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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.](image-url)
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 gone 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 C14 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, *hiss*1520, was examined. This mutant has no detectable synthetase activity (less than 0.2% of the wild-type activity) by this same assay. The

![Diagram](https://example.com/diagram.png)
CONTROL OF THE HISTIDINE OPERON

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 mmol P\textsuperscript{32} pyrophosphate exchanged per min by 1 \mu l of fraction. Transfer activities are presented as counts/min C\textsuperscript{14} histidine (35 mc/m mole) incorporated into tRNA per min by 1 \mu l of fraction.

The 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\textsuperscript{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\textsuperscript{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\textsuperscript{32} pyrophosphate exchange assay at 1.67 mM-histidine (—○—) 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\textsubscript{m} values for the two enzymes. The wild-type enzyme has a K\textsubscript{m} of \(1.5 \times 10^{-4}\) M and the mutant enzyme has a K\textsubscript{m} of \(80 \times 10^{-4}\) 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\textsubscript{m} value for histidine of \(26 \times 10^{-4}\) M when assayed by pyrophosphate exchange. This value is intermediate between the values found for wild-type (K\textsubscript{m} = \(1.5 \times 10^{-4}\) M) and hisS1520 (K\textsubscript{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\textsubscript{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
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 $V_{\text{max}}$ 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 $K_r$ 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 $1.5 \times 10^{-5}$ 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 (Ferro-Luzzi-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 ($K_m = 8 \times 10^{-5}$M) cannot work at the
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rate needed for a maximal growth rate even with the higher intracellular histidine concentration due to 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 hisO3 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 TLA-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 ifA 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 mutant 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

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<th>Extent derepressed</th>
<th>tRNA activity (% wild type grown on M)</th>
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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 m M 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.

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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 araD and the purF loci on the Salmonella chromosome (Roth, Antón, and Hartman, 1966). Since the linkage between araD5 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 araD 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, following lines of evidence suggest that the operator is at the right end of the operon and that translation starts at the G end and continues down the polycistronic messenger which corresponds in size to the histidine operon. (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 hisO1242 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 10⁸ 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>
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<td>Recipients</td>
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<td>G46 O1242</td>
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<td>G46 O1812</td>
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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 1/10^6 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
CONTROL OF THE HISTIDINE OPERON

operon such as histidine, a histidine tRNA repressor (or an uncharged tRNA(his) which is acetylated 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 first enzyme derepress normally (Berkowitz, unpubl.). (4) Nonsense mutants in the gene for the first enzyme derepress normally (Berkowitz, unpubl.).

Acknowledgments

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