

Repression of the Histidine Operon: Effect of the First Enzyme on the Kinetics of Repression

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Kinetic studies on repression of the enzymes for histidine biosynthesis in *Salmonella typhimurium* showed that, upon addition of histidine to a derepressed culture, the enzymes became repressed in a temporal sequence which corresponds with the positional sequence of the genes in the histidine operon. This serial pattern of repression occurred under conditions in which the feedback site of the first enzyme for histidine biosynthesis is intact. When this site was rendered nonfunctional the pattern of repression was changed so that all of the enzymes became repressed concomitantly. These results suggest that the first enzyme for histidine biosynthesis plays a hitherto unrecognized role in control of the histidine system.

The pathway for histidine biosynthesis in *Salmonella typhimurium*, elucidated largely through the work of B. N. Ames and co-workers (2, 5, 22, 27, 28), consists of a series of 10 reactions each catalyzed by a specific enzyme. The genes which specify the structures of these enzymes are located in a small segment of the *Salmonella* chromosome known as the histidine operon (3, 14). The histidine operon functions as a unit in response to the level of histidine available to the organism. As originally shown by Ames and Garry (1), the differential rate of synthesis of the histidine enzymes increases when histidine becomes the limiting growth factor. That is, the operon becomes derepressed. When excess histidine is added to a derepressed culture, the differential rate of synthesis of these enzymes declines. That is, the operon becomes repressed.

Kinetic studies on the repression process are presented here. These studies reveal that the enzyme encoded in the first gene of the histidine operon may be involved in control of the histidine system. This involvement is demonstrated by examining the manner in which translation of the polycistronic histidine messenger ribonucleic acid (mRNA) ceases (19, 30).

MATERIALS AND METHODS

Bacterial strains. The organisms employed in these studies were the LT-2 (wild type) strain of *S. typhimurium*; the nonpolar, "complete" histidine auxo-

trophs *hisG52*, *hisG1102 hisIF135*, *hisG1109 hisIF135*, *hisC2*, *hisA30*, and *hisIF135*; the nonpolar, "leaky" histidine auxotrophs *hisG1306*, *hisB59*, and *hisE11*; the constitutive mutant *hisS1520*; and the polar nonsense mutant *hisG428*. These organisms were obtained from the collection of P. E. Hartman.

Growth conditions. Cells were routinely grown in minimal medium (31) with glucose at 0.5% in 2 liters of culture medium in a 4-liter flask. The cultures were aerated vigorously in a New Brunswick rotary shaker at 37 C. The histidine auxotrophs and the constitutive mutant were grown in the presence of a sufficient amount of L-histidine to support growth to an optical density (OD) at 700 nm of approximately 0.35 (3.4×10^8 cells per ml). After depletion of histidine from the medium, growth was supported by L-histidinol (2.5×10^{-5} M; Cyclo Chemical Co.) in the case of the "complete" auxotrophs and by the endogenous synthesis of histidine in the case of the "leaky" auxotrophs and the constitutive mutant. Derepression in the wild-type organism was obtained when the culture had reached a cell density of 3.4×10^8 cells per ml by the addition of either DL-2-thiazolalanine (Cyclo Chemical Co.; final concentration, 0.1 mM) or 3-amino-1,2,4-triazole (aminotriazole, final concentration, 20 mM; Aldrich Chemical Co.). In all experiments, the growth rate during derepression was the same (doubling time 4 hr), and derepression was allowed to proceed for 30 min. Repression was then effected by the addition of L-histidine at a final concentration of 0.3 mM. The growth rate always returned immediately to that characteristic of repressed cells (doubling time 50 min).

Experimental design. In a typical experiment, samples (50 to 100 ml) were withdrawn from the culture periodically, so that 10 samples were collected during derepression and 10 samples were collected after the addition of histidine. Each sample was immediately mixed with ice and excess histidine. After

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all samples had been collected, they were centrifuged. The pelleted cells were washed with 0.05 M tris (hydroxymethyl)aminomethane (Tris) buffer (pH 8.0), containing histidine at a concentration of 0.1 mM, recentrifuged, and finally resuspended in 4 ml of 0.05 M Tris buffer (pH 8.0). Extracts were prepared from the suspensions with a French pressure cell (American Instrument Co.) at 6,000 psi, clarified by centrifugation, and assayed immediately.

Assays and substrates. Protein was determined by the method of Lowry et al. (15). Assays for the enzymes corresponding to the first gene (*G*), third gene (*C*), fourth gene (*B*), sixth gene (*A*), seventh gene (*F*), and eighth gene (*I*) of the histidine operon were performed as previously described (2, 5, 17, 27, 28). Enzyme levels are expressed as the amount of activity per milligram of protein. Substrates were obtained as follows: L-histidinol phosphate was purchased from Cyclo Chemical Co.; *N*-(5'-phospho-D-ribosylformimino)-5-amino-1-(5'-phosphoribosyl)-4-imidazole-carboxamide and *N*-1-(5"-phosphoribosyl)adenosine monophosphate were synthesized enzymatically (27) from 5-phosphoribosyl-1-pyrophosphate (P-L Biochemicals).

RESULTS

Repression in histidine auxotrophs. We studied the kinetics of repression of the enzymes for histidine biosynthesis in a wide variety of histidine auxotrophs. The results of a typical experiment are shown in Fig. 1. After the addition of histidine to a derepressed auxotroph (*hisA30*), the specific activities of the enzymes began to fall in a definite sequence. The temporal sequence in which the enzymes became repressed corresponds to the positional sequence of the genes in the histidine operon, approximately 10 min intervening between repression of the enzymes corresponding to the first and seventh genes, respectively. Thus, the times at which the various enzymes became repressed are directly related to the distances of the corresponding genes from the operator located at the *G* end of the operon (24). The same result was obtained in all auxotrophs tested (*hisG52*, *hisA30*, *hisIF135*, *hisC2*, *hisB59*, and *hisE11*).

Repression in the wild-type organism. Because the wild-type organism has an intact histidine operon it cannot be derepressed by regulating the supply of exogenous histidine, as in the auxotrophs. Therefore, in experiments with the wild-type organism a limitation of histidine was created by adding an appropriate amount of thiazolalanine to the culture medium (4, 5). Thiazolalanine inhibits the first enzyme of the histidine pathway by binding at the feedback site (21), that is, the same site at which the physiological feedback inhibitor, histidine, binds to the enzyme (18). As a result of this inhibition, the biosynthesis of histidine is impaired, causing the organism to de-

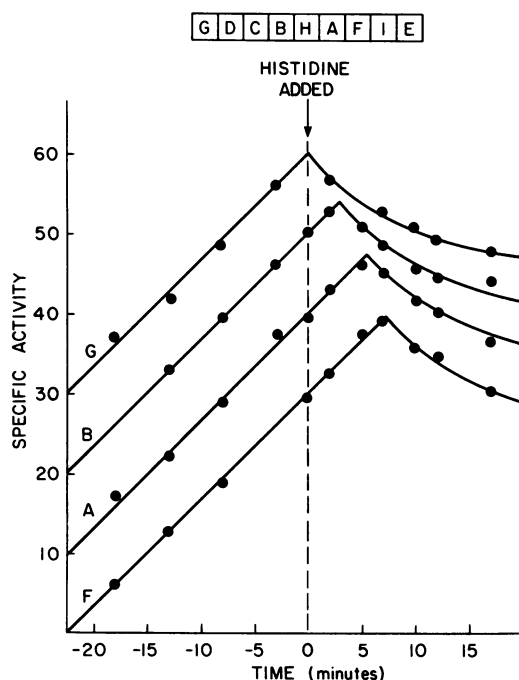


FIG. 1. Kinetics of repression in the histidine auxotroph *hisA30*. The order of genes in the histidine operon is shown at the top. Below this are shown the kinetics of repression of four of the enzymes. These data demonstrate that, when histidine was added to a derepressed culture, repression occurred in a temporal sequence which corresponds with the positional sequence of the genes in the histidine operon. To avoid overlapping of the curves, the specific activity scale for each enzyme was adjusted by adding a constant factor at each point. The rate of decline in specific activity after the addition of histidine is the same for all of the enzymes and is due to dilution resulting from the increased growth rate which occurs upon repression. Only a portion of the data is shown so as to focus on the period around the time when histidine was added.

repress. Once the organism has been derepressed, the kinetics of repression are studied in the same way as in the histidine auxotrophs.

In contrast to the serial pattern of repression seen in all histidine auxotrophs, the pattern of repression in the wild-type organism was concomitant (Fig. 2). We previously reported studies on the kinetics of *derepression* of the enzymes for histidine biosynthesis, using the terms *sequential* and *simultaneous* to describe the two alternative patterns observed. In our studies on the kinetics of repression reported in this paper, we use the terms *serial* and *concomitant* in order to indicate that the phenomena involved in repression are different from those involved in derepression.

Unlike all of the histidine auxotrophs (in which the enzymes became repressed at times which are directly related to the distances of the corresponding genes from the operator), the wild-type organism was characterized by repression of all of the enzymes at about the same time, approximately 3.5 min after the addition of histidine.

To understand the factors responsible for the two patterns of repression (serial in the histidine auxotrophs and concomitant in the wild-type organism), we investigated the differences between the two sets of experiments (the histidine auxotrophs on the one hand and the wild-type organism on the other). These differences were: (i) the

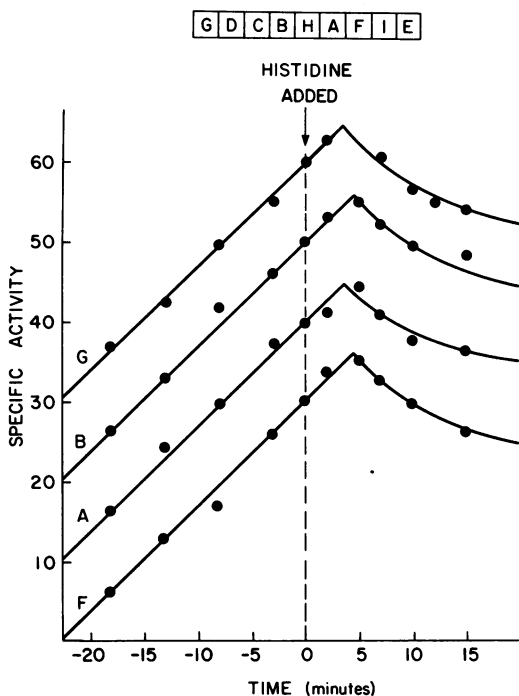


FIG. 2. Kinetics of repression in the wild-type organism (LT-2) which had been derepressed with thiazolalanine. The order of genes in the histidine operon is shown at the top. Below this are shown the kinetics of four of the enzymes. These data demonstrate that, when histidine was added to a culture which had been derepressed by thiazolalanine, repression of all the enzymes occurred concomitantly. To avoid overlapping of the curves, the specific activity scale for each enzyme was adjusted by adding a constant factor at each point. The rate of decline in specific activity after the addition of histidine is the same for all of the enzymes and is due to dilution resulting from the increased growth rate which occurs upon repression. Only a portion of the data is shown so as to focus on the period around the time when histidine was added.

wild-type organism does not have any mutation in the histidine operon, whereas the histidine auxotrophs described above do; and (ii) thiazolalanine was present in the culture medium during derepression in the case of the wild-type organism, but not in the case of the histidine auxotrophs.

Repression in a constitutive mutant. To explore the possibility that the pattern of repression is related to the presence or absence of a mutation in the histidine operon, we examined the pattern of repression in a constitutive histidine mutant (*hisS1520*) which, like the wild-type organism, has a normal histidine operon. As shown by Roth and Ames (23), this organism contains only a mutation in the gene for the histidine-activating enzyme, can be derepressed without the use of thiazolalanine, and can be repressed with histidine. The pattern of repression in this constitutive mutant was identical to the serial pattern seen in all histidine auxotrophs (see Fig. 1). This finding indicated that the pattern of repression is not related to the presence or absence of any mutation in the histidine operon. Therefore, we performed the following experiments to explore the possibility that the pattern of repression is related to the presence or absence of thiazolalanine in the culture medium.

Repression in the wild-type organism in the absence of thiazolalanine. To study the kinetics of repression in the wild-type organism in the absence of thiazolalanine, we utilized another means of causing derepression of the histidine operon. It had been shown by Hilton et al. (12) that aminotriazole causes a histidine limitation in the wild-type organism by inhibiting the enzyme that catalyzes the seventh step of the histidine pathway. As a result of this limitation, the histidine operon becomes derepressed. Thus, the mechanism by which aminotriazole causes derepression of the histidine operon is the same as that for thiazolalanine: both compounds limit the endogenous synthesis of histidine by inhibiting a step in the biosynthetic pathway (aminotriazole, by inhibiting the seventh step; thiazolalanine, by inhibiting the first step).

The results of a representative experiment in which the kinetics of repression were studied in the wild-type organism which had been derepressed with aminotriazole are presented in Fig. 3. It is clear that the pattern was serial, in contrast to the concomitant pattern observed in the same organism when thiazolalanine had been used to effect derepression (cf. Fig. 2 and 3). Thus, it appeared that thiazolalanine was directly responsible for causing the pattern of repression to be concomitant. If this is true, thiazolalanine would be expected to produce the concomitant

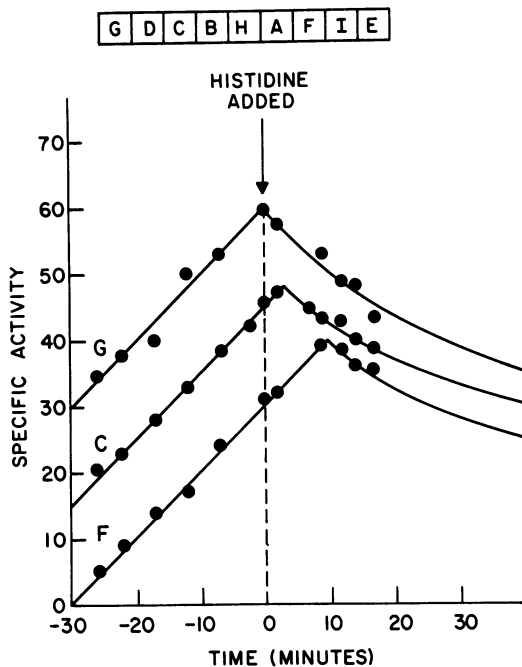


FIG. 3. Kinetics of repression in the wild-type organism (LT-2) which had been derepressed with aminotriazole. The order of genes in the histidine operon is shown at the top. Below this are shown the kinetics of three of the enzymes. These data demonstrate that, when histidine was added to a culture which had been derepressed with aminotriazole, repression of the enzymes occurred in a temporal sequence which corresponds with the positional sequence of the genes in the histidine operon. To avoid overlapping of the curves, the specific activity scale for each enzyme was adjusted by adding a constant factor at each point. The rate of decline in specific activity after the addition of histidine is the same for all the enzymes and is due to dilution resulting from the increased growth rate which occurs upon repression. Only a portion of the data is shown so as to focus on the period around the time when histidine was added.

pattern of repression not only in the wild-type organism but in histidine auxotrophs as well.

Repression in histidine auxotrophs in the presence of thiazolalanine. The kinetics of repression were examined again in the histidine auxotrophs *hisA30*, *hisC2*, and *hisG52*, except that thiazolalanine (final concentration, 0.1 mM) was added to the culture medium. In these experiments, the addition of thiazolalanine had no effect on the derepressed growth rate, since the growth rate was controlled by the concentration of histidinol present in the culture medium. This concentration was adjusted so that the growth rate during derepression was the same as in all other experiments with the auxotrophs and the same as in the

wild-type organism in which the growth rate was limited by the presence of thiazolalanine or aminotriazole. Under these conditions, the pattern of repression was concomitant, confirming that the concomitant pattern of repression is caused by the presence of thiazolalanine. Because thiazolalanine is known to inhibit the histidine pathway by binding to the first enzyme, these results suggest that the pattern of repression is dependent upon whether the enzyme is bound by thiazolalanine. Furthermore, because thiazolalanine is known to act via the feedback site of the first enzyme, not the catalytic site, the findings suggest that the involvement of the first enzyme in determining the pattern of repression is realized through the feedback site. Consistent with this hypothesis is the finding that thiazolalanine changed the pattern of repression to concomitant not only in histidine auxotrophs which have an active first enzyme but also in a missense mutant, *hisG52*, which produces a catalytically inactive first enzyme (14).

Repression of histidine auxotrophs with mutations affecting the feedback site of the first enzyme. If thiazolalanine causes the concomitant pattern of repression by interfering with the function of the feedback site of the first enzyme, then mutations which interfere with the feedback sensitivity of this site would be expected to mimic the effect of thiazolalanine. Therefore, we studied the effect of mutations, described by Sheppard (26), which lead to the production of a catalytically active but feedback-resistant first enzyme. The effect of these mutations was studied in the histidine auxotroph *hisIF135*. Like all histidine auxotrophs, *hisIF135* displays the serial pattern of repression (see Fig. 4A). To determine whether feedback resistance would change the pattern of repression in this auxotroph, we used the two doubly mutant strains *hisG1102 hisIF135* and *hisG1109 hisIF135*, in which the mutations leading to feedback resistance (*hisG1102* and *hisG1109*) are located at widely separated sites in the *G* gene (25). Feedback resistance of the first enzyme was confirmed in these experiments by assays of the extracts for activity of the first enzyme in the presence of histidine at various concentrations. The pattern of repression in these mutants was concomitant (see Fig. 4B). Since the only difference between *hisIF135* (serial) and the double mutants (concomitant) is the feedback sensitivity of the first enzyme, we conclude that it is the defective feedback site which leads to the concomitant pattern of repression in the latter mutants. Thus, when the feedback sensitivity of the first enzyme is impaired by mutation, the pattern of repression is shifted to concomitant just as when it is impaired by thiazolalanine.

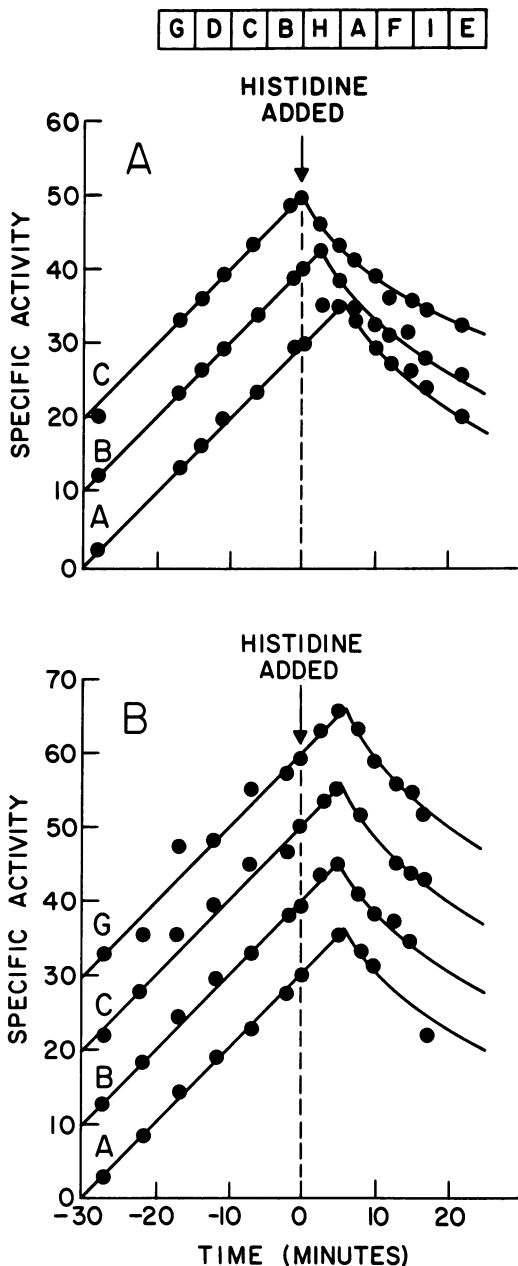


FIG. 4. Effect of a mutation leading to feedback resistance of the first enzyme on the kinetics of repression of the enzymes for histidine biosynthesis. The order of genes in the histidine operon is shown at the top. (A) The kinetics of repression in the histidine auxotroph *hisIF135*. These data demonstrate that, when histidine was added to a derepressed culture, repression occurred in a temporal sequence which corresponds with the positional sequence of the genes in the histidine operon. (B) The kinetics of repression in the double mutant, *hisG1109 hisIF135*, in which all of the

We also studied the effect of a mutation (*hisG1306*) which was shown by St. Peirre (25) to lead to the production of a catalytically active but feedback-hypersensitive first enzyme. In this case, the pattern of repression was serial, demonstrating that a mutation leading to increased sensitivity of the feedback site does not affect the pattern of repression.

Repression in a nonsense mutant of the first gene. The foregoing conclusion, that the serial pattern of repression is observed only when the feedback site of the first enzyme is intact and not occupied by thiazolalanine, led us to the prediction that a mutant missing the first enzyme for histidine biosynthesis would display the concomitant pattern of repression. To test this prediction, we used the nonsense mutant *hisG428* (9). (We chose a nonsense mutant because there is no mutant yet available in which a portion of the first gene of the histidine operon is deleted and in which both the operator and one or more structural genes of the operon are preserved.) This mutant was shown by Fink and Martin (10) to be an amber mutant and to have a polarity value of approximately 50%. The nature of the mutation and its degree of polarity were confirmed in the present study (Fig. 5). As expected, all of the enzymes studied became repressed at the same time, approximately 3 min after the addition of histidine.

Summary of data from kinetic studies. All of the experiments done in the studies discussed above are summarized in Table 1. It is clear from the table that the C, B, and A enzymes were assayed in most experiments; the reason is that these were the assays which could be performed with greatest efficiency. In the eight organisms in which the feedback site of the first enzyme was intact and not occupied by thiazolalanine, the enzymes became repressed in a temporal sequence, which began immediately upon addition of histidine and spanned approximately 11.5 min. In the six organisms in which the sensitivity of the feedback site of the first enzyme was diminished, the enzymes became repressed at essentially the same time, approximately 3.5 min after the addition of histidine. We have described this pattern of repression as concomitant to indicate its striking difference from the serial pattern. However, the concomitance is

enzymes became repressed concomitantly. To avoid overlapping of the curves, the specific activity scale for each enzyme was adjusted by adding a constant factor at each point. The rate of decline in specific activity after the addition of histidine is the same for all the enzymes, and is due to dilution resulting from the increased growth rate which occurs upon repression. Only a portion of the data is shown so as to focus on the period around the time when histidine was added.

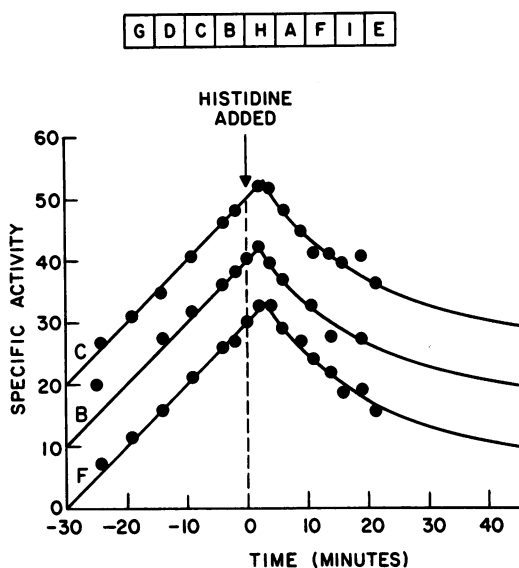


FIG. 5. Kinetics of repression in the nonsense mutant *hisG428*. The order of genes in the histidine operon is shown at the top. Below this are shown the kinetics of repression of three of the enzymes. These data demonstrate that, when histidine was added to a derepressed culture, repression of the enzymes occurred concomitantly. To avoid overlapping of the curves, the specific activity scale for each enzyme was adjusted by adding a constant factor at each point. The rate of decline in specific activity after the addition of histidine is the same for all the enzymes and is due to dilution resulting from the increased growth rate which occurs upon repression. Only a portion of the data is shown so as to focus on the period around the time when histidine was added.

only approximate; in fact, as seen in Table 1, there appear to be small intervals, opposite in temporal sequence to the relatively large intervals observed in the serial mutants. It is difficult to evaluate the significance of this reversed sequence, since it is so small and since the correlation between gene position and time of repression is not perfect (see *A* enzyme data).

DISCUSSION

Because we studied the repression process by measuring changes in enzyme levels, we can interpret our results most directly in terms of the translation process. Our discussion is therefore oriented towards a consideration of the last stage of repression in which mRNA stops being translated. It is entirely possible, however, that the effects we observed in our experiments are secondary to events occurring at the level of transcription.

In serial repression, translation of the polycistronic mRNA of the histidine operon ceases first at the operator end and last at the most distal

TABLE 1. Summary of data from kinetic studies^a

| Enzyme ^b | No. of experiments | Time of repression (min) ^c | |
|---------------------|--------------------|---------------------------------------|--------------------------|
| | | Serial ^d | Concomitant ^e |
| <i>G</i> | 8 5 | 0.125 ± 0.186 | 4.7 ± 1.94 |
| <i>C</i> | 28 15 | 3.34 ± 0.615 | 3.3 ± 0.93 |
| <i>B</i> | 17 12 | 4.8 ± 0.846 | 3.0 ± 0.735 |
| <i>A</i> | 21 10 | 6.2 ± 0.960 | 3.9 ± 0.88 |
| <i>F</i> | 5 9 | 8.2 ± 2.78 | 2.7 ± 1.26 |
| <i>I</i> | 20 | 11.5 ± 1.17 | |

^a The data given here represent the results of over 3,000 assays.

^b Arranged in order of increasing distance of the corresponding genes from the operator.

^c Time of change in differential rate of synthesis after addition of histidine to the culture medium. Expressed in terms of 95% confidence limit.

^d In this category are included experiments with organisms in which there is no known alteration in the allosteric site of the *G* enzyme (with the exception of *hisG1306*, in which the feedback site of the *G* enzyme is hypersensitive to inhibition by histidine). In every experiment, the pattern of repression was serial.

^e In this category are included experiments with organisms in which the allosteric site of the *G* enzyme has been either inhibited with thiazolalanine or altered by mutation. In every experiment the pattern of repression was concomitant.

end. Each portion of the message stops being translated at a time which is directly related to its distance from the operator. This can be explained most simply as follows: at the time of repression, all ribosomes engaged in translating the histidine message continue to the distal end but translation of this message from the operator end by other ribosomes is prohibited. This idea, previously suggested by Ames and Hartman (3), is consistent with the recent findings of Morse, Baker, and Yanofsky (20) on the tryptophan system of *Escherichia coli*.

Concomitant repression apparently represents an alteration of the normal manner in which translation of the histidine mRNA ceases. In this altered form of repression, all cistrons of the message stop being translated at essentially the same time. Our data do not allow us to specify with certainty the mechanism responsible for this concomitant cessation of translation.

Whatever may be the difference between the two mechanisms responsible for the normal, serial pattern and the altered, concomitant pattern of repression, the molecular basis for the difference must involve the first enzyme for histidine biosynthesis. Theoretically, this involvement could be realized either through the catalytic activity of the enzyme or through some other activity of the enzyme. The possibility that the catalytic activity of first enzymes of metabolic pathways may be involved in control has recently been stressed by Tomkins and Ames (29). They discussed the idea, termed "metabolite induction" (29), that in certain bacterial operons derepression can occur only when there is a shortage of the end product of the pathway, when substrate for the pathway is present, and when the first enzyme of the pathway is catalytically active (not inhibited by the end product). However, it appears unlikely to us that the catalytic activity of the first enzyme is involved in determining the pattern of repression, because we do not see a consistent correlation between the level of intermediates of the histidine pathway and the pattern of repression. For example, *hisG52* produces none of the intermediates of the pathway, yet displays either mode of repression, depending upon the presence or absence of thiazolalanine. We favor the view that some other activity of the first enzyme is involved in determining the pattern of repression, an activity which is related to, but not identical with, feedback inhibition. In keeping with this view is the consistent correlation between the pattern of repression and the functional state of the feedback site of the enzyme. Whenever this site is functionally impaired or is occupied by thiazolalanine, the pattern of repression is concomitant; whenever this site is intact and not occupied by thiazolalanine, the pattern of repression is serial.

It is notable that in three of the systems for amino acid biosynthesis (histidine, tryptophan, and leucine), the first structural gene of the operon (that is, the gene closest to the operator) specifies the structure of the first and feedback-sensitive, enzyme of the biosynthetic pathway. This correlation between first gene and first enzyme is unlikely to be fortuitous. It is more reasonable to assume that this correlation is a consequence of evolutionary pressures. Perhaps some survival value was achieved by a regulatory mechanism which requires that the newly synthesized first enzyme be located close to the operator end of the mRNA. Regulatory mechanisms involving the feedback sites of enzymes encoded in the first genes of operons have been discussed on theoretical grounds by Gruber and Campagne (11), Maas and McFall (16), Cline and Bock (8), and Koshland and Kirtley (13). The findings reported here

suggest that the functional state of the feedback site of the first enzyme for histidine biosynthesis influences, directly or indirectly, the mechanism by which translation of the histidine mRNA ceases. Although the physiological significance of this phenomenon is not yet understood, it suggests that the first enzyme may play a previously unrecognized role in regulation of the histidine system.

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