# EVIDENCE OF COMPLEX LOCI IN SALMONELLA*

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The first step in our program to study spontaneous and induced mutability in individual genes of *Salmonella typhimurium* was the accumulation of more than 250 mutants defective in the synthesis of various amino acids or purines or in the fermentation of galactose. All these mutants arose independently of one another. The first few were found in cultures treated with ultraviolet rays, whereas the great majority were obtained from untreated cultures in which they originated as spontaneous mutants. Our collection now includes about 50 strains deficient for cystine, 35 deficient for histidine, 39 for methionine, 22 for proline, 35 for serine, 10 for tryptophan, 13 for adenine, and 12 for adenine and thiamine, as well as 46 strains that are unable to ferment galactose.

As a second step, we undertook to determine which mutants showing the same phenotype were allelic to one another, using a simple and reliable method based on the work of Zinder and Lederberg[1] on transduction. These workers discovered that phage raised on wild-type bacteria is able to induce changes to wild type in a small fraction (one in $10^5$ to $10^3$) of mutant bacteria infected with it but that phage raised on mutant bacteria cannot transduce another population of the same strain of mutant bacteria. All the evidence we have accumulated so far supports the hypothesis proposed by Zinder and Lederberg that a phage particle is able to transfer genetic specificity—presumably by carrying a fragment of a chromosome from the bacterium on which it was raised (donor bacterium) into the bacterium which it infects (recipient bacterium). During subsequent division of the recipient bacterium, this chromosome fragment is incorporated into a chromosome of its offspring, and thus certain genes of the recipient bacterium are replaced by homologous genes of the donor bacterium. With such a mechanism in operation, it is evident that the results of transduction can be detected only if the donor and the recipient bacteria differ in genetic constitution, and therefore transduction experiments can be used to determine whether or not two similar mutants are genetically identical. For example, transduction between two cystineless strains would not be detectable if the deficiencies they carried were due to modifications at identical sites on their chromosomes, but it would be detectable if these modifications were not identical. Therefore, the method of testing similar bacterial mutants for identity consists in growing phage on each of the mutant strains and then using this phage in transduction experiments to infect each of the strains.

*Grouping among Phenotypically Similar Mutants.*—We began transduction tests with a collection of cystineless strains (*cys-1, cys-2*, etc.). No transduction was observed between *cys-20* and *cys-1, -3, -5, -13, -21*, or- *22*, indicating that the last six are allelic to *cys-20*. Also no transduction was discernible between *cys-3* and *cys-5*, indicating allelism between them. Transduction was evident between all the other members of the group *cys-1, -3, -5, -13, -20, -21*, and *-22* (designated group A); but the number of transductions was considerably smaller than the

number observed either when wild-type phage or phage grown on other *cys* strains was used with bacteria of group A or when other *cys* bacteria were used with group A phage (see M. Demerec[2] [Table 4]). A similar relationship was found among *cys-10, -12, -14, -15, -16, -18,* and *-24* (group B), except that in this group a few transductions were obtained in each combination.

Continuing these analyses of transduction between cystine-requiring mutants of independent origin, we were able to place 42 such mutants in three groups, namely, group A, with 8 members; group B, with 20 members; and group C, with 14 members. Similarly, 23 serine deficiencies fell into two groups, 22 proline into two groups, 10 tryptophan into four groups, 23 methionine into five groups, 34 histidine into four groups, 12 adenine into five groups, and 12 adenine-thiamine into four groups. Such grouping has been observed in every case when this type of analysis has been applied to several mutants independent in origin but similar as to phenotype.

Further studies of these groups have revealed that members of the same group may have in common certain properties that are absent, or present to a different degree, in the members of other groups. For example, the cystine deficiency in all but one of the members of group A could be partially satisfied by methionine, and to a somewhat lesser degree by homocysteine or cystathionine, whereas this was the case with only about 50 per cent of the members of group C and only one out of the 20 members of group B.

A more striking example of correspondence between the grouping arrived at by transduction experiments and grouping in terms of some other characteristic was found in studies of galactose-negative mutants carried on by Z. E. Demerec. Forty-six independently originating mutants could be separated into eight classes on the basis of three criteria: appearance of colonies on EMB-plus-galactose medium; amount of residual growth on synthetic medium with galactose as a carbon source; and degree of instability, as shown by the formation of sectors or papillae on colonies grown on EMB medium and by frequency of reversions to wild type on minimal medium. Only two of these classes (the second and third) were suitable for transduction experiments, because the strains belonging to the other six showed such a high degree of residual growth on our minimal medium that the usual techniques for detecting transduction could not be applied. Tests with the 8 mutants of class 2 showed that all belonged to one transduction group and that none of the 5 mutants of class 3 was in that group.[3]

The best evidence of coincidence between groups based on transduction and groups based on some other property, however, is now being accumulated in biochemical studies, carried on by several members of our laboratory, of blocks in the chains of reaction leading to synthesis of the compounds required by phenotypically similar auxotrophs. An investigation of our 10 tryptophan-requiring mutants, made here by Dr. Sydney Brenner, of the department of physiology, Medical School, University of the Witwatersrand, Johannesburg, South Africa, showed agreement between the grouping of these mutants based on transduction tests (groups A, B, C, and D) and that indicated by biochemical analysis. He found that the 1 mutant in group A failed to synthesize anthranilic acid; the 2 in group B were unable to convert anthranilic acid to an as yet unidentified intermediate, called compound "B"; the 1 group-C mutant failed to convert compound B to indole;

and each of the 6 group-D mutants was blocked in the conversion of indole to tryptophan. Dr. Philip E. Hartman's analysis of the 34 histidine-requiring strains shows that the 9 members of group A do not accumulate imidazoles; the 7 group-B mutants accumulate imidazole glycerol; the 6 group-C mutants accumulate substances tentatively identified as imidazole lactic acid, imidazole acetol, and an unknown imidazole; and 11 of the 12 group-D mutants accumulate histidinol, imidazole acetole, imidazole lactic acid, and small amounts of imidazole glycerol. Thus, so far, there is good agreement, in our tryptophanless and histidineless mutant strains, between transduction grouping and grouping in accordance with nutritional requirement or accumulation of certain substances. Biochemical studies of blocks in cystineless strains, carried on by Z. E. Demerec, have indicated a similar pattern with regard to these mutants also. Tests with several sulfur compounds showed that the cystine requirement can be satisfied in members of group A by $Na_2SO_3$ and in members of group C by $Na_2S_2O_3$ and $Na_2S$ but that group B-deficiencies cannot be satisfied by any of these compounds.

The results just described show that phenotypically similar mutants can be separated by transduction tests into well-defined groups. Between members of the same group, transduction to wild type either does not take place or—in the great majority of instances—is significantly less frequent than it is between these members and mutants belonging to another group, or wild-type bacteria. They also show that among these auxotrophs the grouping based on transduction tests coincides with that obtained by biochemical methods which investigate blocks in the chains of reaction leading to synthesis of the compounds required by the auxotrophs.

*Discussion.*—When we began the first transduction tests to determine allelic relationships among 25 cystine-requiring mutants, we were greatly surprised by the results, which showed that transduction to wild type occurred in all but a very few combinations. It did not seem reasonable to suppose that almost twenty gene loci, each playing a major role in the synthesis of cystine, could exist in *Salmonella*. The key to solution of this puzzle was the observation that *cys-20* produced no transductions with *cys-1*, *-3*, *-5*, *-13*, *-21*, or *-22*, indicating allelism between *cys-20* and these other cystineless mutants, but that transductions to wild type were obtained in all combinations between *cys-1*, *-3*, *-13*, *-21*, and *-22*, suggesting no allelism between these mutants and thus contradicting the previous indication. The same experiments, however, revealed that the numbers of transductions between members of this group were considerably smaller than the numbers obtained when any of them was tested either with wild type or with other cystineless mutants of the collection. Subsequent experiments, made with cystineless and other auxotrophic mutants, demonstrated the general presence of such groups among similar mutants and showed that the same groupings could be established by studies of transduction and by studies of biochemical blocks. These results disclosed a considerably higher degree of similarity between mutants belonging to a certain group than between mutants belonging to different groups, and they also reduced the number of major classes within any one mutant phenotype to a level that could reasonably be accounted for by the assumption that all mutants belonging to the same class are nonidentical alleles of the same gene locus. If this is so, then a gene locus extends over a section of a chromosome; changes occurring in different regions of this sec-

tion give rise to different alleles; and the transduction observed between alleles is due to incorporation into the transduced chromosome of a normal, unchanged region brought by phage from the donor to the recipient bacterium.

It is reasonable to assume that the incorporation of a chromosome fragment in transduction is accomplished during bacterial division, at the time of duplication of the bacterial chromosome. It could be brought about either through direct incorporation of the fragment into the newly formed chromosome (Fig. 1, A) or by the fragment's undergoing duplication simultaneously with the bacterial chromosome, replicas of the fragment being incorporated into the new chromosome by a process similar to the one proposed by Belling[4] in his explanation of the mechanism of crossing-over (Fig. 1, B). In the latter case, transduction would require an even number of crossovers between the chromosomal strand derived from the bacterial chromosome and the strand derived from the fragment brought in by the phage. Our experimental evidence favors the last-mentioned possibility.

For consideration of our present problem, it is not essential to know which of these two mechanisms operates in transduction, and therefore discussion of that phase of the problem will be reserved for another paper. We are working on the assumption that the chromosome fragment brought in by phage may exceed the
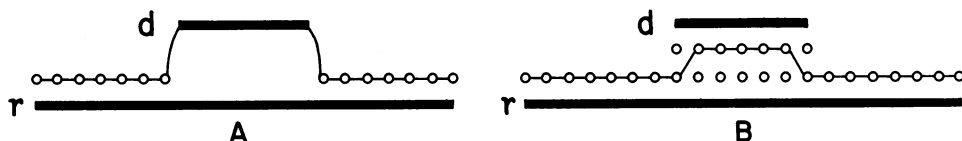


FIG. 1.—Diagram illustrating two possible mechanisms by which a fragment of genetic material brought by phage into a recipient bacterium might be incorporated into the duplicating bacterial chromosome. *A*, direct incorporation; *B*, incorporation by double crossing-over (*r:* chromosome of recipient bacterium; *d:* fragment brought from donor bacterium).

length of a gene locus and that the ends of the incorporated fragment need not correspond with the ends of a locus. As already pointed out, we assume that in the case of two nonidentical alleles the changes (mutations) responsible for the phenotypic difference between wild type and mutants have occurred in different regions of the gene locus. Then transduction to wild type will take place only when the new chromosome derived from chromosomes of the donor and recipient bacteria is composed of segments of the involved locus which do not have mutated regions. It is evident that the frequency with which such combinations arise will be positively correlated with the distance between the sites within a gene locus where changes responsible for alleles have occurred. Thus the frequency of transductions between nonidentical alleles—different members of the same group—might serve as a means of determining the linear order of alleles within a locus and the distances between them. Unfortunately, the efficiency of transduction is affected by several factors that we are not yet able to control, so that quantitative data obtained in different experiments are not always comparable and consequently the values for recombination between nonidentical alleles give only an approximate indication of the distances between these alleles and their order of arrangement within a locus. For example, the data given in Table 1 show that the number of transductions among *cys-14*, *-16*, *-18*, and *-24* is considerably smaller than between any of these

and *cys-10, -12,* or *-15,* indicating that *cys-14, -16, -18,* and *-24* are located close together and some distance from *cys-10, -12,* and *-15.*

Recently we have found linkage between several loci, which makes it possible to use three or four easily distinguishable markers in individual experiments and opens up a more reliable way of determining the order of alleles at these loci and the distances between them.

Among the 28 cystineless mutants of groups A and B, we have found only 6 cases in which transductions to wild type did not occur between certain members of a group: there were none between *cys-20* and any of the other 7 members of the *cysA* group or between *cys-3* and *cys-5* or between *cys-2, -6, -30,* and *-39.* Similarly, Hartman, in analyzing the 34 histidineless mutants, found 9 cases in which transduction was not observed. T. Yura, analyzing 12 adenineless mutants, found that 2 members of the *adA* group and 2 out of 8 members of the *adB* group gave no transductions with each other; and in an analysis of 12 strains requiring adenine plus thiamine he found that 3 of the 8 members of group A and both mem-

TABLE 1

NUMBER OF TRANSDUCTIONS OBSERVED IN EXPERIMENTS IN WHICH ABOUT $3 \times 10^8$ BACTERIA AND $2.3 \times 10^9$ PHAGE PARTICLES WERE USED IN EACH COMBINATION

| | | | | PHAGE RAISED ON | | | | | |
| BACTERIA | cys-10 | cys-12 | cys-14 | cys-15 | cys-16 | cys-18 | cys-24 | Wild | cys-11 |
|---|---|---|---|---|---|---|---|---|---|
| cys-10 | 0 | 47 | 42 | 65 | 26 | 29 | 29 | 1,115 | 1,157 |
| cys-12 | 28 | 0 | 43 | 83 | 33 | 36 | 25 | 2,104 | 2,351 |
| cys-14 | 29 | 38 | 0 | 33 | 2 | 4 | 6 | 1,792 | 2,220 |
| cys-15 | 76 | 95 | 107 | 0 | 54 | 88 | 61 | 954 | 1,019 |
| cys-16 | 15 | 19 | 2 | 34 | 0 | 2 | 3 | 979 | 1,223 |
| cys-18 | 5 | 20 | 1 | 23 | 3 | 0 | 3 | 771 | 856 |
| cys-24 | 25 | 47 | 14 | 84 | 10 | 8 | 0 | 1,617 | 528 |
| cys-23 | 1,879 | 791 | 2,296 | 1,399 | 951 | 860 | 1,616 | 1,592 | 742 |

bers of group D failed to show transductions with each other. With regard to the 10 tryptophanless strains, we found that transduction was not observed between 2 of the 6 members of group *tryD.* In the great majority of cases, however, mutants belonging to any particular group produce wild-type transductions with the other members of that group, showing that they are genetically different. This suggests the existence of many sites within a gene locus where changes responsible for the appearance of nonidentical alleles may occur. In cases in which a mutant fails to produce transductions with several nonidentical alleles of the same locus, we may be dealing with some chromosomal aberration like a deficiency or an inversion rather than with a more common change involving a single site.

*Summary.*—By the use of transduction tests, phenotypically similar auxotrophic mutants of *Salmonella* can be separated into well-defined groups, and this grouping corresponds with the grouping arrived at by studies of their biochemical blocks. It is assumed that each such group represents a gene locus and that different members of a group are "nonidentical alleles (pseudo-alleles)," which have originated through mutations occurring at different sites in a gene locus.

[1] N. D. Zinder and J. Lederberg, "Genetic Exchange in Salmonella," *J. Bacteriol.,* **64,** 679–699, 1952.

[2] M. Demerec, "What Is a Gene?—Twenty Years Later," *Am. Naturalist,* **89,** 5–20, 1955.

[3] M. Demerec, H. Moser, J. Hemmerly, I. Blomstrand, Z. E. Demerec, P. L. FitzGerald, S. W. Glover, J. F. Hanson, F. J. Nielsen, and T. Yura, "Bacterial Genetics—I," *Carnegie Inst. Wash. Year Book*, **53**, 225–241, 1954.

[4] J. Belling, "Crossing Over and Gene Rearrangement in Flowering Plan ts," *Genetics*, **18**, 388–413, 1933.

# GENETICALLY CONDITIONED METABOLIC ANTAGONISM AND SUPPRESSOR ACTION IN NEUROSPORA*

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A mutant strain of *Neurospora crassa*, T77, which grows at a rate equivalent to that of the wild-type strains at 35° C. but is markedly inhibited in growth by the amino acid *l*-threonine has been previously described.[1, 2]  By inhibition analysis it was demonstrated that the threonine inhibition of this strain could be explained as a competitive antagonism of threonine to homocysteine metabolism which results in the interruption of the biosynthesis of methionine, the thiazole portion of thiamine, and, at higher threonine concentrations, adenine and serine.[2]  Wagner and Bergquist[3] have shown that this strain accumulates $\alpha$-ketoisovaleric, $\alpha$-keto-$\beta$-methylvaleric, and pyruvic acid in its growth medium when growing in the presence of threonine, but not in its absence.  The accumulation of these keto acids is accompanied by a rapid decrease in the threonine concentration during a period of time in which there is only a small increase in the weight of the mycelium.  Wild-type strains do not accumulate keto acids, except for a very small amount of pyruvate when growing in the presence of threonine.  The data suggest that the inhibitory effect of threonine on homocysteine metabolism in this strain is in some way related to the rapid disappearance of threonine and the consequent accumulation of keto acids which can be presumed to be in part, at least, products of threonine metabolism,[3] though the biochemical mechanism involved cannot be resolved on the basis of present evidence.

The early analysis of this strain led to the suggestion[1] that the inability of an organism to mediate a chemical synthesis because of a genetic block may not necessarily be the result in every case of the absence or inactivation of an enzyme required for that synthesis but may be caused by some sort of inhibition at the metabolic level, resulting from the mutation.  The studies described here were undertaken with the objective of testing this hypothesis by the use of strain T77.  If the aberrant metabolic mechanism responsible for the complex nutritional requirements of T77 in the presence of threonine could be shown to be operative in the absence of threonine under altered environmental conditions, thus causing a requirement for exogenous sources of metabolites normally synthesized by the wild-type strain under similar environmental conditions, then this hypothesis would receive some support.  The results described below demonstrate that T77 requires either the thiazole portion of thiamine or both homocysteine and threonine for growth at lower temperatures of incubation, indicating the operation of the inhibitory mechanism in growth failures at lower temperatures.