Correlation between Location and Time of Expression for Genes in a Single Operon

Abstract. *When the histidine operon in* Salmonella typhimurium *becomes derepressed in the presence of 4-amino-5-imidazole carboxamide ribonucleoside, derepression of the enzymes for histidine biosynthesis occurs in a temporal sequence which corresponds with the positional sequence of the genes in the histidine operon, approximately 20 minutes intervening between the derepression of the first and that of the last enzyme studied. In the case of the deletion mutant,* hisH,B22, *the interval between the derepression of the first and that of the last enzyme was reduced from 20 minutes to 10 minutes. This reduction was due exclusively to the fact that the interval between the derepressions of the two enzymes, the structural genes for which are located on either side of the deletion, was almost completely eliminated. The results support the genetic evidence for the physical absence of genetic material situated between these two genes.*

The studies of Nomura and Benzer (*1*) on the rII region of the bacteriophage T4 have provided genetic evidence that the physical basis for a deletion mutation is the absence of a segment of chromosomal DNA. Lengthy deletions of DNA in bacteriophages can be detected by physical techniques (*2*). More recently, hybridization techniques have been used to demonstrate that deletion mutations involve the loss of a segment of DNA and an absence of the corresponding messenger RNA (*3*). We now report studies on the kinetics of derepression of the histidine operon in a deletion mutant of *Salmonella typhimurium, hisH,B22*, indicating that in this organism a segment of genetic information is missing.

The structural genes for the enzymes involved in histidine biosynthesis in *Salmonella typhimurium* are localized in a small segment of the chromosome, the histidine operon, which functions as a single unit in response to the level of histidine available to the organism. Genetic and biochemical studies with this system have recently been reviewed (see *4, 5*).

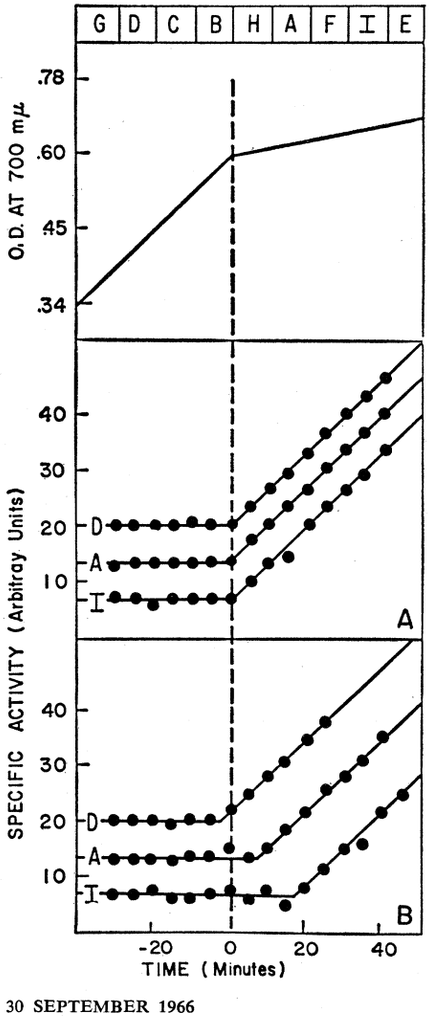

Fig. 1. Alternative modes of derepression of the histidine operon. At the top is shown the order of the genes in the operon. Below this is a typical curve demonstrating the change in growth rate caused by histidine limitation. The histidine auxotrophs were grown in the presence of an amount of L-histidine (20 μm) sufficient to support growth to an optical density at 700 mμ of 0.6 (5.6×10^8 cells/ml). After histidine was depleted, growth was supported by L-histidinol (10 μm), and the growth rate changed from a doubling time of approximately 50 minutes to one of approximately 4 hours. Portions were removed from the cultures periodically before and after the change in growth rate (derepression). Extracts prepared from these samples were assayed for protein and for five of the enzymes of histidine biosynthesis. A, the pattern obtained in a mutant characterized by the simultaneous mode of derepression (*8*). B, the pattern obtained in a mutant characterized by the sequential mode of derepression (*8*). Although not shown in this figure, the other two enzymes examined (*G* and *C*) in each case followed the characteristic pattern. The vertical reference line denotes the time of change in the growth rate caused by the limitation of histidine. For the sake of clarity, overlapping of the curves has been avoided by adding a constant factor to each set of points.

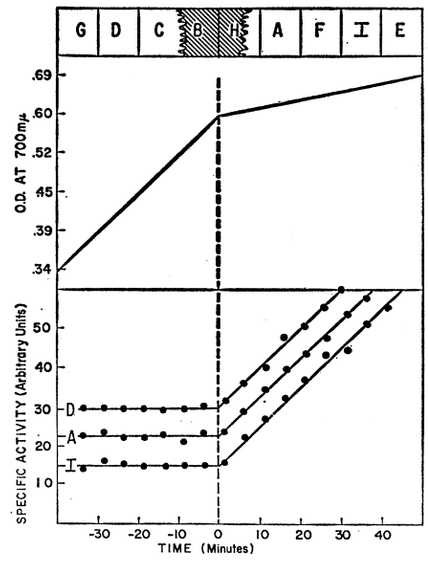

Fig. 2. Simultaneous mode of derepression observed in hte deletion mutant, *hisH,B22*. See legend of Fig. 1 for details of sampling and assay.

Ames and Garry (*6*) showed that the activities of four of the enzymes increase (derepress) in a coordinated way when histidine becomes the limiting growth factor. Subsequently, the activities of the enzymes corresponding to the other genes of the histidine operon have been characterized, and have also been shown to obey the principle of coordinate repression (*7*).

We have reported studies on the kinetics of derepression of five of the enzymes for histidine biosynthesis in *S. typhimurium* (*8*) demonstrating that, on the basis of the kinetics of derepression, histidine auxotrophs may be divided into two groups. In one group derepression of all the enzymes occurs simultaneously (Fig. 1A); in the other, derepression of the enzymes occurs in a temporal sequence corresponding to the positional sequence of genes in the histidine operon. Approximately 20 minutes intervene between the derepression of the first and that of the last enzymes studied (Fig. 1B). The mode of derepression can be shifted from simultaneous to sequential by the addition of 4-amino-5-imidazole carboxamide ribonucleoside (ribosyl-AIC) to the culture medium, and from sequential to simultaneous by the addition of adenine.

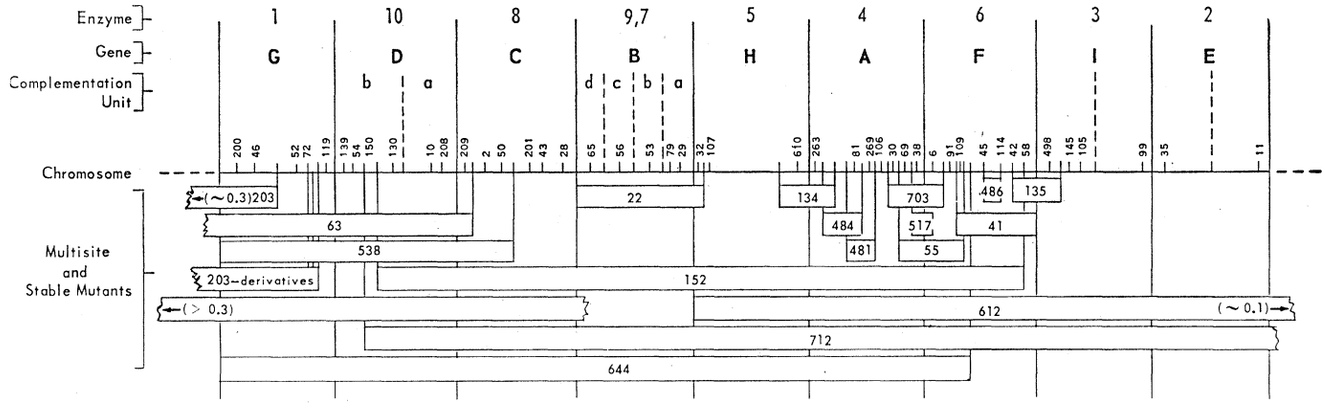

Fig. 3. A genetic map of the histidine operon, constructed by Dr. P. E. Hartman, showing the position of the deletion in *hisH, B22*. No attempt has been made to indicate the different sizes of the various genes.

It seemed likely that a significant shortening of the operon by loss of a segment of its DNA would result in a shorter interval between the derepressions of the two enzymes controlled by the structural genes located on either side of the deletion. However, if the genetically defined deletion were caused by inversion, rather than by the loss, of a segment of DNA, no alteration in the kinetic pattern of derepression would be expected.

The histidine auxotroph used in our study, *hisH,B22*, is one which displays the simultaneous mode of derepression (Fig. 2) and is a spontaneous, nonpolar, deletion mutant from the collection of Dr. P. E. Hartman. The deletion extends through the *B* gene and into the *H* gene (*5*) (Fig. 3). In our experiments a shift in the mode of derepression in *hisH,B22* from simultaneous to sequential by the addition of ribosyl-AIC was used as a basis for examining the effect of the deletion in the histidine operon on the kinetics of derepression of the histidine enzymes. Figure 4 shows the specific activities of three enzymes, obtained in a typical experiment with *hisH,B22* in the presence of ribosyl-AIC. The interval between the derepression of the *A* and that of the *I* enzyme was approximately 10 minutes whereas the *D* and *A* enzymes, the genes for which are located on either side of the deletion, derepressed essentially at the same time. These results should be compared with those in Fig. 1B, in which the intervals obtained with a point mutant are illustrated; in that case, the intervals between derepressions of the *D* and *A* enzymes and between those of the *A* and *I* enzymes were both approximately 10 minutes.

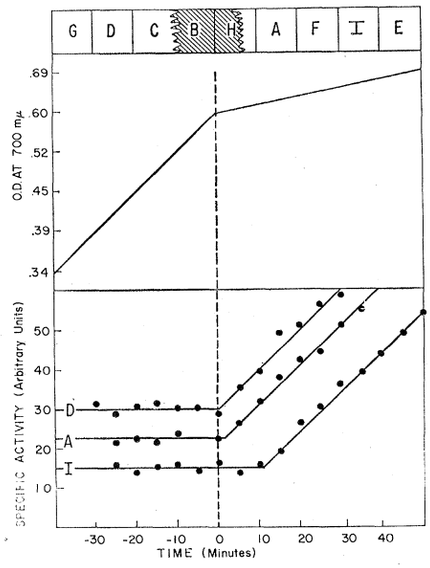

Fig. 4. Sequential mode of derepression observed in the deletion mutant, *hisH, B22*, when ribosyl-AIC was present in the culture medium at a concentration of $3.5 \times 10^{-5} M$. For details of sampling and assay, see legend, Fig. 1.

When the mode of derepression is sequental, kinetic studies on the derepression of the enzymes for histidine biosynthesis may be used to roughly correlate genetic map distances with "temporal distances." There was no interval between the derepression of the *D* and that of the *A* enzyme in the deletion mutant *hisH,B22* in the presence of ribosyl-AIC. This result suggests that genetic material situated between the two corresponding structural genes in the histidine operon is physically absent.

The amount of time eliminated is larger than would be expected simply on the basis of genetically defined map distances; almost the entire interval between the derepressions of the *D* and *A* enzymes was eliminated, whereas less than half of the DNA intervening between the two genes appears to have been deleted. However, it is possible that the rate-limiting step in the utilization of genetic information is the process by which the borders between cistrons are crossed—that is, the termination and initiation of polypeptide chains. In that case a much more drastic reduction in the interval between derepression of genes *D* and *A* of *hisH,B22*, such as that observed in this study, would be expected.

DIANA MARVER*
MARY ANNE BERBEVICH†
ROBERT F. GOLDBERGER
National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

References and Notes

1. M. Nomura and S. Benzer, *J. Mol. Biol.* **3**, 684 (1961).
2. L. A. MacHattie and C. A. Thomas, *Science* **144**, 1142 (1964); E. Burgi, *Proc. Nat. Acad. Sci. U.S.* **49**, 151 (1963).
3. F. Imamoto, N. Morikawa, K. Sato, S. Mashima, T. Nishimura, A. Matsushiro, *J. Mol. Biol.* **13**, 157 (1965); E. K. F. Bautz and E. Reilly, *Science* **151**, 328 (1966).
4. B. N. Ames and P. E. Hartman, *Cold Spring Harbor Symp. Quant. Biol.* **28**, 349 (1963).
5. J. C. Loper, M. Grabnar, C. Stahl, Z. Hartman, P. E. Hartman, *Brookhaven Symp. Biol.* **17**, 15 (1964).
6. B. N. Ames and B. Garry, *Proc. Nat. Acad. Sci. U.S.* **45**, 1453 (1959).
7. D. W. E. Smith and B. N. Ames, *J. Biol. Chem.* **239**, 1848 (1965); B. N. Ames, R. G. Martin, B. Garry, *ibid.* **236**, 2019 (1961); D. W. E. Smith and B. N. Ames, *ibid.* **240**, 3056 (1965).
8. R. F. Goldberger and M. A. Berberich, *Proc. Nat. Acad. Sci. U.S.* **54**, 279 (1965); M. A. Berberich, P. Venetianer, R. F. Goldberger, *J. Biol. Chem.*, in press.

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* Present address: Argonne Cancer Research Hospital, Department of Medicine, Chicago, Ill.
† Postdoctoral fellow of Damon Runyan Memorial Cancer Fund.

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