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Transfer RNA and the Control of the Histidine Operon

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The biosynthesis of histidine in *Salmonella typhimurium* involves ten enzymes. A cluster of the structural genes for these enzymes, the histidine operon (Fig. 1), is regulated as a unit (Ames and Garry, 1959; Ames, Garry, and Herzenberg, 1960; Ames and Hartman, 1962; Ames and Hartman 1963; Ames, Hartman, and Jacob, 1963). This paper reviews recent work on the regulation of this operon.

The specific activity of each of the ten enzymes can vary from 1, the repressed level, to about 25 under conditions of histidine starvation. Regulatory mutants have been obtained in which the histidine operon is not fully repressed; the mutants having high levels of the histidine biosynthetic enzymes (Roth, Antón, and Hartman, 1966). These regulatory mutants have been classified into four genetic groups, which have been located on the Salmonella chromosome (Roth et al., 1966).

Mutants of one class, *hisO*, are located at one end of the histidine operon. This class may be similar to operator constitutive mutants studied in other systems. Preliminary experiments on the fine structure analysis of the *hisO* gene indicate that recombination occurs between different *O* mutants. A second class, *hisS*, is linked, in transduction tests, to the *guaA* and *strB* loci. The *hisS* gene is the structural gene for the histidyl-tRNA synthetase (the histidine activating enzyme). The *hisS* mutants that have been examined have an altered activating enzyme with a decreased affinity for histidine (Roth and Ames, 1966; Roth, Antón, and Hartman 1966). Mutations of a third class, *hisR*, are linked in transduction tests to the *ilv* and *metE* loci (Roth and Hartman, 1965) and these mutants have only about 55% of the normal amount of the transfer-RNA for histidine (Silbert, Fink, and Ames, 1966). A fourth class of mutants, *hisT*, is linked to the *purF* and *aroD* loci. The function of the *hisT* gene has not been determined.

The properties of the various mutants suggest that histidine-tRNA, and not free histidine, is important in repression. The results are discussed in terms of models of regulation of the histidine operon.

ISOLATION OF TRA-RESISTANT MUTANTS

The regulatory mutants were selected for resistance to the histidine analogue, 1,2,4-triazole-3-alanine (TRA), which acts as a false co-repressor

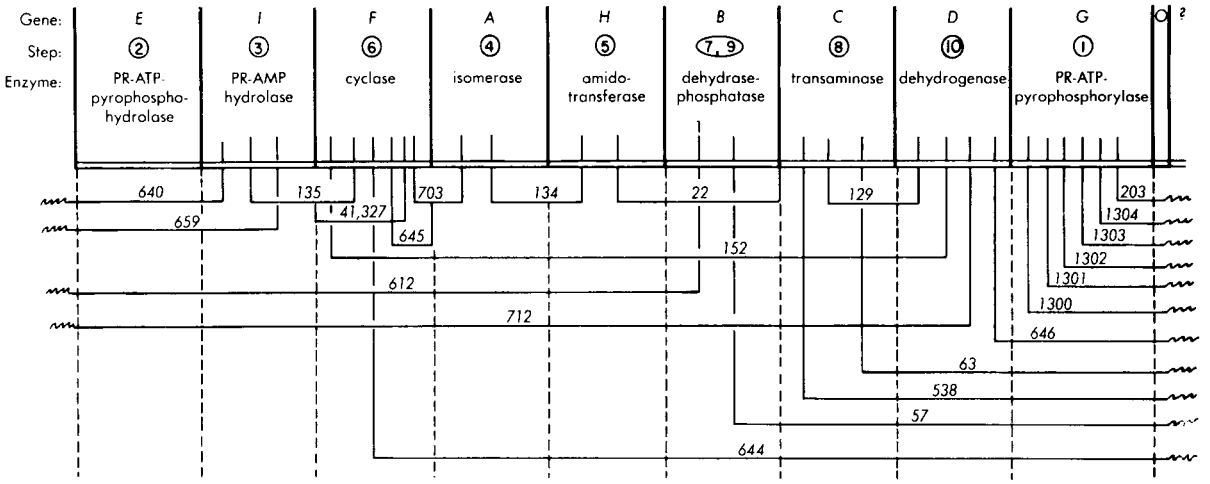

FIGURE 1. General map of the histidine operon as worked out by Hartman and co-workers (Loper, Grabnar, Stahl, Hartman, and Hartman, 1964). The capital letters refer to the gene loci. The steps in the reaction sequence controlled by each gene are circled and the trivial name of each enzyme is listed. The extent of some deletion mutants along with their isolation numbers are shown. About 1,000 point mutants have been mapped in the operon but these are not shown in the figure.

of the histidine operon and is incorporated into protein (Levin and Hartman, 1963). The TRA-resistant mutants cannot be isolated directly. TRA does not inhibit the wild-type Salmonella growing on minimal medium because of its fully repressed levels of the histidine biosynthetic enzymes (Ames and Garry, 1959). However, TRA does inhibit Salmonella having a partial block in the histidine pathway. These bacteria are able to grow slowly on minimal medium only when they produce high levels of the histidine biosynthetic enzymes, and require derepression for growth under these conditions. Thus, TRA does not inhibit the repressed wild type, but does inhibit cells with a partial histidine requirement which must derepress to grow. The partial block in the pathway, required for TRA sensitivity, can be introduced genetically or physiologically. A partial genetic block is present in strains with "leaky" histidine mutations. Such strains grow slowly on minimal medium only if they derepress the enzymes of the histidine operon. A partial block in the pathway can be physiologically introduced into wild-type cells by the addition of the herbicide aminotriazole. This compound is an inhibitor of one of the histidine biosynthetic enzymes in Salmonella, (imidazole glycerol phosphate dehydrase; Hilton, Kearney, and Ames, 1965). Wild-type bacteria overcome inhibition by aminotriazole by increasing the levels of the histidine biosynthetic enzymes, and therefore are sensitive to inhibition by TRA. Each of the above methods allows selection of TRA-resistant mutants.

DETECTION AND PROPERTIES OF DEREPRESSED MUTANTS

Several classes of mutations could be responsible for resistance to TRA. We were interested in mutants with an altered regulatory mechanism, in which repression by TRA no longer occurred. We have been able to distinguish these regulatory mutants by their unique morphology from the other classes of mutants resistant to TRA (or aminotriazole), such as permease mutants. It had been observed a number of years ago (by B.N.A.) that the bacteria growing with derepressed histidine enzyme levels form colonies with wrinkled surfaces (see Roth and Hartman, 1965, Figure 1). This characteristic morphology depends on elevated concentrations (1 to 2%) of glucose or other fermentable carbon sources in the medium. The wrinkled phenotype is not expressed on 0.2% glucose, although the concentration of glucose has no significant effect on the levels of histidine biosynthetic enzymes. Mutants selected for resistance to TRA were examined for colony morphology on medium containing 2% glucose. Only those mutants that formed wrinkled colonies were studied further. In every case tested, the bacteria forming wrinkled colonies had high enzyme levels for the histidine biosynthetic enzymes and carried an altered regulatory element for the histidine operon (Roth et al., 1966).

The TRA-resistant, derepressed mutants were found to be divisible into four distinct classes on the basis of map position (Roth et al., 1966).

All mutants tested were able to derepress further when grown on limiting histidine. This suggested that the mutants tested possessed altered regulatory mechanisms and that in none of these cases had a regulatory element been completely destroyed.

THE *HisS* GENE

Mutations in the *hisS* gene, which maps near *strB*, far from the histidine operon (Fig. 2), result in high levels of the histidine biosynthetic enzymes. This derepression of the histidine operon is caused by a reduction in the activity of histidyl-tRNA synthetase (Roth, Anton, and Hartman, 1966). The *hisS* gene is the gene for the histidyl-tRNA synthetase as *hisS* mutants have a synthetase with altered kinetic properties, such as a decreased affinity for histidine (Roth and Ames, 1966).

The *hisS* mutants examined by Roth et al. (1966) for histidyl-tRNA synthetase activity (by transfer of C^{14} histidine to tRNA) had about 10% of the wild-type activity. These mutants have a normal growth rate on minimal medium. In a later investigation by Roth and Ames (1966) a new *hisS* mutant, *hisS1520*, was examined. This mutant has no detectable synthetase activity (less than 0.2% of the wild-type activity) by this same assay. The

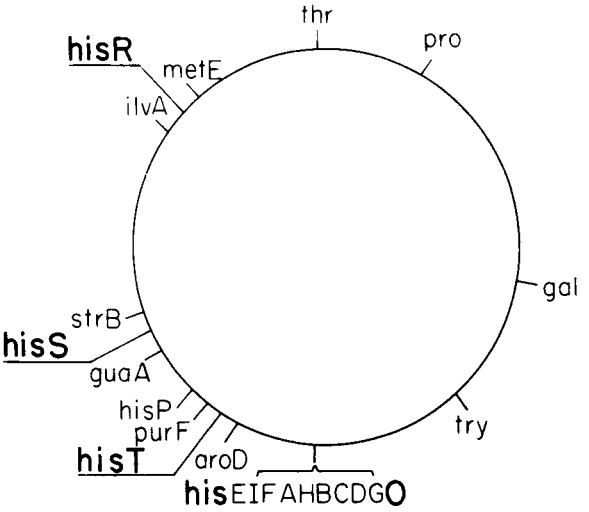

FIGURE 2. Chromosome map of *Salmonella typhimurium* showing approximate locations of *his* regulator genes (Roth, Antón, and Hartman, 1966).

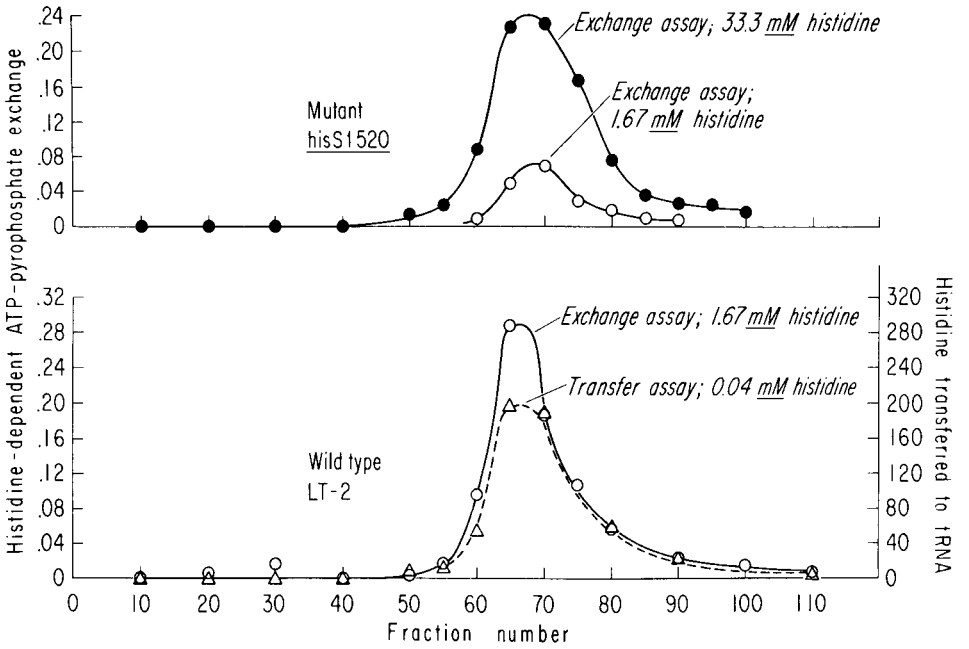

FIGURE 3. DEAE-cellulose column chromatography of histidyl-tRNA synthetase from mutant *hisS1520* and wild-type LT-2 (Roth and Ames, 1966). Protein is distributed from tubes 10–110. Pooling fractions 60–80 yields approximately 5-fold purification with 80% recovery of activity. Exchange activity is presented as mμmoles P^{32} pyrophosphate exchanged per min by 1 μl of fraction. Transfer activities are presented as count/min C^{14} histidine (35 mc/mmole) incorporated into tRNA per min by 1 μl of fraction.

growth rate of this mutant on minimal medium is severely impaired, presumably because of the shortage of charged histidyl-transfer RNA necessary for protein synthesis. It seemed probable that, in order to grow, the mutant must have some histidyl-tRNA synthetase activity. This was shown by changing to the P^{32} pyrophosphate exchange assay in which a much higher histidine concentration could be used. The following experiments indicate that mutant *hisS1520* has the same amount of synthetase as the wild-type, but that it is an altered enzyme with a poor affinity for histidine.

(a) Chromatography of Histidyl-tRNA Synthetase

Histidyl-tRNA synthetase from the *hisS1520* mutant and from the wild type were eluted from DEAE-cellulose as a single peak at the same salt concentration. The elution patterns for the two activities are compared in Fig. 3. In the transfer assay (0.04 mM C^{14} histidine) (—Δ—) the wild type enzyme shows a peak of activity, but the mutant shows no detectable activity (not shown in figure), the peak tube having less than 0.2% of the wild-type activity. In the P^{32} pyrophosphate exchange assay at 1.67 mM-histidine (—o—) the mutant has a peak corresponding in position to the wild-type peak but with only about one-third the activity. In the exchange assay at high histidine (33.3 mM) (—●—) the mutant enzyme peak is approximately the same as that of the wild type. The wild-type peak assayed at 33.3 mM-histidine (not shown) was the same as at 1.67 mM.

(b) Affinity of the Synthetase for Histidine

The affinity of histidine for the histidyl-tRNA synthetase from both wild type and *hisS1520* was measured (Fig. 4). When the pyrophosphate exchange assay was used, a 50-fold difference was obtained between the K_m values for the two enzymes. The wild-type enzyme has a K_m of 1.5×10^{-4} M and the mutant enzyme has a K_m of 80×10^{-4} M. The two enzymes have the same maximal velocity.

Another *hisS* mutant, *hisS1210*, was also investigated. Histidyl-tRNA synthetase from this mutant had a K_m value for histidine of 26×10^{-4} M when assayed by pyrophosphate exchange. This value is intermediate between the values found for wild-type ($K_m = 1.5 \times 10^{-4}$ M) and *hisS1520* ($K_m = 80 \times 10^{-4}$ M) enzymes. Thus two mutations, *hisS1210* and *hisS1520*, both mapping in the same region of the chromosome, affect the K_m of histidyl-tRNA synthetase to different extents.

(c) Action of Histidine in Repression and Growth Stimulation of *HisS* Mutants

Mutant *hisS1520* grows with a prolonged doubling time (185 min) on minimal medium and the

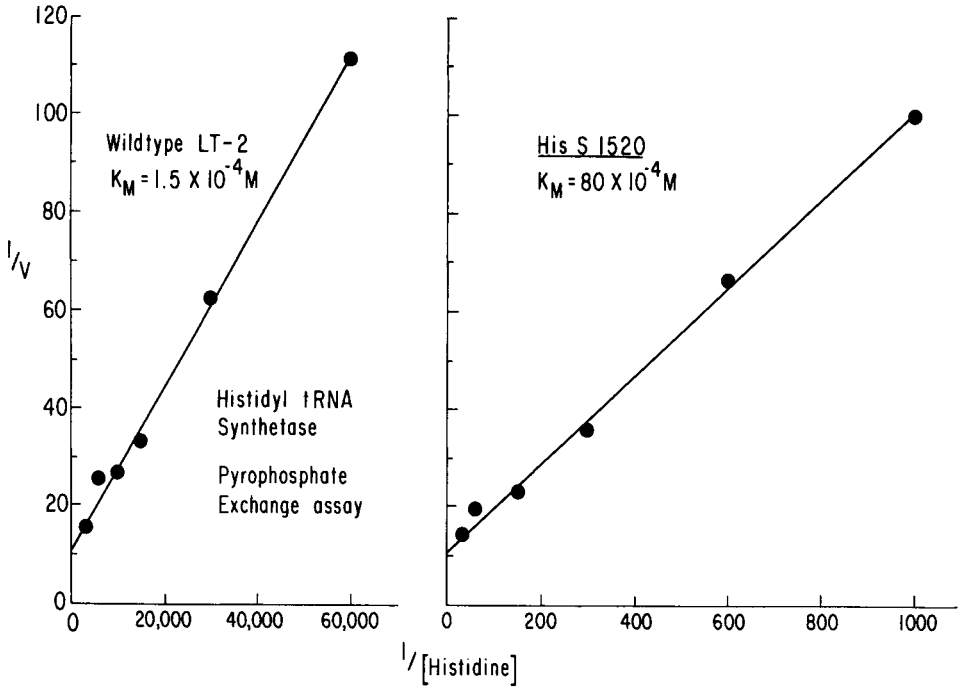

FIGURE 4. Dependence of pyrophosphate exchange reaction on histidine concentration (Roth and Ames, 1966). Velocity (V) is mμmole P^{32} pyrophosphate exchanged per minute. Enzyme used is from peak fractions of DEAE columns; 11 μg of mutant protein and 14 μg of wild-type protein were used.

histidine biosynthetic enzymes are derepressed 17-fold. However, when excess histidine is added to the growth medium the mutant has a normal doubling time (48 min) and repressed levels of the histidine biosynthetic enzymes. Added histidine is able to overcome the damaging effects of the *hisS* mutation (Roth and Ames, 1966).

Of 7 *hisS* mutants isolated, only *hisS1520* has a very slow growth rate on minimal medium. However, two other mutants, *hisS1209*, and *S1210*, which have normal doubling time, are also repressed by histidine. Four mutants, *hisS1211*, *S1213*, *S1219*, and *S1259* are only partially repressed by histidine, and we suspect that these mutants have a synthetase which might be altered in its affinity for tRNA, or has a V_{max} alteration, though this has not been investigated as yet.

Our interpretation of the findings with mutant *hisS1520* is that both growth of the mutant and repression of the histidine operon are limited by the supply of charged histidyl-tRNA and that addition of histidine allows increased synthesis of histidyl-tRNA by the mutant enzyme. A more detailed analysis is presented in the following sections.

The histidine pool and feedback inhibition. The size of the histidine pool in the wild-type organism, growing on minimal medium, is limited by feedback inhibition (the inhibition of the first enzyme of the pathway by histidine). The K_i of the first enzyme (Martin, 1963a; Voll, unpubl.) is about the same as the concentration of histidine in the pool in Salmonella, which is about 1.5×10^{-5} M (Ferro-Luzzi-Ames, 1964). This pool can be increased in a number of ways. (1) When histidine is added to the minimal medium it is taken up rapidly and the pool increases about 5-fold (FerroLuzzi-Ames, 1964). (2) When the level of the histidine biosynthetic enzymes is increased, as in the various classes of histidine regulatory mutants, the histidine pool seems to increase. (3) Feedback resistant mutants have a first enzyme insensitive to histidine inhibition, and have a greatly increased histidine pool as shown by their excretion of a considerable amount of histidine into the growth medium (Sheppard, 1964).

Increasing the histidine pool by endogenous histidine. The partial block in the *hisS1520* mutant of histidyl-tRNA synthesis can be overcome by an increased pool due to endogenously synthesized histidine in a feedback-resistant mutant. A double mutant was constructed (Roth and Ames, 1966) which carried both the *hisS1520* mutation and a mutation conferring feedback resistance, *hisG1924*. This strain excretes a large amount of histidine and thus presumably has a markedly increased internal pool of endogenously synthesized histidine. Consequently, even on minimal medium, this strain has a repressed operon and a normal doubling time.

Our interpretation of the slow growth of mutant *hisS1520* on minimal medium is that the mutant synthetase ($K_m = 8 \times 10^{-3}$M) cannot work at the

rate needed for a maximal growth rate even with the higher intracellular histidine concentration due to the derepression. The external histidine can swell the pool sufficiently so that the synthetase can make enough histidyl-tRNA for maximal protein synthesis. It also appears that when the tRNA for histidine is charged then the histidine operon is repressed, and when it is not charged the operon is derepressed.

(d) Mapping of the *HisS* Gene

The mutation *hisS1520* is co-transducible (14%) with the *strB* locus (far removed from the histidine region of the Salmonella chromosome) (Roth and Ames, 1966), as are six other *hisS* mutants that have been examined previously (Roth et al., 1966). Mutations in the *strB* locus confer low-level streptomycin resistance and simultaneous requirements for thiamine and nicotinic acid (Demerec, Lahr, Balbinder, Miyake, Ishidsu, Mizohichi, and Mahler, 1960). Phage P22 grown on *hisS1520* can be used to transduce a *strB* mutant to wild type by selection for growth on minimal medium. The *hisS1520* mutation is received by 14% of the recombinants from such a cross.

(e) Control of the Histidyl-tRNA Synthetase

Histidyl-tRNA synthetase is not regulated in parallel with the enzymes of the histidine operon. This conclusion stems from three lines of evidence (Roth and Ames, 1966). (1) Three classes of histidine regulatory mutants, *hisR*, *hisT*, and *hisO*, have 5- to 10-fold derepressed levels of the histidine biosynthetic enzymes, while the level of histidyl-tRNA synthetase is unaffected (Roth et al., 1966). (2) Physiological derepression of the histidine operon also fails to affect the synthetase. Histidine-requiring mutants were grown under conditions of limiting histidine (Roth and Ames, 1966); regardless of the extent of derepression of the operon, the specific activity of histidyl-tRNA synthetase remained essentially unchanged. This conclusion had been previously reached by Ames and Garry (unpubl., summarized in Ames and Hartman, 1962). (3) The large deletion *his63* has a deleted operator region and no detectable histidine biosynthetic enzymes and has normal levels of synthetase. This suggests that no subunit of the synthetase is coded for by a gene in the histidine operon.

THE *HisR* GENE

A second class of TRA-resistant mutants, *hisR*, has been mapped (Roth and Hartman, 1965; Roth and Sanderson, 1966; Roth et al., 1966) and found to lie between the *metE* and the *ilvA* loci (Fig. 2). *HisR* mutants have high levels of the histidine biosynthetic enzymes and are able to derepress further when grown under conditions of limiting histidine (Roth, Antón, and Hartman, 1966). Their growth rate is normal.

The nature of the *hisR* gene product, and its role in repression have been investigated by Silbert, Fink, and Ames (1966). They found that *hisR* mutants have approximately 55% of the normal level of histidine-specific tRNA acceptor activity, whereas none of the other classes of regulatory mutants shows such a reduction (Table 1). This suggests that the *hisR* gene is involved, directly or indirectly, in the production of a histidine-specific tRNA.

This decrease in tRNA(his) in the *hisR* mutants seems to be due to an actual decrease of the tRNA (his) species relative to the total tRNA, rather than some decreased specific activity due to contaminating nucleic acid that is not tRNA. This conclusion is based on chromatography of tRNA from wild type and from the *hisR* mutant on various types of columns. Chromatography showed that the acceptor capacity of the *hisR*-tRNA(his) is only 55% of that of the wild type, although the general tRNA absorbancy profiles are the same. The chromatography of equal amounts of tRNA from a *hisR* mutant and from wild type on a G100 Sephadex column and then on a DEAE-Sephadex column, yielding an 8-fold purification of the tRNA(his), preserved the *hisR* to wild type ratio of about 0.55 in all the fractions. A double-labeling experiment, comparing *R* and wild type histidyl-tRNA(his) on a MAK column, showed that this ratio was preserved in the region where only tRNA is eluted.

In addition to the decrease in tRNA(his), the *hisR* mutants have somewhat increased activity of tRNA for several other amino acids. The small increase of tRNA species other than histidine in the *hisR* cannot account for the marked decrease in the specific activity of tRNA(his). A doubling of all the other tRNA species would be necessary to lower the specific activity of tRNA(his) by half. In addition, the yield of tRNA isolated from the *hisR* mutants was identical to that from wild type, indicating no general increase in cellular tRNA.

Chromatographic fractionation of tRNA on MAK, DEAE-Sephadex, and hydroxylapatite, and gel filtration on G100 Sephadex did not reveal two tRNA(his) species or show a qualitative difference between mutant and wild type tRNA(his) accounting for the difference in specific activity. There is evidence of only one tRNA(his) in *E. coli* by countercurrent fractionation.

Another approach to the problem of whether

TABLE 1. tRNA ACTIVITY IN REGULATORY MUTANTS

Strain	Growth condition	Doubling time	Extent derepressed	tRNA activity (% wild type grown on M) His	Tyr	Gln	Asp	Ile	Pro
Wild type									
LT-2	M†	48	1	100	100	100	100	100	100
	H†	48	1	100					
Regulatory mutants									
hisR1813	M	48	6	54	134	128	132	115	93
				55	128				99
				57	126				
				60	125				
				51					
				50					
				48					
	H	48		58					
hisR1203	M	48	8	56					
hisR1200	M	48	8	57		124	117		
	H		7						
hisT1501	M	58	6	102	104			95	
				112					
				97					
hisO1202	M	48	6	104					
hisO1202	H	48	6	98	98				
hisH107				103					
				96					
hisS1520	H	48	1	103					
hisS1210	M	48	8	101					
				107					
				93					
	H	48	2	102					

From Silbert, Fink, and Ames (1966). † M, and H stand for minimal medium supplemented respectively with (1) 0.5 g % glucose, (2) 0.5 g % glucose and 0.1 mM-*L*-histidine.

tRNA activity was determined in duplicate. The scatter of values obtained from assays on different days or with different preparations is given for tRNA(his) in the several classes of derepressed mutants and tRNA(tyr) and tRNA(pro) in the *hisR* class. The extent of derepression was measured by the activity of histidinol dehydrogenase and/or histidinol phosphate phosphatase.

there are two histidine-tRNA species is the comparison of the tRNA(his) from *hisR* and wild type by a codon binding analysis. This has been done by Dr. M. Nirenberg (unpubl. observations) on preparations furnished by this laboratory. He has found no differences between *hisR* and wild type. Triplets CAU and CAC stimulated ribosome binding of histidyl-tRNA from unfractionated tRNA, and the extent of binding was the same for the tRNA from mutant or wild type. In addition, histidyl-tRNA(his) from both sources responded with moderate ambiguity to the polynucleotide UC. Thus, if there are two species, they probably have similar if not the same anti-codons, and similar structures.

The failure to demonstrate more than one histidyl-tRNA by these several procedures, nevertheless, does not eliminate the possibility that there are two very similar species, not easily separable by available methods, and that one of these is inactivated by the *hisR* mutation.

In conclusion, in the *hisR* class of regulatory mutants the level of tRNA(his) is reduced and tRNA activity for several amino acids (though not all) is simultaneously increased. These findings could be explained by one of the following hypotheses: (1) the *hisR* gene codes for tRNA(his). If there were two very similar species of tRNA(his), one of them (a 45% component) could be coded for by the *hisR* gene and be missing in the *hisR* mutants. On the other hand, if there was only one species, then it would also be necessary to say that the altered tRNA(his) was inactivated partially so that only 55% remained in the several mutants. Neither of these alternatives would easily explain the increases in the other tRNA species occurring along with the decrease in tRNA(his). (2) The *hisR* gene codes for an enzyme involved in the biosynthesis of tRNA(his). To convert virgin tRNA to its final mature structure involves many enzymes (Hurwitz, Gold, and Anders, 1964; Srinivasan and Borek, 1964; Comb and Katz, 1964). If some of these enzymes work on a number of different tRNA species, it is possible that an altered or missing enzyme could affect several different tRNA types.

The isolation and characterization of tRNA(his) should help to decide between these various alternatives.

THE *HisT* GENE

The *hisT* mutants have high levels of the histidine biosynthetic enzymes and normal specific activity

of histidyl-tRNA synthetase. The growth rate of the mutants is close to normal. The *hisT* locus maps between the *aroD* and the *purF* loci on the *Salmonella* chromosome (Roth, Antón, and Hartman, 1966). Since the linkage between *aroD5* and *purF145* (8.1%) is weaker than the linkage of any *hisT* mutant to either of them, we infer that the *hisT* gene is located between the *aroD* and the *purF* loci. This conclusion is substantiated by three point tests. The histidine permease (*hisP*) gene is also linked to *purF* but on the side opposite from *hisT* (Shifrin, Ames, and FerroLuzzi-Ames, 1966; FerroLuzzi-Ames, unpubl.).

As yet no biochemical alteration has been detected in *hisT* mutants. All strains tested have normal levels of histidyl-tRNA synthetase. No change in the amount of histidine-specific tRNA acceptor capacity has been detected in a *hisT* mutant (Silbert, Fink, and Ames, 1966).

THE *HisO* MUTANTS

Three of the TRA-resistant mutants have been designated *hisO* on the basis of their high repressed-enzyme levels and map position adjacent to the structural genes of the histidine operon. From three point crosses (Roth, Antón, and Hartman, 1966), it was inferred that all three operator mutations were located either to the right of all known histidine mutations, or close to those known sites lying at the extreme right end of the gene *hisG*. These results are consistent with a location of the operator region at the extreme right end of the operon either within or beyond the structural gene proper. The following lines of evidence suggest that the operator is at the right end of the operon and that translation and transcription start at the right end (the *G* gene): (1) Studies on polarity (Ames and Hartman, 1963; Martin, Silbert, Smith, and Whitfield Jr., 1966; Whitfield Jr., Martin, and Ames, 1966) indicate that translation starts at the *G* end and continues down the polycistronic messenger which corresponds in size to the histidine operon. (2) Analysis of deletions, which turn off the operon by deleting the right-hand end of it (the *G* gene) (Ames, Hartman, and Jacob, 1963). (3) The studies of sequential synthesis of the histidine enzymes on derepression (Goldberger and Berberich, 1965; Goldberger, Berberich, and Venetianer, 1966).

Recombination between hisO *mutations.* Since all three *hisO* mutations seemed to be located at the same place, the right-hand end of the *hisG* gene, crosses were made (Roth, Antón, and Hartman, 1966) to determine whether recombination between *hisO* mutations could be detected. Such recombinants were sought using the *hisO* mutations as unselected markers in transductional crosses between a double mutant recipient, *hisG46 hisO*, and a donor strain carrying a different *hisO* lesion, and selecting for the loss of the histidine requirement. Recombinations were inspected for colony morphology.

A smooth colony is the phenotype of a $hisO^+$ and a wrinkled colony for *hisO*. Results of these crosses are presented in Table 2. The smooth colony recombinants had wild type enzyme levels. It is concluded that recombination can occur between *hisO1812* and the other two *hisO* mutations, but not between *hisO1202* and *hisO1242*. Thus, at least two sites are present in the *hisO* region; if the lesions in these strains are deletions, they do not overlap each other. The order of the mutations is G46-(O1202,O1242)-O1812.

The basal (repressed) enzyme levels for *hisO1202* and *hisO1242* are quite different. Therefore, all three *hisO* mutations can be differentiated either on genetic or on biochemical grounds.

Test for reversion of hisO1242. It has been observed that the four classes of constitutive mutants do not grow at 42°, a temperature at which the wild type grows readily (Voll and Fink, unpubl.). Therefore, derepression of the histidine operon results in inability to grow at high temperature. If about 10^8 cells of *O1242* are plated on a minimal plate at 42°, only about 400 colonies will appear. Of these 400 colonies there are usually only about 7 smooth colonies. (The *O1242* colonies have a wrinkled surface because of derepression.) These smooth colonies have been examined to see if they are true revertants of the original *O1242* by assaying the enzyme levels (Voll, unpubl.). Out of 15 smooth colonies examined there is none

TABLE 2

Recipients	Donors					
	hisO1202		*hisO1242*		*hisO1812*	
G46 O1202	0/5310	<0.02%	0/7302	<0.01%	13/18,813	0.07%
G46 O1242	0/4535	<0.02%	0/1534	<0.07%	38/12,705	0.30%
G46 O1812	1/1983	0.05%	1/7023	0.01%	0/8412	<0.01%

Number of $hisO^+$ recombinants per number of colonies scored. The smooth prototrophic recombinants were selected on a minimal plate. All of them had repressed enzyme levels. Mutation *hisG46* causes a histidine-requirement while the *hisO* mutations do not.

which appears to be a true revertant of *hisO1242*. Thus the reversion rate of *O1242* is probably less than $1/10^8$ cells. This could be consistent with the *O* mutants being small deletions. Some of the smooth colonies have a second mutation causing polarity in the histidine operon without causing a histidine requirement, while others have a second mutation outside the histidine operon.

HISTIDYL-tRNA RATHER THAN HISTIDINE IS IMPORTANT IN REPRESSION

The question of whether the amino acid or the aminoacyl-tRNA is the "co-repressor" has always been an open one. Although the question had not been clearly formulated until recently, Boman, Boman, and Maas (1961) were certainly thinking along these lines. Our interest in the role of the histidyl-tRNA in repression (Ames and Garry, 1959; Ames and Hartman, 1962, 1963) prompted an attempt to find an inhibitor of the histidine activating enzyme in order to answer this question. Histidinol was a good competitive inhibitor (Ames and Hartman, 1962), but attempts to use histidinol to derepress the histidine biosynthetic enzymes in the wild type organism were inconclusive because of its conversion to histidine by the bacteria.

The first clear evidence that an amino acyl-tRNA rather than the amino acid was involved in derepression came from the important work of Schlesinger and Magasanik (1964). They showed that in Aerobacter and in *E. coli*, upon addition of α-methyl histidine to the bacterial culture, the histidine biosynthetic enzymes were derepressed and histidine accumulated. This analogue was shown to inhibit the histidine-activating enzyme. They explained their data by suggesting that the pool of charged histidine transfer-RNA and not the pool of free histidine is responsible for repression. This conclusion was strongly supported (in valine synthesis) by the work of Eidlic and Neidhardt (1965) on temperature-sensitive amino acid activating enzymes. The work (Roth, 1965; Roth et al., 1966; Roth and Ames, 1966; also discussed in Ames and Hartman, 1963) on the *hisS* mutants gives more direct support for this conclusion. On the other hand, Ravel, White, and Shive (1965) have shown that some tyrosine analogues that repress are not activated. It is not clear how to resolve this apparent disagreement.

The *hisS* mutants studied were isolated as histidine regulatory mutants. The fact that a partial block in histidyl-tRNA synthesis by the action of a defective synthetase can cause derepression strongly suggests that this product is important in the regulation of the histidine operon. This conclusion is also reinforced by the finding that another type of regulatory mutation, *hisR*, affects in some way the synthesis of active histidine-specific tRNA (Silbert, Fink, and Ames, 1966).

However, several alternative explanations are not ruled out. One is that the activating enzyme itself is the repressor when it has histidine bound to it. Another is that histidyl-tRNA is a precursor of some other substance which is the repressor.

MODELS OF REPRESSION

The Jacob-Monod model. To interpret our results in terms of the Jacob-Monod model we would say that the *hisR* and *hisS* genes are not true regulatory genes, but only necessary in synthesizing histidyl-tRNA, which is the true "co-repressor." The *hisT* gene then would make an allosteric protein influenced by charged histidine tRNA, and this protein would interact with the operator gene at the end of the histidine operon. There are, however, a few minor problems with the model. (1) The *hisT* mutants can be further derepressed. This would be different from the behavior of the *i* gene mutant in the lactose operon and hard to fit with a dispensable regulatory gene. (2) Histidine tRNA is a very large molecule and therefore it is difficult to imagine that it acts as an allosteric modifier.

Translation control by histidine tRNA. One can also think of a number of models of regulation in which histidine tRNA is involved directly in a control at the translation level (Ames and Hartman, 1963; Stent, 1964). Thus the operator region could be in RNA. It could be a control region near (or at) the beginning of the large message corresponding to the histidine operon (Martin, 1963b) which is translated unidirectionally from the operator end (Ames and Hartman 1963; Martin, Whitfield, Berkowitz, and Voll, this volume). The postulate, (Ames and Hartman, 1963; Stent, 1964) that the messenger RNA is stripped off the gene by the translation process, has received recent support (e.g., Bautz, Kasai, Reilly, and Bautz, 1966) and would make a translational control consistent with the various studies of messenger RNA during induction. One type of model that seems attractive is to have the initiation of reading controlled by having a histidine codon in some critical spot of the RNA operator so that charged tRNA(his) causes a block while the uncharged tRNA(his) allows initiation to occur. It may be that the RNA operator is before a critical AUG (formylmethionine) that is necessary to start the first enzyme, and thus the operator is transcribed, but not translated as part of the first enzyme, or it may be that the operator is actually part of the first enzyme and is both transcribed and translated. For an amino acid

operon such as histidine, a histidine tRNA repressor (or an uncharged tRNA(his) which is acylated by something such as a formylmethionine and thus is converted to a translation inducer), has a number of attractive features. More specific models for translational control may be possible as soon as the biochemistry of protein synthesis and initiation becomes better understood.

Translational control by folding of the protein. A control by folding of the first enzyme (the G enzyme which is feedback inhibited by histidine), influenced by the histidine concentration, does not seem likely in the histidine system. (1) The feedback insensitive mutants derepress normally (Sheppard, 1964) and feedback supersensitive mutants derepress normally (O'Donovan and Ingraham, 1965; Ames, Goldberger, Hartman, Martin, and Roth, 1966). (2) Thiazolealanine is a good feedback inhibitor and does not repress, and triazolealanine is a good repressor and does not cause feedback inhibition (Martin, 1963a). (3) Histidine is involved in feedback inhibition and histidine tRNA in repression. Histidyl-tRNA is not active in feedback inhibition at concentrations at which it is present in the cell (Fink and Berkowitz, unpubl.). (4) Nonsense mutants in the gene for the first enzyme derepress normally (Berkowitz, unpubl.).

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