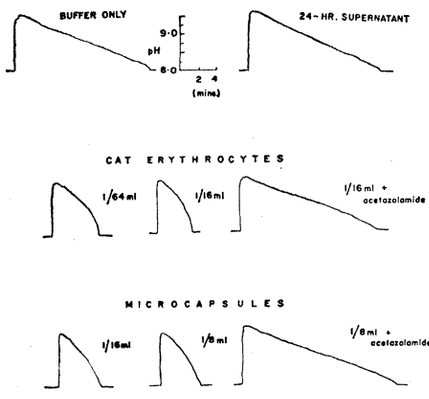

Fig. 2. Carbonic anhydrase activity, measured as the rate of fall of p*H* while CO_2 bubbles through 12 ml of alkaline buffer: method as in (*3*), but with 0.15*M* NaCl added to the medium. Fall of *p*H is accelerated by the presence of erythrocytes or of nylon microcapsules containing erythrocyte hemolysate diluted 1 : 1 (nylon microcapsules made from the same volume of hemolysate solution); no acceleration occurs in the presence of acetazolamide or when the microcapsules are replaced by supernatant from a 50 percent suspension of microcapsules stored for 24 hours.

in seconds or minutes if the solute species is one that penetrates the membranes: for a given osmolarity, the duration of crenation rises steeply with the molecular weight of the solute in the case of nonelectrolytes, and with the radius of the largest hydrated ion in the case of electrolytes. With small water-soluble species such as urea, crenation is not detected at concentrations below 3*M*, but large hydrated ions such as $SO_4^=$ and $HPO_4^=$ and large nonelectrolyte molecules such as sucrose cause long-lasting crenation.

One would expect the activity of an encapsulated enzyme, tested on a substrate present in the external phase, to be less than that of the enzyme in free solution, since the diffusion of its substrate or product, or both, would usually be the rate-limiting process. In favorable cases the activity in vivo is approached. Figure 2 shows that carbonic anhydrase from a red cell hemolysate, enclosed in nylon microcapsules of 10-μ mean diameter, did not leak out but catalyzed the hydration of CO_2 nearly as efficiently as before the cells were hemolyzed, and was sensitive to the inhibitor acetazolamide. Trypsin (on dipeptide but not on protein substrates) and urease also acted fairly efficiently after incorporation into microcapsules.

The stability of enclosed enzymes is variable: the carbonic anhydrase of an encapsulated hemolysate retained its activity after weeks of storage, while an encapsulated solution of crude urease lost most of its activity in a day if a preparation of soya bean trypsin inhibitor was not included. Some enzymes and proteins probably would be partly or wholly denatured when encapsulated by these procedures; this is so with hemoglobin which, however, retains more than 50 percent of its oxygen-carrying capacity if a favorable environment is specially provided for preparation and storage of the microcapsules.

I have occasionally tested in vivo the toxicity and the enzymatic activity of injected microcapsules. The toxicity of subcutaneously or intraperitoneally administered microcapsules is low and significant enzymatic activity is retained. Injection of encapsulated urease can markedly increase the concentration of ammonia in the blood. Intravenously injected microcapsules are not toxic provided they are washed free of Tween 20 and are administered in small doses; those prepared by the methods described herein usually produce some circulatory disturbance if the dosage exceeds 1 to 2 ml per kilogram of body weight, and like other foreign particles they do not survive for long in the blood stream. Some progress has been made toward a procedure for making microcapsules with a longer lifetime in the circulation: small negatively-charged capsules seem to survive longest.

It seems very likely that a number of applications, both in vitro and in vivo, will be found for these small objects. An obvious but not an immediate possibility is the therapeutic replacement of an enzyme lost in a genetic accident.

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Gene-Enzyme Relations in Histidine Biosynthesis in Yeast

Abstract. *Each of the histidine mutants of* Saccharomyces cerevisiae *has been correlated with a particular step in histidine biosynthesis. The relations of the genes controlling histidine biosynthesis in yeast appear to be identical with those found in* Neurospora *and clearly distinct from those found in* Salmonella.

Although the original theory, now more than 20 years old, which suggests that genes control enzymes came largely from observations on biosynthesis in *Neurospora* (*1*), the identification of genes with their enzyme products has proceeded slowly. Until recently the assignment of a gene for every enzyme in a biosynthetic pathway had been achieved only in bacterial systems where the genetic material, unlike that of higher organisms, is contained in a single chromosome. However, application of the detailed methods of Ames and his collaborators (*2–4*) on the histidine pathway recently permitted the correlation of each of the histidine mutants of a eucaryotic organism, *Neurospora crassa*, with a particular step in histidine biosynthesis (*5*). The pathway of histidine biosynthesis is the same in *Neurospora* and *Salmonella*, but the relations of the genes controlling the enzymes in the pathway are different. To determine whether the gene-enzyme relations in the histidine pathway of other fungi also differ from *Salmonella* and whether they resemble *Neurospora* the present work was undertaken with the histidine mutants of *Saccharomyces cerevisiae*.

The mutational blocks were determined by enzyme assays and by characterization of accumulation products. Since some of the substrates before imidazole-glycerol phosphate (IGP) are not available, the yeast mutants were assayed by incubating them with adenosine triphosphate (ATP), 5-phos-

Table 1. The accumulations characteristic of the histidine mutants of yeast and the enzyme defects responsible for the accumulation. Ascending chromatography was carried out for 3 hours on Whatman No. 1 paper (23 × 18 cm) at 25°C. The solvents were mixtures of isopropanol, water, and ammonia (70:40:10 or 70:20:10). Imidazoles were detected on air-dried chromatograms sprayed with a diazo reagent (*11*). Bound Bratton-Marshall compounds (BBM) were detected by hydrolysis of filtrates or lyophilized cells with 0.1 ml of 1*N* HCl at 100°C, subsequent diazotization of the free amino group with nitrous acid, and finally coupling the diazo product with *N*-(1-naphthyl)-ethylenediamine.

Mutant	Accumulation	Enzyme defect
hi-1	None	PR-ATP-pyrophosphorylase
hi-2	Histidinol phosphate	Histidinol phosphate phosphatase
*hi-3**	Imidazole glycerol	IGP dehydrase
hi-4 ABC	None	PR-AMP pyrophosphorylase PR-AMP hydrolase Histidinol dehydrogenase
hi-4 B		PR-AMP pyrophosphorylase
hi-4 C	Histidinol	Histidinol dehydrogenase
hi-5	Imidazole acetol	IAP transaminase
hi-6	BBM	*F, A, H* of *Salmonella*
hi-7	BBM	

* *Hi-3, -8,* and *-10* have been redesignated *hi-3* since they all accumulate imidazole glycerol and since the genetic evidence indicates that they are alleles of the same gene. All strains were obtained from the collection of Dr. R. Mortimer of the University of California or were derived from these strains.

phoribosyl-1-pyrophosphate (PRPP), Mg^{++}, and NH_4^+ and extracts of *Salmonella* mutants with known enzymatic defects. If the mutants are defective in the same enzyme there will be no formation of 5-amino-1-ribosyl-4-imidazolecarboxamide-5′-phosphate (AICAR). The formation of AICAR therefore means that the two mutants are defective for different enzymes.

All the mutants were assayed for the first enzyme (*2*), and activity was found in all but *hi-1* mutants (Table 1 and Fig. 1). Furthermore, of all the yeast mutants which did not accumulate imidazoles, *hi-1* mutants were the only ones which did not form AICAR when mixed with extracts of *Salmonella G* mutants. The *hi-4 ABC* mutants were incapable of providing either *E* or *I* mutants (Fig. 1) with active enzyme to produce AICAR. These as well as the *hi-1* mutants accumulate neither imidazole nor Bratton-Marshall (*6*) derivatives. Both *hi-6* and *hi-7* accumulate a bound Bratton-Marshall compound in the cells and can supply *E* and *I* mutants of *Salmonella* with enzymatic activity to produce AICAR. Therefore, *hi-6* and *hi-7* of yeast seem to correspond to *A* and *H* of *Salmonella*, although exact designations have not yet been made. Mutants for the steps after IGP accumulate imidazoles which are readily identified by paper chromatography. *Neurospora* mutants whose enzymatic defects are known were used as standards for comparison (*3*). In addition, all mutants were assayed for histidinol phosphate phosphatase and histidinol dehydrogenase (*7*). For all the mutants except the *hi-4 ABC* mutants, the results of these enzyme assays were in complete agreement with the data on accumulation products, namely, *hi-2* lacked phosphatase activity, and both *hi-4 C* and *hi-4 ABC* lacked dehydrogenase activity.

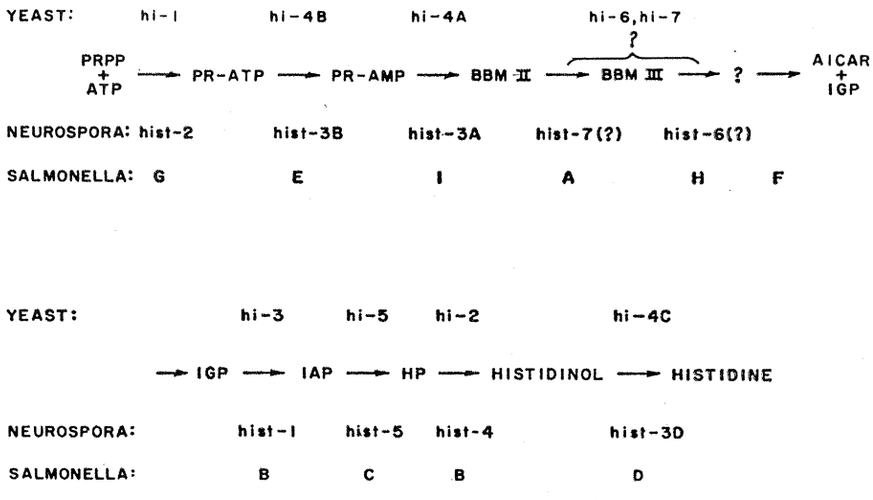

Fig. 1. A comparison of the mutational blocks in histidine biosynthesis of yeast, *Neurospora*, and *Salmonella*. Although the pathway is schematically divided at IGP, the pathway is, of course, continuous. Abbreviations used: P = phospho, R = ribose (PR-ATP = phosphoribosyl ATP); IAP = imidazoleacetol phosphate; HP = histidinol phosphate. All other abbreviations are explained in the text. Data for *Neurospora* from references *9* and *10*; those for *Salmonella* from references *4* and *12*.

The *hi-4 B* mutants have lost *E* activity and retain both *I* and histidinol dehydrogenase activity, whereas *hi-4 C* mutants have lost only histidinol dehydrogenase activity. (The *hi-4 ABC* mutants are point mutations which are defective for three activities: *E, I,* and histidinol dehydrogenase.) As expected, these *hi-4 ABC* mutants, due to their pleiotropic defect, accumulate no histidinol (Table 1). Whether this represents a loss of three enzymes organized into an operon or of a single multifunctional enzyme is under investigation.

Klopotowski's results (*8*) suggested that the early part of the histidine pathway in yeast is the same as it is in *Salmonella*. My results indicate that the reactions for the entire pathway, so far as it is known, are the same as those in *Salmonella*. In terms of metabolic regulation, the first enzyme in the histidine pathway, phosphoribosyl-ATP-pyrophosphorylase, is inhibited by low concentrations of histidine in both *Salmonella* and *Neurospora* (*2, 9*). That this enzyme is also under feedback inhibition in yeast is demonstrated by the fact that $10^{-5}M$ L-histidine causes 50 percent inhibition of its normal activity. The relationship of the genes controlling histidine biosynthesis in yeast resembles that in *Neurospora*, another eucaryote, more closely than it resembles that in the procaryotic *Salmonella* system. The most striking difference is that the genes controlling the histidine enzymes are scattered throughout the genome in *Neurospora* and yeast, whereas in *Salmonella* they are all closely linked. In addition, the phosphatase and dehydrase activities in *Salmonella* appear to be associated with one enzyme and one genetic region. In yeast and *Neurospora*, by contrast, the phosphate and dehydrase mutants are unlinked, and mutants affecting the dehydrogenase have full phosphate activity. The most note-

worthy homology indicated by these results is that the *hi-4* region of yeast appears to have the same functional complexity as the *hist-3* region of *Neurospora* (*5, 10*).

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Replication of the RNA of Bacteriophage R17

Abstract. Escherichia coli *cells were irradiated with ultraviolet light to stop ribosomal RNA synthesis. After infection of such cells with the single-stranded RNA phage R17, the RNA most rapidly labeled by a pulse of tritiated uridine sedimented in a broad band in the 16S region of sucrose gradients. The effect of ribonuclease on this material and its behavior during a "chase" period in nonradioactive medium suggest that it consists of a core of double-stranded RNA, one strand of which—the viral strand—is continually displaced by a growing strand forming single-stranded tails and ultimately free 27S viral RNA.*

Molecules with some of the properties of double-stranded RNA (*1*) have been detected in both mammalian (*2, 3*) and bacterial (*4*) cells infected with RNA viruses, as well as in the product in vitro of virus-induced RNA polymerases from both bacterial and mammalian cells (*5*). That this double-stranded ribonuclease-resistant RNA is probably relevant to virus growth is implied by the observations that it is infective (*2*) and that much of the RNA of the the originally infecting virus is transformed to such a form (*4*). Our study concerns the formation of double-stranded RNA in *Escherichia coli* cells infected with the RNA phage R17, and the part played by it in the replication of the phage RNA.

Bacteriophage R17 and its host, *E. coli* K12 strain Hfr$_1$ (*6*), were grown in TPG minimal medium (*7*) supplemented with 0.5 percent glucose and 0.1 percent Bacto-tryptone (*8*).

Because these studies involve extraction of RNA from bacterial cells, it was first necessary to show that newly formed viral RNA is not degraded during lysis of the cells and extraction of the RNA. A sample of labeled R17 RNA was added to a suspension of *E. coli* cells, and RNA was extracted from the mixture and centrifuged through a sucrose gradient (Fig. 1). The viral RNA formed a peak of radioactivity just ahead of the 23*S* ribosomal RNA, at about 27*S*, in agreement with Ellis and Paranchych (*9*). There was very little radioactivity higher up the tube, an indication that the viral RNA was not appreciably degraded.

In cells infected with R17 a high rate of normal cellular RNA synthesis persisted. When tritiated uridine was added in 1- or 5-minute "pulses" 30 minutes after infection, there were labeled bands in the sucrose gradients at 4*S*, 16*S*, and 23*S* and a small additional peak or shoulder at 27*S*, indicating synthesis of viral RNA. However, when RNA from cells labeled between 30 and 35 minutes after infection was treated with pancreatic ribonuclease (1 μg/ml for 10 minutes at 37°C in 0.1*M* NaCl) and then analyzed in a sucrose gradient, a labeled peak remained which centered at fractions 17 to 18 (about 10*S*). There was no such material in uninfected cells.

Since we were interested in virus-specific labeled materials which might have been obscured in the gradients by the labeling of ribosomal RNA, we attempted to reduce the background rate of cellular RNA synthesis by irradiating the cells with ultraviolet light before infection, a technique which had been used previously to study RNA synthesis in animal cells infected with poliovirus (*10*). With increasing doses of ultraviolet light, the decrease in yield of infectious virus was about equal to the decrease in rate of synthesis of RNA (measured by the radioactivity in the fraction precipitable by trichloroacetic acid after a 5-minute pulse of tritiated uridine). Nevertheless, cells whose rate of total RNA synthesis had been reduced to about 5 percent of normal by ultraviolet irradiation

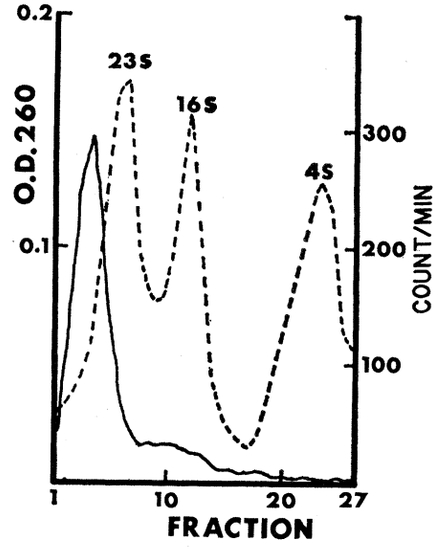

Fig. 1. Sedimentation pattern of a mixture of R17 and *E. coli* RNA. P^{32}-labeled R17 phage was shaken with phenol at 20°C and the phenol was removed by two extractions with ether. A sample of this labeled phage RNA was added to a suspension of *E. coli* (about 10^{10} cells) in 1 ml of solution (0.1*M* NaCl, 0.001*M* magnesium acetate, 0.05*M* tris buffer, *p*H 7.2). The cells were lysed by adding 1 percent sodium dodecyl sulfate, and then alternately frozen and thawed four times. The lysate was shaken vigorously with phenol for 1 minute at 20°C and centrifuged; RNA and DNA in the aqueous phase were precipitated by adding two volumes of ethanol at 0°C. The nucleic acids were sedimented (15 minutes at 5000 rev/min), redissolved, precipitated again with ethanol, and finally dissolved in 0.2 ml of a solution of 0.1*M* NaCl and 0.001*M* magnesium acetate. The nucleic acid solution was layered on a 4-ml sucrose gradient (7 to 20 percent) containing 0.1*M* NaCl and 0.001*M* magnesium acetate, and centrifuged in a Spinco SW 39 rotor for 4 hours at 37,500 rev/min at the 30°F temperature setting. Equal fractions of about 0.15 ml were then collected from the bottom of the tube, and 0.1 ml of each was transferred to 12 ml of scintillation fluid containing methanol and toluene at 3:10 and counted in a Packard Tricarb scintillation counter. The remainder of each fraction was diluted with 0.5 ml of water, and the optical density was measured at 260 mμ. Solid line: radioactivity (R17 RNA). Broken line: optical density (*E. coli* RNA).