Bipolarity of Information Transfer from the Salmonella typhimurium Chromosome

Abstract. *Apparently the leucine and tryptophan gene clusters are polarized in opposite directions on the* Salmonella *chromosome. Either the direction of polarity is independently determined for each operon or the bacterial chromosome may consist of more than one multi-operon unit of DNA, each such unit determining the direction of polarity of its constituent genes. Each hypothesis results in a different prediction concerning the distribution of operon polarity directions on the chromosome and the functional status of chromosomal inversions.*

There is evidence that the transfer of information carried by a gene or an operon is polarized. This implies that information transfer is initiated at a specific end of the operon and progresses along the linear array of nucleotides to the other end. Such polarity apparently exists for both transcription of the information into messenger RNA and translation into polypeptides. The polarity is most readily detectable when a cluster of genes functions as a single operon, and therefore mutations which occur at only one end of the cluster affect the functioning of physically intact genes toward the other end. Evidence for polarity forms a basis for the models of regulation of protein synthesis such as the operon hypothesis of Jacob and Monod (*1*) as well as the modulator hypothesis of Ames and Hartman (*2*). Furthermore, the concept of polarity is implicit in the notion of the requirement of a reading frame alignment for the effective transfer of information from a single cistron (*3*).

The direction of polarity is known for only a very few operons on any one bacterial chromosome. There is now evidence that in strain LT2 of *Salmonella typhimurium* the polarity of the tryptophan genes is clockwise, whereas that of the leucine operon is counterclockwise. To demonstrate these opposite polarities both the direction of polarity within each operon and the orientation of each operon within the chromosome must be determined.

The direction of polarity of the leucine operon was determined by identification of the operator end with respect to a closely linked arabinose marker (*4*). There is a point mutation with strong polarity effects at the end most distant from the arabinose genes. The orientation of the leucine-arabinose region with respect to other genes on the chromosome has been more difficult to demonstate. However, Calvo (*5*) has obtained data from Hfr crosses which are consistent with the orientation shown in Fig. 1. Furthermore, the order of genes in the *Escherichia coli* and *S. typhimurium* chromosomes are identical in most respects (*6*), and Gross and Engelsberg (*7*) have shown in *E. coli* the orientation of the leucine-arabinose region with respect to a threonine marker (Fig. 1). Therefore, the leucine operon is polarized in a counterclockwise direction as the chromosome is usually drawn (Fig. 1).

The orientation of the tryptophan cluster within the chromosome appears well established. Stocker, Smith, and Subbaiah (*8*) have interpreted their recombination data from colicin matings of *S. typhimurium* as indicating that the orientation of the tryptophan-cysteine B (*try-cysB*) region of the chromosome is that shown in Fig. 1. I have examined Sanderson's (*9*) data on interrupted Hfr matings, and these data unequivocally confirm the interpretation of Stocker *et al.*

The determination of the direction of polarity of the tryptophan genes resulted from the selection of apparent deletion mutations originating outside the cluster, near the *cysB* region of the chromosome (*10*). The degree to which these extended into the tryptophan gene cluster was ascertained from the production of try^+ recombinants in transduction-mediated crosses with 5 *tryA*, 16 *tryB*, 12 *tryD*, and 4 *tryC* point mutant strains. All of the deletions appeared to terminate at or near the ends of genes, since similar results were obtained with all point mutations belonging to one class. That is, any one deletion produced try^+ recombinants from crosses either with all of the point mutants of one class or with none of them.

Table 1. The nutritional phenotypes of five different deletion mutant strains and their capacity to produce try^+ recombinants from crosses with the four classes of *try* point mutant strains. None of these deletion mutants can utilize anthranilic acid for growth. *I*, indole; *T*, tryptophan; try^+ recombinants produced; 0, no try^+ recombinants produced.

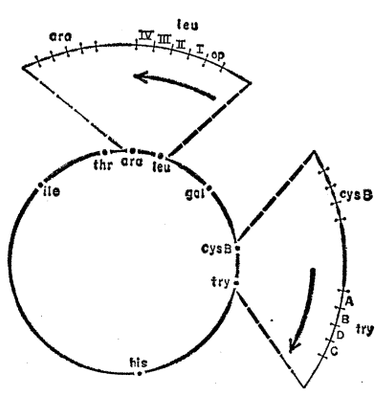
Deletion mutants	Classes of *try* point mutants			
	tryA	*tryB*	*tryD*	*tryC*
Phenotype: grows on I or T				
8T1	+	+	+	+
5T1	0	+	+	+
4CT1*	0	+	+	+
Phenotype: grows on T only				
3T1	0	0	+	+
6T1	0	0	0	+

* This deletion mutant strain had a cysteine auxotrophy as well as a tryptophan auxotrophy.

There is a disparity between the extents of the physical deletions (indicated by the recombination data) and the effects on gene function (indicated by the nutritional phenotypes) (Table 1). Demerec and Hartman (*11*) had noted that mutations leading to nonfunction of *tryA* produced an auxotrophy which could be satisfied by anthranilic acid, indole, or tryptophan; failure of the *tryB* or *tryC* genes to function caused a nutritional requirement which could be satisfied by indole or tryptophan; *tryD* mutants could be satisfied only by tryptophan (*12*). Table 1 shows that the deletion mutations 8T1, 5T1 and 4CT1, which do not physically affect the *tryB* region, cause a phenotype which would result from *tryB* nonfunction. Similarly, mutation 3T1, which does not appear to physically delete *tryD*, produces a nutritional phenotype resulting from *tryD* inactivity. These polarity effects are diagrammed in Fig. 2.

Evidence of the physical integrity of the genes whose failure to function is here ascribed to polarity effects was obtained by selection for secondary mutations that restored functioning. Deletion strain 8T1 gave rise to prototrophic secondary mutants as an apparent result of a return to function of *tryA* and *tryB*. Strains 5T1 and 4CT1 produced secondary mutants which could utilize anthranilic acid as a result of the renewed activity of *tryB*. Similarly, from strain 3T1 secondary mutants could be selected which utilized indole for growth, an indication that *tryD* was again functioning. In each case the inactive genes which were considered physically intact on the basis of recombination data proved capable of renewed function. In no case was it possible to select secondary mutations that restored functioning of genes which the recombination data had indicated were deleted. Regardless of the physical basis of the inactivity of the intact gene, their existence gives evidence of the polarity.

The recombination data from the crosses with deletion 6T1 confirm the revised order of the *tryD* and *tryC*



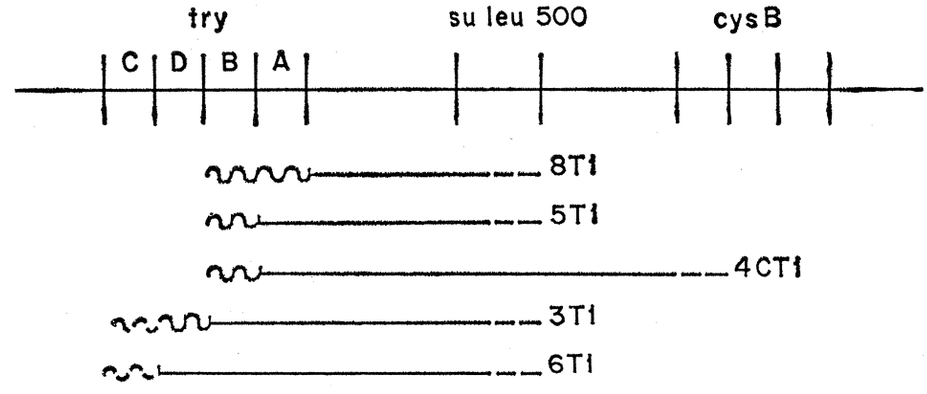


Fig. 1 (left). A map of the *Salmonella typhimurium* chromosome showing details of the organization of the leucine and tryptophan gene clusters and the relative positions of other marker genes that provide orientation. The arrows indicate the polarities of the leucine and tryptophan gene clusters. Gene symbols: *ara*, arabinose; *cysB*, cysteine B; *gal*, galactose; *his*, histidine; *ile*, isoleucine; *leu*, leucine; *thr*, threonine; *try*, tryptophan. Fig. 2 (right). The organization of the *cysB-try* portion of the *Salmonella typhimurium* chromosome. The extents of the five deletion mutations are shown below the chromosome segment. The solid straight lines represent the extents of the physical deletions as determined by recombination tests. The solid wavy lines indicate the extents of the polarity effects upon function as determined by nutritional tests. The broken lines indicate that the tests provided no information about polarity effects or deletion limits or both with regard to the portions of the chromosome which they underlie.

genes, with respect to *tryA* and *tryB*, which Balbinder (*13*) found by using three-point transduction tests. The polarity effects demonstrated by the nutritional phenotypes have been interpreted as a possible indication of more than one operon (*14*). Furthermore, the polarities of the tryptophan genes are clockwise as the chromosome is usually drawn (Fig. 1). This interpretation is consistent with the results obtained on *E. coli* by Yanofsky and Lennox (*15*), who reported no evidence of polarity effects resulting from deletions originating on the other side of the *try* gene cluster. Matsushiro *et al.* (*16*) find evidence for the same direction of polarity within the tryptophan gene cluster in *E. coli* as that described here for *S. typhimurium*. There remains the possibility that the orientation of the *cysB-try* region of the *E. coli* chromosome is the reverse of that in *S. typhimurium*.

The difference in direction of polarities of the leucine and tryptophan genes suggests that the strands of DNA to which messenger RNA is complementary is not the same for all of the *Salmonella* chromosome. This interpretation is based on the assumptions that the polarity described here reflects mechanisms which function in transcription and that the polarity of transcription determines which of the DNA strands is copied. Polarity effects could also result from mutant alterations affecting the process of translation from messenger RNA into polypeptide chains, if polycistronic messenger normally occurs (*2, 17, 18*). Evidence for mechanisms which control translation, and which when altered by mutation might result in polarity effects, has been presented by Ohtaka and Spiegelman (*19*). Within one operon all mutations that produce polarity effects have always displayed the same direction of polarity, even when some were considered to result from effects at translation and others from effects at transcription (*2, 20*). Thus the directions of polarity described for the leucine and tryptophan genes would presumably be valid for transcription polarity, even though the mutations which revealed them were, in fact, affecting translation mechanisms.

The prediction that, in the *S. typhimurium* chromosome, both strands of DNA will contain regions which are complementary to some messenger RNA sharply contrasts with the evidence, described by Spiegelman and Hayashi (*18*), that all the messenger RNA of bacteriophage φX174 is complementary to a single DNA strand. Similar experiments with other bacteriophages (*21*) lead to the same conclusion reached with φX174.

The evidence from the *S. typhimurium* chromosome alone suggests that the direction of polarity of each operon may be an individual affair. In other words, the operator end of each operon initiates the progressive transfer of information in the proper direction along the associated structural genes. In this case the direction of polarity of each operon would show no special relation to that of operons close to it. If an inversion of a complete operon occurred, the operon would remain functional. This hypothesis does not explain the lack of agreement with the evidence from studies of bacteriophage. One must further assume that either some special mechanism acts to restrict the direction of operon polarities on the phage chromosome or that all the genes on the chromosomes of the phages studied were polarized in the same direction by chance.

Another, simpler hypothesis reconciling the data from the studies with phage and bacteria is that there exists a genetic unit (such as a DNA molecule) larger than an operon but not necessarily as large as a chromosome and that all genes within such a unit are polarized in the same direction. The phage genome is relatively small compared to that of *S. typhimurium*. Therefore, the phage chromosome may consist of a single such unit or molecule whereas that of *S. typhimurium* may be made up of two or more molecules. If this is the case the tryptophan genes would be located on a different molecule from that of the leucine operon and as the directions of polarity of more operons are determined there should become evident a clustering of operons exhibiting the same direction of polarity. In addition, inversion of a complete operon should result in nonfunctioning of the inverted genes.

On the basis of either hypothesis the inversion of a complete cistron within an operon would probably result in physiological nonfunctioning of the cistron. This had been suggested by Hayashi, Hayashi, and Spiegelman (*22*)

as a result of their earlier studies demonstrating restriction of genetic transcription in vivo to one of the complementary DNA strands of bacteriophage φX174. There is evidence which is consistent with this prediction. A leucine auxotroph, *leu 39*, appears to result from inversion of a large portion of the leucine operon containing at least one complete cistron (leu III) which by complementation tests is physiologically nonfunctional (*4*). This suggests that whatever serves in the genetic code as a punctuation mark to designate the end of one cistron and the beginning of the next cannot correct for a reversed polarity.

Either of the foregoing hypotheses concerning the mechanism by which polarity of information transfer is determined can explain the conclusion (*23*) that in *Bacillus subtilis* the genetic information for the enzymes concerned with histidine synthesis is carried on the strand of the DNA molecule opposite to that carrying the information for the tryptophan-synthesizing enzymes. Technical difficulties make this conclusion somewhat uncertain. However, if correct, the work (*23*) indicates that (i) the histidine and tryptophan genes are on two different units of DNA each having opposite polarities within the *B. subtilis* chromosome (second hypothesis) or (ii) the histidine and tryptophan genes are merely located within operons having opposite polarities (first hypothesis).

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Staphylolytic Substance from a Species of Pseudomonas

Abstract. *A substance that rapidly lyses living cells of* Staphylococcus aureus, S. roseus, Gaffkya tetragena, *and* Sarcina lutea *has been partially purified. The substance is produced extracellularly by a species of* Pseudomonas *which was isolated from soil by an enrichment procedure.*

Certain investigations into the physiology and genetics of *Staphylococcus aureus* have been restricted by the lack of a lytic method for isolating intracellular macromolecular components. Recently, enzymes which lyse *S. aureus* and several other Gram-positive bacteria have been isolated (*1*). We have now isolated another staphylolytic substance which differs from the enzymes described earlier in origin, spectrum of activity, and optimum *p*H.

A microorganism which produces a staphylolytic substance was isolated by means of the following procedure. A sample of soil was added to a sterile enrichment medium consisting of basal salts and lyophilized cells of *S. aureus* strain U9 (*2*) in distilled water. After the mixture had been incubated at room temperature for 7 days, a sample was inoculated by means of a wire loop onto plates of nutrient agar rendered turbid by the addition of lyophilized cells of *S. aureus* strain U9 at the time the plates were poured. These plates were incubated for 24 hours, and after this incubation clear zones were present around several colonies of a bacterium that produced a green pigment. When one of these colonies was subcultured into brain-heart infusion broth, a highly active staphylolytic substance was produced. The bacterium has been identified as a member of the genus *Pseudomonas* because it is oxidative, it has polar flagella, it is a Gram-negative rod, and it produces a green, water-soluble pigment.

The substance produced by the *Pseudomonas* sp. shows, toward cells of *S. aureus*, lytic activity which decreases as the salt concentration increases; in a phosphate buffer at concentrations above 0.07*M*, no lytic activity could be detected. When the salts were removed by dialysis, the activity was fully restored. A similar relation between salt concentration and lytic activity was observed by Hash in his study of the *Chalaropsis* B enzyme (*1*). The maximum lytic activity of the substance produced by the *Pseudomonas* sp. was obtained at a *p*H of 8.5 in phosphate and acetate buffers of low ionic strength.

All assays of the staphylolytic substance were performed at 25°C in 0.01*M* ammonium acetate adjusted to *p*H 8.5 with NH_4OH. A sufficient number of lyophilized cells of *S. aureus* was suspended in the buffer to obtain an optical density reading of 0.50 at a wavelength of 535 mμ. After addition of a sample of the staphylolytic substance to this suspension, changes in optical density were recorded at 1-minute intervals. Low concentrations of the substance produced nearly linear results over 5-minute periods (Fig. 1). At these low concentrations and at time periods of 5 minutes or less, one unit of the staphylolytic substance was defined as the amount of the substance required to reduce the optical density of a suspension of lyophilized cells of *S. aureus* strain U9 by 0.001 optical-density units per minute. Different cultures varied slightly in sensitivity when viable cells of *S. aureus* were tested as substrate in lysis experiments. Reproducible results were obtained with lyophilized cells of *S. aureus* strain U9, and therefore these were used in the standard assay of the substance.

The staphylolytic substance is colorless and precipitable with either acetone or ammonium sulfate. The activity of the substance is completely destroyed by incubation for 30 minutes at 56°C, but dialysis for 24 hours against 0.01*M* ammonium acetate results in no loss of activity. These re-