit{his4B-331} is a nonsense mutation in the second step of histidine biosynthesis, controlling the hydrolysis of N-1-(5'-phosphoribosyl)-adenosine triphosphate (PR-ATP) to N-1-(5'-phosphoribosyl)-adenosine-5'-monophosphate. The assay for the first enzyme being dependent upon the production of PR-ATP, a block in the second step is necessary to prevent its disappearance. The \textit{holl} mutation enables the strain to slowly take up and utilize histidinol in the production of histidine. Both mutations cause a derepression of the first enzyme by limiting histidine production (G. FINK, personal communication, 1969).

The yeast strain was grown in YEPD medium with vigorous shaking for two days at 30°C to a density of about 10\(^8\) cells/ml; then the cells were shifted to an equal volume of either M or
MHA medium. Shifting to M or MHA medium for about 21 hours at 29°C caused a 5.3- and 4.7-fold derepression of the transferase, respectively. Enzyme extracts were prepared by suspending the harvested cells in a volume of extraction buffer equivalent to their wet weight and disrupting them in a Model MSK Mechanical Cell Homogenizer (Bronwill Scientific, Rochester, New York) by shaking with glass beads (0.45-0.50 mm) for 1 minute at 4000 rpm with cooling by liquid CO₂. A colloid mill and freeze-thawing with liquid nitrogen were tried as methods to disrupt the cells but were found unsatisfactory—especially the latter, which destroyed all enzyme activity. Subsequently, the extract was readjusted to pH 7.5 with 1M Tris base and was centrifuged free of cell debris at 27,000 g for 25 minutes in a Sorvall RC-2B centrifuge at 0°-5°C. Ribosomes and minute debris were removed by centrifugation at 105,000 g at 1°C for 1½ hours. Prior to this step it is not possible to detect enzymatic activity because of the high absorbance at 290 nm.

The sedimentation behavior of phosphoribosyltransferase was studied in linear sucrose density gradients by the method of Martin and Ames (1961). The gradients were run in a Spinco SW50L swinging bucket rotor in a Beckman L2-65B ultracentrifuge at 3° ± 1°C for 13 hours plus about 8 minutes for acceleration and deceleration without braking. The transferase was run alone and the standards (catalase, alcohol dehydrogenase, and alkaline phosphatase) were run at the same time in the other tubes of the centrifuge rotor. Seven drop fractions were collected from the bottom of the tube, with an average of 283.5 drops collected per sample.

The phosphoribosyltransferase was also chromatographed in the extraction buffer on a Sephadex G-200 column prepared as suggested by Pharmacia Fine Chemical Co. Samples with sucrose added were layered under the buffer on the top of the gel bed. Blue Dextran B-2000 (0.13%) and H₂O were added to samples as markers of the void volume (Vᵥ) and of the total bed volume (Vᵥₚ), respectively. Fractions (1.0 ml) were collected at 2°-3°C and the absorbance at 280 and 230 nm, the enzymatic activity of the transferase and standards, and cpm (in a Packard Tri-Carb Scintillation Counter) were measured. Phosphoribosyltransferase extracts were run alone and together with several standards on the column: ferritin, catalase, aldolase alcohol dehydrogenase, and ovalbumin.

Sources of chemicals: ATP and PRPP were obtained from P.L. Biochemicals, Inc., Milwaukee, Wisconsin. E. coli alkaline phosphatase, inorganic pyrophosphatase, yeast alcohol dehydrogenase, rabbit muscle aldolase, bovine liver catalase, chicken ovalbumin, hydrazine sulfate, Tris base ("TRIZMA"), L-histidinol, and Blue Dextran B-2000 were obtained from Sigma Chemical Co., St. Louis, Missouri. Sephadex G-200 (fine) was obtained from Pharmacia Fine Chemical Co., Piscataway, New Jersey.

RESULTS

Of the 1953 possible pairwise combinations involving 62 hisI alleles, both heteroallelic and homoallelic, 1,800 (about 92%) were made using one or both methods of testing for complementation. Some crosses were tested as many as thirteen times. The final judgment as to complementation response is given in Table 1, which was used to construct the complementation map. Complementation response was scored as positive if the score was a + or ++ for method I or a value of 2 or greater for method II. Noncomplementation was indicated if the score was 0 by method I, or 0 or 1 by method II, or if no complementation was observed when the diploid was used in the genetic mapping studies. This latter observation was considered definitive of noncomplementation over any other responses observed with either methods I or II. The cutoff levels were chosen for two reasons. First, they were found to be the only ones by which a complementation map could be constructed, since the lower or higher cutoff level produced chaotic results when the construction was attempted. Second, using the
results of the last day of scoring, in order to allow weak complementation to be scored as well as strong, did not provide a workable separation of responses among the strongly complementing his1 mutations. Consequently, the clearest separation between noncomplementation and complementation occurred between 0/1 and 2/3/4 for method II, and between 0 and ++/++ for method I. Any ambiguities caused by mixed positive and negative results were resolved by the complementation mapping rules described above.

Eighteen his1 alleles did not complement any allele at the above cut-off levels. These alleles are 1F, 2, 4, 15, 17, 19, 26, 31, 35, 39, 41, 45, 47, 49, 52, 53, 54, and 68 and are excluded from Table 1. Those alleles with the same pattern of complementation responses at the above cut-off levels are grouped. Alleles 28, 32, and 42 are leaky at the temperature used in these crosses, giving a colony size of 1 or perhaps 2 in certain crosses as well as with each other, which made scoring difficult. Because of this, these alleles were not included in the complementation matrix shown in Table 1, but were added to the complementation map last.

The complementation map shown in Figure 1 was constructed from the responses of the 44 complementing his1 alleles by the rules described above. There are no exceptional responses to the way the complementation map is drawn. The map has 31 complementation groups and is divided into 18 complementation units (“complons”). The complementation groups vary in size, con-
FIGURE 1.—Complementation map of the hist gene.

taining from one to six alleles. Branching points of complementation groups are indicated by intersecting lines with a large dot at the intersection (e.g., allele 18 and alleles 9 and 27). Complementation groups which behave the same from a branching point are joined together by radial lines with large dots joining appropriate groups. For example, the three groups formed by alleles 20, 37, 46, and 69 respond in the same manner with respect to the mutants in the small outside ring and hence are linked by a radial. The other two examples are the two groups formed by allele 20 and alleles 21, 48, 63, and 65 that complement allele 42 but not allele 28; and the 8 groups formed by alleles 18, 8, 16, 34, 37, 50, 51, and 66 that do not complement alleles 20 or 42. A radial line which intersects a complementation group without a large dot at the intersection is not a branching point for that group—e.g., the radial line joining alleles 20, 21, 48, 63, and 65, which crosses the complementation group of alleles 9 and 27. Allele 61 complements all 44 complementing hist mutations but not the 18 noncomplementing mutations.

GILLIE (1966, 1968) has made a good case or considering the clustering of com-
Allelic complementation maps as representing the points of close interaction between subunits of multimeric protein. Therefore, clustering of complementation groups was investigated in the *hist* complementation map. Gillie (1968) defined clusters as consisting of groups "of similar, mutually noncomplementing mutants," which "are chosen such that their total number is the least possible with any given set of mutants." He suggested two practical ways of identifying complementation clusters, one of which was used here. A histogram in the shape of the complementation map was made by plotting the sum of the number of complementation groups whose center points lie within each complementation unit without regard to the length of the line. To determine the center point of the group that extended into the interior of the map, the center of the longest possible line was determined. Such a histogram is shown in Figure 2.

Two clusters of complementation groups are evident, one in compleons XIII, III, IV, V, and VI, the other in compleons IX, X, and XI. These clusters are labelled A and B, respectively. The center of cluster A is between compleons IV and V, and the center of cluster B is in compleon X. The five single groups with centers in I, between I and II, XV and XVI, XVI and XVII, and in XVIII do not belong definitely to any one cluster, although the former two single groups may belong in cluster A and the latter three may be part of cluster B.

Gillie and Peto (1969) have developed a computer program which performs a cluster analysis on complementation data in the form of a matrix. This computer program provides an objective analysis of clustering. They very kindly performed a computer analysis on the complementation data of 39 complementing *hist* alleles (excluding alleles 20 and 37 which complemented only allele 61
and the three temperature-sensitive alleles which, because of their inherent leakiness, did not elicit clear-cut responses). The data they analyzed contained the responses of 717 of the possible 741 crosses. Six analyses of clustering structure were performed. In five of the six analyses two clusters were consistently detected. Cluster A contained the his1 alleles 3, 6, 40, 7S, 8, 27, 16, 21, 48, 63, 65, 30, 34, 50, 51, and 66. Cluster B contained alleles 23, 25, 14, 22, 38, 43, 67.

### TABLE 2

**Complementation matrix of thirty-nine his1 alleles ordered by the computer program of Gillie and Peto (1969)**

| 1S | 6 | 7S | 9 | 16 | 48 | 65 | 34 | 51 | 5 | 25 | 18 | 38 | 67 | 59 | 24 | 23 | 46 | 55 |
|----|---|----|---|----|----|----|----|----|---|----|----|----|----|----|----|----|----|----|----|
| 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0 | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  | 0  |

0 = no growth
1 = weak growth
2 = intermediate growth
3 = Wild-type growth
* = not tested
ALLELIC COMPLEMENTATION IN *histidine-1* 315

56, 59, 10, 24, 29, 33, 44, 46, and 69. This assignment of alleles to clusters agrees with that seen by visual inspection of the complementation map. In Table 2, the complementation matrix used by Gillie and Peto has been reordered by the computer and one can easily see the clustering of mutants in such a presentation. For this table, all complementation responses of 2, 3, and 4 were considered as positive. In the sixth analysis, based only on the very strong responses, the computer split cluster B into two subclusters. The following exceptions were noted. Allele 61 was never a member of any cluster because it complements all of the complementing alleles. Occasionally, alleles 18, 5, and 36 were put in cluster A and alleles 18 and 55 in cluster B. Although these alleles were not unequivocally classified by this method, it did show to which cluster, if any, they belonged. In the data originally sent to Gillie and Peto, there were two errors made in copying. These were detected by their analyses as exceptional responses for the particular alleles.

Weak positive complementation responses made it easier for the computer to detect the clustering structure. Weak responses were not misleading in the determination of cluster grouping and, in fact, the weak responses allowed the recognition of allele 5 as being atypical. On the basis of strong responses only, it was classified as belonging to cluster A. They found also that the most reliable criterion of complementation was the choice of responses of 2, 3, and 4 (and + and ++ ) as indicating complementation and 0 and 1 as indicating noncomplementation. It was concluded that “the most important aim of experimental technique was to avoid missing weak positive without introducing false positives, the grade of positivity being less important.”

The molecular weight and sedimentation coefficient (s30,w) of the yeast phosphoribosyltransferase were estimated by its centrifugation sedimentation in a linear sucrose density gradient relative to the sedimentation of proteins with known molecular weights and sedimentation coefficients. The standard curve of the distance traveled by the standard proteins versus their sedimentation coefficients was constructed by linear regression analysis of the data. The relative distances the various enzymes sedimented were measured by the difference between the total number of seven drop fractions (B) and the fraction number with the peak of the particular enzymatic activity (A), i.e., (B-A) or (B/A).

The data from three sedimentation experiments are summarized in Table 3. Because the average number of rpm and the total times for the three runs were essentially the same, the data were considered as a unit. The fraction number of peak activity and the total number of fractions were consistent for the three experiments. Using these data, the regressions of (B−A) and (B/A) versus s30,w are very linear (P < 0.01). Assuming that the partial specific volume (v) of the transferase is approximately the same as that of the standard proteins (i.e., between 0.70 and 0.77), the s30,w of this enzyme is estimated by the two above regressions to be 8.6 ± 0.1 s and 8.5 ± 0.1 s, respectively. These values are midway between the values of 8.42 s and 8.83 s (average 8.63 s) reported by Voll, Apella and Martin (1967) for the transferase of *Salmonella typhimurium*, which was a v of 0.747.
### TABLE 3

Sucrose density gradient sedimentation data

<table>
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<tr>
<th>Protein</th>
<th>Experiment</th>
<th>Average rpm</th>
<th>Peak fraction number (A)</th>
<th>Total number of fractions (B)</th>
<th>B/A</th>
<th>Average B/A</th>
<th>B—A</th>
<th>Average B—A</th>
<th>$s_{20,w}$</th>
<th>Molecular weight $\times 10^3$</th>
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<tbody>
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<td>38,846</td>
<td>11</td>
<td>39 3/7</td>
<td>3.584</td>
<td>28.4</td>
<td></td>
<td></td>
<td>11.3$^a$</td>
<td>2.323$^d$</td>
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<tr>
<td></td>
<td>2</td>
<td>38,526</td>
<td>11</td>
<td>40 3/7</td>
<td>3.675</td>
<td>3.452</td>
<td>29.4</td>
<td>28.4</td>
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<tr>
<td></td>
<td>3</td>
<td>38,585</td>
<td>13</td>
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<td>3.099</td>
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<tr>
<td>Alcohol dehydrogenase</td>
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<td>38,846</td>
<td>21</td>
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<td>Alkaline phosphatase</td>
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<td>23</td>
<td>39 4/7</td>
<td>1.720</td>
<td>1.686</td>
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<td>16.45</td>
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<td>0.800$^d$</td>
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<td>38,585</td>
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<td>1.651</td>
<td>16.3</td>
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<td>Phosphoribosyltransferase</td>
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<td>39 6/7</td>
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<td>21.9</td>
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<td>2.158</td>
<td>22.0</td>
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The $s_{20,w}$ value for alkaline phosphatase was assumed to be 6.2, the average of the two known values. The linear regression of B/A and B—A against $s_{20,w}$ of the standards gave a regression with $P < 0.01$. (a) Martin 1963; (b) Schlesinger 1964; (c) Garen and Levinthal 1960; and (d) Klotz and Darnall 1969.
ALLELIC COMPLEMENTATION IN *histidine-1* 317

Martin and Ames (1961) suggested that the sedimentation coefficient of a protein is directly proportional to its molecular weight raised to the two-thirds power. But in these studies it was found that the sedimentation coefficient was not proportional to $(\text{molecular weight})^{2/3}$ for the various standard enzymes used here. Therefore, a smooth curve was fitted to the different values of $s_{20,w}$ corresponding to different molecular weights. Assuming the $s_{20,w}$ of the yeast transferase is $8.55 \, s$ the molecular weight is $1.8 \cdot 10^5$. Martin and Ames (1961), using their relationship, found the molecular weight of the phosphoribosyltransferase from Salmonella to be about $1.7 \cdot 10^5$. Upon recalculation of their data using the above graphical method of molecular weight estimation together with more recent estimates of the molecular weight of their standard proteins, the molecular weight of the Salmonella transferase is also $1.8 \cdot 10^5$.

These molecular weight estimates for the enzyme from the two organisms differ from the estimate for the Salmonella transferase measured by sedimentation equilibrium studies of $2.15 \cdot 10^5$ (Voll, Appella and Martin 1967; Bell 1970). Since the yeast transferase has not been completely purified, chromatography on calibrated Sephadex G-200 columns was used as an independent estimate of its molecular weight by the method of Andrews (1965). In Table 4 the results from

<table>
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<th>Protein</th>
<th>Experiment</th>
<th>$V_e$ (ml)</th>
<th>$V_o$ (ml)</th>
<th>$V_e/V_o$</th>
<th>Average $V_e/V_o$</th>
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Ferritin was taken to have the same molecular weight as apoferritin (Andrews 1965). The regression of $V_e/V_o$ against log (molecular weight) for the protein standards gave a regression with $P < 0.001$. (a) Hofmann and Harrison 1963; (b) Schroeder et al. 1969; (c) Harris 1964; (d) Kawahara and Tanford 1966; (e) Castelline and Barker 1968.
five such experiments are summarized. The molecular weight for the yeast phosphoribosyltransferase is \((2.38 \pm 0.09) \cdot 10^4\) \(\pm\) S.E.M.). This value is considerably larger than the molecular weight of \(1.8 \cdot 10^4\) determined by sucrose gradient analysis. This difference is real because in the sucrose gradients the transferase always sedimented between alcohol dehydrogenase and catalase, whereas on Sephadex G-200 it consistently eluted before both of these enzymes. It suggests that either the transferase interacts with the Sephadex or it is a very asymmetrical molecule. This discrepancy in molecular weight estimates of the yeast enzyme was also observed for the Salmonella transferase. Robert M. Bell (personal communication, 1970) determined by sedimentation equilibrium the molecular weight of the Salmonella enzyme to be \(2.17 \cdot 10^4\) and on Sephadex G-200 found it to be \(2.2\) to \(2.4 \cdot 10^4\).

**DISCUSSION**

This study was undertaken with the hope that genetic investigations of the his1 gene of *S. cerevisiae*, coupled with an investigation of the physical properties of the enzyme encoded by this gene, would provide insights into the tertiary and quaternary structure of the subunits. In the discussion, after a consideration of the pertinent data on protein interactions, an evaluation of the different models of complementation will be made. With this background I then will consider (1) the locations on the genetic map which may correspond to the functionally active regions of the polypeptide, (2) how the colinearity of the genetic and complementation maps might be used to determine the placement of the functionally active regions of the enzyme with respect to the points of contact between subunits, (3) a proposed model of the tertiary structure of the polypeptide chains within the multimeric protein, (4) the possible arrangement of the functionally active regions within the multimeric enzyme, and (5) possible experimental tests of the proposed structural and functional model of phosphoribosyltransferase.

X-ray crystallographic and electron microscopic studies of multimeric proteins (enzymes, viruses of bacteria, plants, and animals) have shown that a basic property of subunit interaction is that it occurs along rotational axes of symmetry, as distinct from mirror planes of symmetry. Subunits, in general, do not intertwine as do strands of rope, but maintain a semi-autonomous state somewhat like a number of balls stuck together. (See Monod, Wyman and Changeux [1968] for summaries of X-ray crystallographic studies of enzymes and Valentine and Pereira [1965], Valentine et al. [1966] and Valentine, Shapiro and Stadtman [1968] for beautiful electron micrographs of viruses and enzyme molecules, respectively, showing this property of subunit interactions.) A second principle of subunit interaction which comes from these studies is that subunits aggregate in a finite manner such that they are a closed system, i.e., have a fixed number of subunits per functional unit.

Perutz and Lehmann (1968) summarized studies of variants of the hemoglobin molecule and concluded that conformational correction can and probably does occur by side chain interactions, and that pathogenic amino acid replacements occur at points of contact between subunits and at the active site where the
heme moiety is located. These changes decrease the stability of the hemoglobin molecule by altering the nonpolar contacts between subunits and the relationship of the polypeptide chain to the heme moiety. Also nonpathogenic amino acid replacements, detected as electrophoretic variants, occur at the external surface of the hemoglobin molecule (i.e., not at the points of contact between subunits nor at the active site) and do not affect its activity. These authors also present evidence that mutations are usually deleterious in the mutant protein but not in the hybrid protein of the heterozygote. Bernhard (1968) reviewed the crystallographic studies of various enzymes (carbonic anhydrase, carboxypeptidase, \( \alpha \)-chymotrypsin, egg white lysozyme, hemoglobin, and s-ribonuclease) which show that the active site lies in a cleft that extends deep into the interior of the protein and not at the surface of the enzyme molecule (a cleft being necessary for a correct orientation between enzyme and substrate).

The basic premise of the models of complementation of Crick and Orgel (1964) and Kapuler and Bernstein (1963) is that a mutation results in a misfolding of the polypeptide chain and complementation occurs when the misfolded region is corrected by interacting with the homologous, undistorted region of another subunit (encoded by the same gene but with the wild-type or the complementing mutant sequence) when they associate in a multimeric protein. Mutants with defects in the same homologous region, i.e., which are overlapping, are not expected to complement. This is formally equivalent to the accepted method of representation of complementing mutations by nonoverlapping lines and noncomplementing mutations by overlapping lines in the complementation map. From this it follows that complementation should occur mostly between mutants well separated on the genetic map and this has been obseved by Kapuler and Bernstein (1963) and in this study. This hypothesis of homologous correction also predicts that points of contact between subunits, where complementation occurs, are in isologous binding associations, in the terminology of Monod, Wyman and Changeux (1965). Heterologous associations are not excluded; but by the hypothesis, complementation occurs primarily where subunits are associated isologously. These points of contact would lie along rotational axes of symmetry, mirror planes of symmetry being excluded since proteins are composed only of L-amino acids.

The model of Crick and Orgel embodies all of the above premises. However, they reason that because of possible interactions between different parts of a polypeptide chain (via sulfhydryl bonds and side chain interactions), one would observe many exceptions to colinearity between the genetic and complementation maps of a gene. They suggested that exceptions would occur when a mutation in the polypeptide affects a genetically distant region of the polypeptide that is in close proximity (because of folding) to the mutated region in the active protein molecule.

There are several deficiencies and unlikely predictions in Kapuler and Bernstein's model (1963). They proposed an open, infinite structure for multimeric proteins without any limitations as to size, analogous to a stack of coins. Such open structures are known not to occur in normally active proteins, and are only
observed in the crystallization of a protein (e.g., VALENTINE, SHAPIRO and STADTMAN 1968) or in special cases such as the polysheath and polyhead mutants of bacteriophage T4 (EPSTEIN et al. 1963). Also, their model as drawn inadvertently suggests either complementation between mutants in the same homologous region or isologous associations which should have closed at the dimer stage but did not. Rotational axes of symmetry are not considered in their model.

McGavin (1968) suggested a model in which a mutant with an altered active site and one with an altered allosteric site can complement each other by supplying an unaltered allosteric and an unaltered active site, respectively, to the complementation product. In this model the active site and the allosteric site would necessarily be in close proximity to each other. That is, complementation would occur where the subunits are associated heterologously. Such an arrangement could explain feedback control by the allosteric site on the active site, but the allosteric inhibitor would act as a competitive inhibitor, which by definition it is not. The model also implies that the active and allosteric sites would lie at the periphery of the subunits at the points of contact. This seems unlikely since from X-ray crystallography of various enzymes the active sites have been found to lie deep within the protein subunits.

Gillie (1966, 1968) has taken the ideas of CRICK and ORGEL (1964) and constructed a model of complementation which can be tested. He suggests from his analysis of the complementation data in the literature that the points of contact between subunits are reflected in the clusters of complementation groups that appear in complementation maps. This is based on the hypothesis that complementation occurs where subunits interact. Since they interact at discrete regions of the polypeptide chains and not over their whole length (as suggested by KAPULER and BERNSTEIN 1963), one would expect to observe clustering of mutants with similar complementation responses and for those mutants in a cluster in general to map in the same region of the gene. In this model, the number of clusters indicates the minimum number of contact points each subunit has with others in the multimeric protein. For instance, a protein with four subunits and two clusters in its complementation map might be expected to be shaped as a square (flat tetrahedron).

A possible way to localize the regions of the his1 protein that are important functionally is to analyze the distribution of alleles with such properties as unusually high revertibility, noncomplementation, and reversion to feedback resistance. Korch (1970) and Korch and Snow (1973) have made such observations and they are as follows. First, the mutants which can revert to feedback resistance (alleles 3, 7S, 315) map in a small region at the hom3 end of the gene. Perhaps this region of the gene codes for a region of the enzyme involved in its feedback control. Interestingly, almost all such mutants in the analogous gene in Salmonella (hisG) map in a small region and in a similar relative position on the genetic map as do these his1 mutants (SHEPPARD 1964). Second, the noncomplementing alleles map only in the arg6 half of the gene. This part of the gene might code for the portion of the polypeptide chain that comprises the active site. Mutations near the active site would probably be very difficult to correct by comple-
allelies with relatively high homoallelic reversion rates were not distributed at random throughout the gene but mapped only at the hom3 end (with one exception, the leaky allele 18, which is discussed later). Many missense mutations can probably occur in this region of the gene without loss of protein function. Reversion of a mutant in this region would occur either by restoration of the original wild-type base sequence or by mutation to an acceptable missense sequence, implying that this half of the molecule is less critical for activity than the arg6 half, yet is still necessary for enzymatic function. The suggestion is that the hom3 end of the gene contributes the major portion of the feedback site and the arg6 end contributes the principal portion of the active site.

The degree of colinearity between complementation and genetic maps is variable. CRICK and ORGEL (1964) predicted some colinearity but also expected many exceptions. KAPULER and BERNSTEIN (1963) found a great deal of colinearity for the ad-8 gene of Neurospora crassa. They based their interpretations on the data of ISHIKAWA (1962a, b), who has since (ISHIKAWA 1965) interpreted his data more conservatively than they did, but has found a very complex relationship between the two maps. There are several other studies correlating the recombination and complementation maps in various organisms (BERNSTEIN, EDGAR and DENHARDT 1965; CASE and GILES 1960; DORFMAN 1964; LEUPOLD and GUTZ 1964; and RAMIREZ, FRIS and LEUPOLO 1963). But the complementation maps are linear in some of these cases (perhaps involving several cistrons at a locus) and the genetic maps do not have a well-established order. Thus a more useful estimate of colinearity must be obtained by a comparison of a complex complementation map with a series of well-mapped alleles.

To determine colinearity between the genetic and complementation maps for the his1 gene, the rule presented by KAPULER and BERNSTEIN (1963) was followed. Colinearity was determined by beginning at one end of the gene and drawing radial lines from the mutations on the genetic map to the complementation group containing them. Following this procedure, the genetic map of the his1 gene (KORCH and SNOW 1973), beginning at the hom3 end with allele 40, can be drawn colinearly with the complementation map. A two-turn spiral is the simplest way to to illustrate the colinearity, as shown in Figure 3. The alleles were not lined up with the center of their complementation groups because very complex foldings would be introduced. Such foldings may reflect the real situation, but were not included for the sake of simplicity. This map in Figure 3 was not drawn to genetic scale; if it is, as shown in Figure 4, the genetic map linking the mutants of the two clusters as well as the region around alleles 18, 5, and 15 must be shortened. The fold caused by allele 5's being in cluster A allows alleles 23, 25, and 29 to overlap alleles 10, 24, and 33, all six of which belong in the same complementation group. Note that all of the noncomplementing mutations lie in or
near cluster B, and the three mutations which can revert to feedback resistance are in cluster A.

At present there is no direct biochemical data on the number of subunits in yeast phosphoribosyltransferase. The enzyme from Salmonella has six subunits and a molecular weight for the multimer of $2.17 \cdot 10^5$ (Voll, Appella and Martin 1967; Whitfield 1971; Bell 1970). The molecular weight of the yeast phosphoribosyltransferase is by sucrose density gradient analysis $1.8 \cdot 10^5$ and by Sephadex G-200 chromatography $2.4 \cdot 10^5$. Since, as mentioned above this discrepancy in estimates of molecular weight by these two methods has been observed also for the Salmonella enzyme, the molecular weight for the yeast enzyme when determined by sedimentation equilibrium studies will probably be, about $2.1$ to $2.2 \cdot 10^5$. From the length of the hist gene map (7.6 X-ray map units) and from the finding of Parker and Sherman (1969) that there are about 43 amino acids per X-ray map unit, the molecular weight of a polypeptide chain would be about $3.6 \cdot 10^4$ (assuming a molecular weight of 109 per amino acid residue, on the average). Using these assumptions the implication is that the yeast enzyme also has six subunits.
Figure 4.—The his1 complementation map with the composite genetic map drawn colinearly, with correction for genetic map distances.

On the hypothesis of Gillie (1966, 1968), the two clusters of complementation groups in the his1 complementation data imply the presence of two points of isologous interaction between subunits of the active protein. Only a hexagonal ring or chair-like arrangement of the six subunits would satisfy this condition.

With the colinear relationship between the genetic and complementation maps, perhaps a crude approximation of the folding of the polypeptide chain in each of the six subunits can be made. In order to draw this approximation, three premises of the Crick-Orgel model (1964) as restated by Gillie (1966, 1968) are used. These premises are:

1. The clusters of complementation groups represent the points of contact between subunits in the active molecule, and the regions of the polypeptide chains involving mutations in these clusters are assumed, in general, to lie at the points of contact;
2. Complementary mutants lie at a point of contact and noncomplementary mutants may lie away from a point of contact in the internal regions of the subunits;
3. The subunits of a protein in which complementation occurs are associated
with each other in an isologous manner, which allows for homologous correction.

Figure 5 shows a model of how the polypeptide chains of phosphoribosyltransferase may be arranged in the active molecule. The hom3 end of the gene associated with cluster A (contact point A) is represented as a large circular loop to indicate the large genetic region (about 3.0 X-ray map units, i.e., about 130
allellic complementation in histidine-1

amino acids [Korch and Snow 1973; Parker and Sherman 1969]) containing mutants that belong in this cluster. At about 3.0 X-ray map units from the hom3 end, the genetic map moves away from cluster A and towards cluster B (contact point B). The center of the map, 3.7 to 4.3 X-ray map units from the hom3 end, is associated with cluster B. Alleles 1S and 5 are associated with cluster A, suggesting a folding back of the polypeptide from point B to point A. This suggested fold-back is supported by the fact that the intervening alleles, 19, 68, and 2, are noncomplementing and, according to premise number 2, may lie away from an axis of symmetry. Alleles 23, 29, and 44 are associated with contact point B, suggesting that the polypeptide folds from point A back again to point B. Allele 15, which intervenes between allele 5 and alleles 23, 29, and 44, does not complement and presumably lies between points A and B. The group of noncomplementing alleles, 47, 1F, 41, 54, 31, 52, 53, and 26, suggest a region of the polypeptide that folds away from point B. This folding is most likely in the form of a loop because of the subsequent complementing alleles, 25 and 38, which lie at point B. The polypeptide may then loop away from point B again, because of the noncomplementing alleles 45 and 17, which are bracketed by alleles 25 and 38 on one side, and 61 on the other. The alleles 4, 35, 39 and perhaps 49 suggest that the polypeptide chain now forms a tail which lies away from a point of contact. Allele 18 is interesting because it has a branched complementation group showing that it interacts with both contact points A and B. By computer analysis it was classified as an irregular mutant which most likely belonged to cluster B. This agrees with the above colinear relationship between the genetic and complementation maps. And yet, it shows high homoallelic revertibility typical of cluster A mutants (Korch 1970; Korch and Snow 1973). Perhaps it is a mutant which strongly effects both points of contact of the subunits as, for example, by a side chain interaction.

Certain features of this model are interesting because they suggest the distribution of the active and allosteric sites within the protein as well as a mechanism by which the allosteric site can influence the active site. As noted before, the unusual distribution of the noncomplementing mutants suggests that the active site is formed by the region of the gene containing them. These regions may lie away from the points of contact and be folded to form an active site, a cleft formation being necessary to provide the correct orientation of the substrate(s) to the enzyme. The two clefts may be where the substrates ATP and PRPP interact with the enzyme subunits. The foldback owing to alleles 1S and 5 and the interaction with allele 18 may be the means by which the feedback site influences the active site, through conformational changes induced by L-histidine's interacting at contact point A.

The active molecule predicted by this molecule is a ring of six subunits with the feedback sites (FB) of two adjacent subunits near a common point of contact and the active sites (AC) of two adjacent subunits near another common point of contact. This distribution of regulatory and active sites in the protein is diagrammed in Figure 6. There is a maximum of six active sites and six feedback sites predicted by this model. In agreement with this prediction, Bell and Kosh-
LAND (1971) have shown that there are six active sites in the Salmonella phosphoribosyltransferase. This model has three two-fold axes of symmetry (in the plane of the ring) and one three-fold axis of symmetry (perpendicular to the plane of the ring). To maintain this symmetry, all six subunits are identical; but note that when they lie adjacent to each other, they are not mirror images but are related to each other along the upper surface of one subunit, they are then related to each other along the under surfaces of the two adjacent subunits. This is best shown in Figure 5.

The obvious experiment needed to check this model is an X-ray crystallographic study of yeast phosphoribosyltransferase. However, the protein has not been purified and crystallized. Studies with the electron microscope of the purified enzyme may shed some light on the number of subunits and their arrangement in the active enzyme.

Another approach to determining the subunit arrangement would be that of LANGRIDGE (1968a,b,c; 1969) who has acquired mutants affecting feedback inhibition, the $K_m$, the $V_m$, the temperature sensitivity, and the urea sensitivity of an enzyme. Since altered urea sensitivity is caused by mutants which affect the aggregation of subunits, such mutants for the his1 gene should map in the region corresponding to complementation clusters A and B. This type of experiment, together with an “in vitro” complementation assay for the his1 gene.
ALLELIC COMPLEMENTATION IN *histidine-1* (similar to that discussed earlier; Korch, 1970) would provide another system in which to test the hypothesis that clusters of complementation groups reflect the points of interaction between subunits. Furthermore, finding and mapping mutants which alter the $K_m$ and $V_m$ of the transferase would provide strong evidence as to whether the position of the noncomplementing mutants is at the active site. Mutants with an altered $K_m$ would presumably be altered in their ability to bind either the substrates or the products. Those having an altered $V_m$ presumably would be those having an alteration of the reactive amino acid residue(s) catalyzing the reaction or be in close proximity to this reactive site. Additional temperature-sensitive mutants should be obtained and their position determined correlated with urea sensitivity, altered $K_m$ and $V_m$. In this model the close proximity of the feedback sites to each other and of the active sites to each other suggests that cooperation effects may be noted in the kinetics of substrate and feedback inhibitor concentration. This also can be tested.

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**LITERATURE CITED**


ALLELIC COMPLEMENTATION IN histidine-1


