Hybridization experiments involving *E. coli* & S. *typhimurium*

D.W. Stetter

ABSTRACT

Integration of an Escherichia coli tryptophan operator into a Salmonella typhimurium tryptophan operon

In hybridization experiments involving E. coli males and S. typhimurium females results are sometimes inconclusive due to ambiguities concerning integration of the male genes into the female chromosome. The formation of partial heterozygotes is common; furthermore, if recombination does occur, it may take place at a novel location on the chromosome. The focus of this study was the production of a hybrid tryptophan operon by preparing a cross between E. coli and S. typhimurium which would indicate by the utilization of proper genetic markers precisely if and where recombination had occurred in the female chromosome. In the cross an E. coli chromosomal segment containing a tryptophan operator-promotor and an anthranilate synthetase gene, the first structural gene of the tryptophan operon, integrated adjacent to the four operator-distal structural genes of a S. typhimurium tryptophan operon. The strains containing the hybrid operons were analyzed by transduction and enzyme assay. The transduction test confirmed the existence of a hybrid operon; the enzyme assay measured the ability of the E. coli operator-promotor to derepress the S. typhimurium structural genes.

Integration of an *Escherichia coli* Tryptophan Operator into a *Salmonella typhimurium* Tryptophan Operon

by

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I. Introduction

A. Hybridization in Enterobacteriaceae

This investigation focusses on an aspect of hybridization between two species of enteric bacteria, Escherichia coli and Salmonella typhimurium, the genetically best known bacterial species (Taylor 1970; Sanderson 1970). The remarkable similarity of the genetic maps of these species (Sanderson 1971) may facilitate the formation of viable interspecific hybrid recombinants.

Genetic recombination in matings between mutant strains of E. coli was discovered by Tatum and Lederberg (1947). Rapidly after this discovery the fertility system involving an episomal DNA structure termed a fertility factor or sex factor (F factor) was proposed as the basis for the fertility of such conjugation crosses (Hayes 1952). A bacterium containing the F factor may be a contributor of chromosomal genes in bacterial mating if the F factor contains bacterial genes as a consequence of recombination with the donor's chromosome (F-prime or F') or if the F factor has integrated into the chromosome and has mobilized the entire donor chromosome at the time of mating (high frequency of recombination or Hfr). There is a unilateral contribution of genetic material from the donor (Hfr or F') to the cell receiving genetic material (F-minus or F-).

Luria and Burrous (1957) reported recombination between E. coli and many Shigella species which acted as F-minus strains. Attempted crosses between E. coli and Salmonella were unsuccessful until Baron et al. (1958a) detected recombination at low frequency between E. coli and S. typhimurium strain TH-9. Subsequently, a streptomycin-resistant mutant of TH-9 was isolated prior to mating, and this strain (TH-9 Sr-2) acted as a high frequency recipient strain

in matings with E. coli. Two more S. typhimurium strains (LT-7 and HB) were subsequently found to be fertile. No recombinants were obtained from approximately 70 other species or strains of Salmonella with the same or similar serotypes. It was therefore concluded that populations of Salmonella cells generally are unable to act as recipients. Occasionally some cells in the generally infertile population mutate to a fertile recipient state. If a hybridization experiment were performed and a hybrid could be isolated using selective markers, it would be F-minus and capable of being mated again as a recipient strain to yield hybrids at a high frequency. This was demonstrated in a conjugation cross between an E. coli Hfr met strain and a S. typhimurium met^+ lac ara strain at a ratio of 1:20. Hybrid recombinants were isolated which were lac^+ met^+ ara. A second conjugation between the E. coli Hfr and the ara hybrids resulted in a high yield of ara^+ hybrids (Baron et al. 1958b).

With the deveolpment of protocols for hybridization between species of enteric bacteria, it was possible to look at homologies of the genetic material in these related organisms. For studying the macrohomology with respect to chromosomes or segments of chromosomes, recombination itself was available. For studying the microhomologies, fine structure genetic analysis by means of transduction was available (Zinder and Lederberg 1952). Zinder (1960) found that the genes for the receptors of bacteriophage T-4, resistance to sodium azide, fermentation of lactose and arabinose, and synthesis of leucine were in the same map order in both species which indicated that the gross features of the two genetic maps were the same. When transducing phage P22 was grown on an E. coli - S. typhimurium hybrid, the transduction efficiency of alleles in the hybrid region was generally low which suggested that evolutionary diversity had occurred within individual genes. Characteristic differences in the

transduction efficiency of the various genes do exist, some genes being more easily transduced than others.

Another interesting observation was the isolation by Baron et al. (1960) of diploid heterozygous hybrids from matings between E. coli and Salmonella typhosa. The strains employed were an E. coli Hfr met and various S. typhosa strains with a variety of sugars markers. Hybrids were selected which possessed a gene from the male parent for the utilization of the sugar. When positive hybrids clones were restreaked on nutrient agar, two colonial forms were observed: a small, unstable, dense colony which gave rise to translucent sectors and a large, stable, translucent colonial form. Upon restreaking single dense colonies which were not yet sectored, it was determined that the translucent colonial form actually was segragated from the clones. The translucent forms were always negative for the sugar on which the particular hybrid was originally selected, whereas the small dense colonies observed on nutrient agar retained their fermentative capabilities.

The dense colonies cross-reacted with the antiserum prepared against E. coli K-12, whereas the translucent colonies did not. Both dense and translucent forms were agglutinated by S. typhosa antiserum. It was concluded the dense colonies were unstable diploid heterozygotes which frequently reverted to the translucent colonial form. A relatively stable and presumably diploid colony, however, was later. Unlike the highly unstable dense colonies which could not be retained for more than a few transfers, a fairly uniform appearance of this hybrid could be kept by the careful selection and restreaking of typical colonies. Occasionally a met colony would be isolated which indicated integration of the male chromosomal segment into the female chromosome to result in haploidization (Falkow et al. 1962). There was also a continual low level of reversion in the

diploid to S. typhosa parental form. Similar phenomena in matings between E. coli and S. typhimurium will be described later in Chapter II of this report.

Demerec and Ohta (1964) observed that the presence of E. coli genetic material increases the frequency of integration of nearby S. typhimurium genes on a transducing fragment in an E. coli recipient. Demerec and Ino (1968) extended this observation and also pointed out a reciprocal effect. That is, the presence of E. coli genetic material decreases the frequency of integration of nearby S. typhimurium genes on a transducing fragment in a S. typhimurium recipient. In the latter cases, it was impossible to assign the precise origin, E. coli or S. typhimurium, of the genetic material near the juncture of the contributions of the two species. Other methods, such as analysis of protein primary structure, would be necessary for such assignment.

The depression of transduction frequencies of S. typhimurium material in such composite transducing fragments occurred in the analysis of E. coli - S. typhimurium hybrids of the argB-cysC region (Demerec and Ohta 1964). This may have been due to the differing levels of homology studied in the two chromosomal regions, one at the level of nucleotide sequences within genes or operons, the other for the gross ordering of genes on the map. In both E. coli and S. typhimurium the gene order appears to be trp-cysB-pyrF; however, this group of genes is inverted between the two species (Sanderson and Hall 1970). The operator-distal end of the trp operon is oriented toward cysB in both species, and the ordering of the five genes within the trp cluster is the same in the two species. It was therefore concluded that, even if the two species have considerable homology at the genetic fine-structure level, this homology might be masked by chromosomal rearrangement (Balbinder 1962).

Incorporation of a small portion of the E. coli pyrF locus into the

S. typhimurium chromosome resulted in a partial requirement for uracil. Substitution of the entire pyrF locus of E. coli for that of S. typhimurium allowed formation of phenotypically prototrophic bacteria. The factor controlling a partial uracil requirement is located in the pyrF gene and is not due to an extragenic suppressor. The pyrF gene in S. typhimurium appears by complementation tests to be a single cistron, the structural gene for the decarboxylase involved in the conversion of orotidine-5'-phosphate to uridine-5'-phosphate (Yan and Demerec 1965). These pyrF hybrids point out how an evolutionary system of single-gene isolation may be initiated, setting the stage for rapid divergence in the nucleotide sequences of that gene.

Another approach to the analysis of enterobacterial hybrids is electrophoretic analysis of hybrid gene product. Atkins and Armstrong (1969) first demonstrated this approach using the product of the ilvC gene in S. typhimurium - S. montevideo hybrids. The gene product, reductoisomerase, has different electrophoretic mobility on starch gel electrophoresis in these two species of Salmonella. From an available collection of hybrids selected for the ilvC locus, 31 hybrids were analyzed to determine the electrophoretic mobility of the reductoisomerase of each strain. The observed mobility of the reductoisomerase of each strain agreed with the species origin that had been assigned to its ilvC locus by previous transductional analysis.

An enzyme assay approach was utilized in the analysis of the hybrids in this present study which concerned genetic recombination in the tryptophan operon region of the chromosome.

B. The Tryptophan Operon and Biosynthetic Pathway

The biosynthesis of tryptophan in enteric bacteria, which consists of five steps and requires at least five enzymes, begins with chorismic acid which contains a benzene ring and is an intermediate common to the synthesis of many aromatic amino acids (Figure 1). Chorismic acid is converted to anthranilic acid (p-amino benzoate) by anthranilate synthetase (AS). Anthranilic acid is used as a phenyl base from which the indole portion of the tryptophan molecule is synthesized. There is feedback inhibition of anthranilate synthetase by tryptophan, the end product of this metabolic pathway. Anthranilic acid is converted to anthranilate-N-riboside-5-phosphate with the addition of phosphoribose by phosphoribosyl transferase (PRT). Actually the phosphoribose is transferred from riboside-triphosphate; the products of the reaction are phosphoribose and pyrophosphate. After attachment of the ribose compound, the ribose ring is split open between carbons 2 and 3 with the addition of hydrogens by the enzyme phosphoribosyl isomerase (PRAI). The gene which produces PRAI does not map within the tryptophan operon (Chakrabarty 1968). PRAI has only been isolated from Pseudomonas putida. The next step produces the indole ring which is formed by the reaction of the carboxyl group on the phenyl portion with the hydroxyl group in carbon 2 of the ribose portion. This reaction is mediated by the enzyme indoleglycerol phosphate synthetase (InGPS) resulting in the formation of indoleglycerol phosphate and carbon dioxide.

The synthesis of tryptophan from indoleglycerol phosphate is catalyzed by an enzyme consisting of two sub-units: tryptophan synthetase A (TS-A) detaches

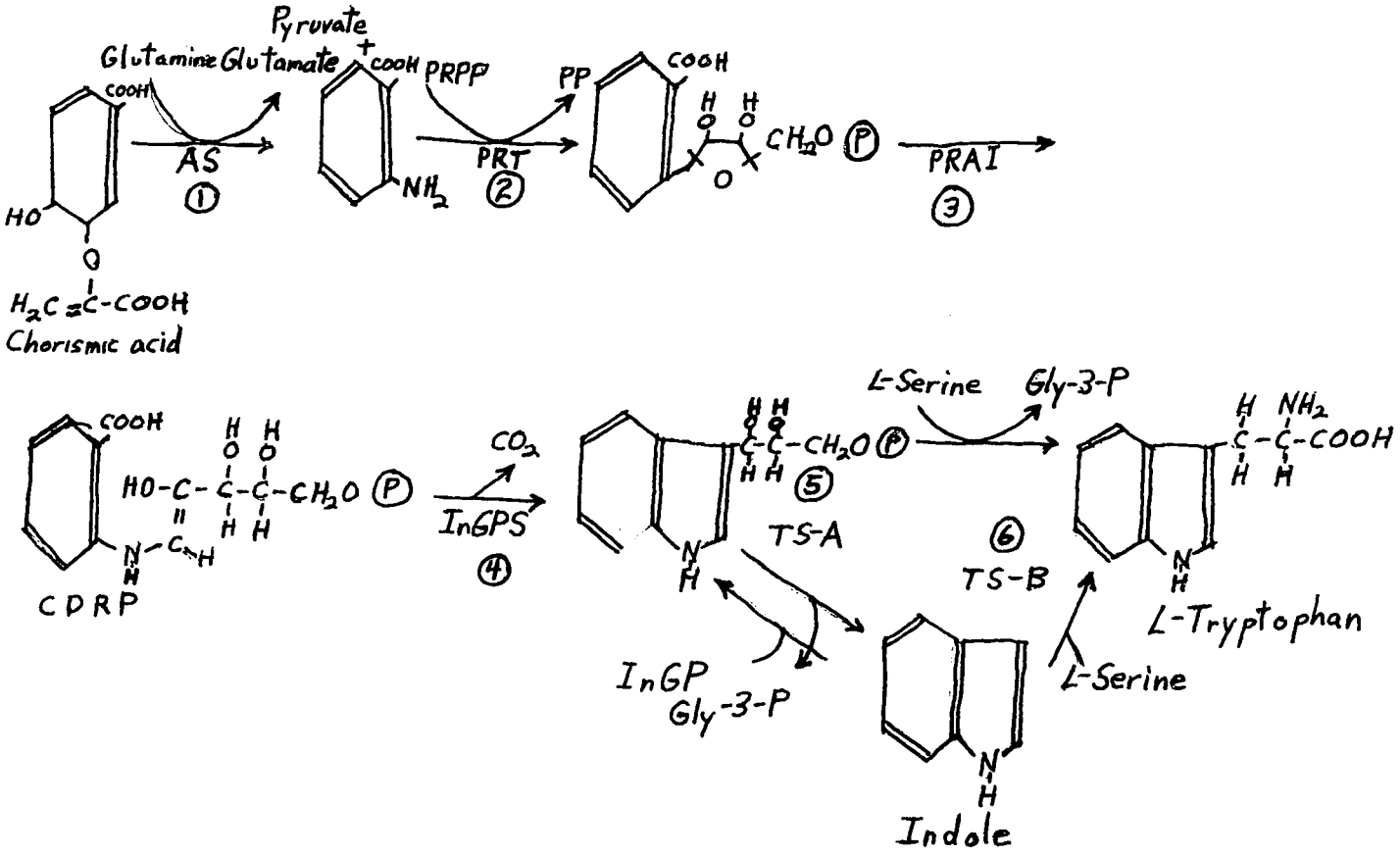

Figure 1. Tryptophan biosynthetic pathway (after Chakrabarty 1968)

glycerol-3-phosphate from indole glycerol phosphate leaving a pure indole structure. Tryptophan synthetase B (TS-B) mediates the reaction of the indole with L-serine. The hydroxyl group on the serine residue combines with a hydrogen on the indole forming a water molecule and a union between the serine residue and the indole creating L-tryptophan. It is important to note that subunits A and B combine.

Separated tryptophan synthetase A protein has been shown to be a single polypeptide chain (Carlton and Yanofsky 1962) termed the α subunit. Separated tryptophan synthetase B protein is believed to be a dimer of identical polypeptide chains for the following reasons: (a) it yields two C-terminal leucine residues per mole of protein, (b) there are forty-five peptides in the peptide pattern of tryptic digests and eighty-one lysine and arginine residues per mole, and (c) it binds two moles of the cofactor pyridoxal phosphate per mole of protein. The B subunit is therefore termed β_2 (Wilson and Crawford 1964).

Combination of the α and β subunits results in a striking enhancement (30 to 100 fold) of the rates of the partial reactions of the individual subunits. This effect is not dependent on the enzymic activity of the complementary chain as shown by the properties of α and β_2 subunits from certain mutant strains (Yanofsky and Crawford 1964). These mutant proteins are themselves enzymatically inactive but nevertheless retain the capacity to

combine with the complementary subunit and to enhance its catalytic activity.

The α : β combining ratio of 0.64 agrees fairly well with the ratio of molecular weights $\alpha : \beta$ = 29,500 : 49,500 = 0.60, as expected for a 1:1 complex. The molecular weight for the complete complex, 159,000, agrees very well with that expected for two α (59,000) plus two β (99,000) chains. The determination of the molar combining ratio of α and β polypeptide chains can be verified by measurements of enzymatic activity using either of the partial or over-all reactions. For example, the indole to tryptophan reaction, which is catalyzed by the β_2 subunit, is much faster in the presence of the α subunit only when the same amount of α is combined with β_2 into $\alpha_2 \beta_2$. Thus one can use the indole to tryptophan reaction to measure the activity of either the α or the β_2 subunits since the complex containing both subunits (in definite proportions) is much more active than β_2 alone. The amount of each subunit in a mixture is determined by measuring enzymatic activity in the presence of an added excess of the other subunit. Since the same active complex is being measured in either assay, the units of α and β_2 subunit activity are equivalent in the fully associated complex (Goldberg et al. 1966).

It is interesting to compare E. coli tryptophan synthetase with the Neurospora enzyme. The latter enzyme has also been found to have four polypeptide chains, two each of two types, and it has a molecular weight of 135,000 to 150,000. The subunit structure and sizes of these two tryptophan synthetases support the view that they have a common evolutionary origin (Carsiotis et al. 1965).

The altered tryptophan synthetase A proteins produced by a group of mutants of E. coli were examined for primary structure, and each mutant protein was found to differ from the wild type protein by a change of a single amino acid. By showing that the order of the positions at which these single amino

acid changes occurred in the A protein was the same as the order of the respective mutational sites on the genetic map, the colinear relationship between gene structure and protein structure was established (Yanofsky 1963). The distance on the genetic map also is reasonably representative of distance in the polypeptide chain. Colinearity of gene structure and protein structure has also been convincingly demonstrated using different experimental material such as nonsense mutants and frame shift mutants (Fowler and Zabin 1966).

The complete sequence of the 267 amino acid residues in the E. coli tryptophan synthetase A protein has been determined and consequently the relationship between the genetic map of the A gene and the changes in mutationally altered A proteins can be reconsidered in terms of the primary structure of the entire protein. The amino acid sequence of the A protein shown in Figure 2 was determined by analysis of fragments derived by treating the protein with various proteolytic enzymes or with cyarogen bromide (Yanofsky et al. 1967).

C. Purpose of This Investigation

At the onset of this study experiments in this laboratory on hybridization of E. coli and S. typhimurium produced inconclusive results when selection was made for tryptophan recombinants. By using female strains with special genetic properties or by treating female strains with heat or mutagenic agents, hybrids could be readily produced, but the question of whether or not integration into the recipient chromosome of the male material had taken place could not be easily resolved. Hybrids diploid in the chromosomal region under study (partial heterozygotes) were able to replicate and form full-sized colonies. Furthermore, if integration of male genes into the female chromosome took place, it could not be discerned exactly where the integration took place in the chromosome. The question of integration is important because the frequency of recombination between

Met-Gln-Arg-Tyr-Glu-Ser-Leu-Phe-Ala-Gln-Leu-Lys-Glu-Arg-Lys-Glu-Gly-Ala-Phe-Val-
Pro-Phe-Val-Thr-Leu-Gly-Asp-Pro-Gly-Ile-Glu-Gln-Ser-Leu-Lys-Ile-Asp-Thr-Leu-Ile-
Glu-Ala-Gly-Ala-Asp-Ala-Leu-Glu-Leu-Gly-Ile-Pro-Phe-Ser-Asp-Pro-Leu-Ala-Asp-Gly-
Pro-Thr-Ile-Glu-Asn-Ala-Thr-Leu-Arg-Ala-Phe-Ala-Ala-Gly-Val-Thr-Pro-Ala-Gln-Cys-
Phe-Glu-Met-Leu-Ala-Leu-Ile-Arg-Gln-Lys-His-Pro-Thr-Ile-Pro-Ile-Gly-Leu-Leu-Met-
Tyr-Ala-Asp-Leu-Val-Phe-Asp-Lys-Gly-Ile-Asp-Glu-Phe-Tyr-Ala-Gln-Cys-Glu-Cys-Val-
Gly-Val-Asp-Ser-Val-Leu-Val-Ala-Asp-Val-Pro-Val-Gln-Glu-Ser-Ala-Pro-Phe-Arg-Gln-
Ala-Ala-Leu-Arg-His-Asn-Val-Ala-Pro-Ile-Phe-Ile-Cys-Pro-Pro-Asn-Ala-Asp-Asp-Asp-
Leu-Leu-Arg-Gln-Ile-Ala-Ser-Tyr-Gly-Arg-Gly-Tyr-Thr-Tyr-Leu-Leu-Ser-Arg-Ala-Gly-
Val-Thr-Gly-Ala-Glu-Asn-Arg-Ala-Ala-Leu-Pro-Leu-Asn-His-Leu-Val-Ala-Lys-Leu-Lys-
Glu-Tyr-Asn-Ala-Ala-Pro-Pro-Leu-Gln-Gly-Phe-Gly-Ile-Ser-Ala-Pro-Asp-Gln-Val-Lys-
Ala-Ala-Ile-Asp-Asp-Gly-Ala-Ala-Gly-Ala-Ile-Ser-Gly-Ser-Ala-Ile-Val-Lys-Ile-Ile-
Glu-Gln-His-Asn-Ile-Glu-Pro-Glu-Lys-Met-Leu-Ala-Ala-Leu-Lys-Val-Phe-Val-Gln-Gln-
Met-Lys-Ala-Ala-Thr-Arg-Ser

Figure 2. Amino acid sequence of the E. coli tryptophan synthetase A (Yanofsky et al. 1967)

analogous genes of E. coli and S. typhimurium at a specific site on the chromosome is an indication of the degree of genetic homology at that site. Since the tryptophan gene cluster comprises a single operon in each of these species, it is of interest to determine if a hybrid operon which contains E. coli and S. typhimurium tryptophan genes could be controlled by a single operator. The focus of this study was therefore to devise an analytical procedure which would indicate if and where genetic recombination had taken place in a hybrid cross. An E. coli operator gene would be placed in an S. typhimurium operon where ability of the operator to derepress the complete operon could be measured. This required a special combination of markers in the male and female strains.

II. MATERIALS AND METHODS

A. Media

Nutrient broth (8 g Difco nutrient broth and 5 g NaCl per 1000 ml distilled water) and nutrient agar (15 g Bacto agar per 1000 ml nutrient broth) were used as complete media. The minimal medium (Demerec and Ozeki 1959) contained 10.5 g of K_2HPO_4, 0.05 g of $MgSO_4$, 1 g of $(NH_4)_2SO_4$, and 0.47 g of sodium citrate $2H_2O$ per 1000 ml of distilled water. Minimal agar contained in addition 15 g of agar per 1000 ml minimal medium. Amino acid supplements were added to minimal medium when needed at 20 mg per 1000 ml of minimal medium.

B. Nomenclature and Abbreviations

Standard nomenclature (Demerec et al. 1966) was followed throughout this report with E. coli convention for trp genes: trpABCDEO (Taylor 1970). The corresponding nomenclature for the trp operon of S. typhimurium is trpCDEBAO (Sanderson 1970). The SQ prefix for bacterial strains' numbers was assigned to the laboratory of R. B. Middleton by K. E. Sanderson, Salmonella Stock Center, University of Calgary, Alberta. The style for standard abbreviations and citation of references follows Conference of Biological Editors, Committee on Form and Style (1960). Abbreviations are as follows: arg for arginine, AS for anthranilate synthetase, cys for cysteine, DNA for deoxyribonucleic acid, gua for guanine, InGPS for indoleglycerol phosphate synthetase, ilv for isoleucine-valine, MA for minimal medium agar, NA for nutrient agar, NB for nutrient broth, NNG for N-methyl-N'-nitro-N-nitroso guanidine, PRAI for phosphoribosyl isomeras, PRT for phosphoribosyl transferase, pyr for pyrimidine, RNA for ribonucleic acid, sup for suppressor, trp for tryptophan, TS for tryptophan synthetase, and WT for wild type.

C. The Male Strain

Table 1 lists all bacterial strains utilized in this study. The E. coli Hfr strain (SQ996) used was originally prototrophic (SQ995). It was therefore necessary to mutate the male before making the cross in order to select against male cells on the medium producing hybrid recombinants. A mutation in the trpB gene of the E. coli Hfr male would be useful for selecting a hybrid operon with the E. coli parent supplying the operator-promotor and perhaps two or three operator-proximal structural genes and the S. typhimurium parent supplying the operator-distal two or three genes. A trpB male would help insure selection for recombination within the operon.

The mutagenic agent used to mutate the trpB marker in the E. coli strain was N-methyl-N'-nitro-N-nitroso guanidine (NNG)(Adelberg 1965). NNG can disrupt the backbone of a DNA helix by removing phosphonucleotides to result in a deletion mutation. Deletion mutations are preferable to point mutations in which single bases are replaced by other bases along a single strand of DNA, eventually to result in a single base change, since deletion mutations do not revert.

NNG may be applied in aqueous solution or, as in this study, as crystals. The desired mutant strain would grow only on MA supplemented with tryptophan. 0.1 ml of an overnight culture in NB at 37C of the prototroph E. coli Hfr was spread onto MA supplemented with tryptophan in a Petri dish with a sterile glass spreader. A small crystal of NNG was immediately placed in each quadrant of an inoculated Petri dish.

The Petri dishes were incubated right side up for 48 hours at 37C. At the periphery of the clear zones caused by the NNG crystals, individual colonies would be either trp$^+$ or trp; all other mutants would fail to grow. With sterile toothpicks, individual colonies were sampled (20 from each treated plate) and transferred to an NA master plate (40 on each). After overnight incubation

Table 1. Bacterial Strains

Strain	Characteristic	Source
SQ835	*E. coli* wild type	A.T.C.C. #10536
SQ995	" Hfr wild type AT2570	A. Taylor
SQ996	" Hfr *trpB*	D. W. Stetter
SQ737	" F- *trpA*	A. Taylor
SQ975	" F- *trpB*	"
SQ976	" F- *trpC*	"
SQ977	" F- *trpD*	"
SQ978	" F- *trpE*	"
SQ979	" F- *trpBCD*	"
SQ980	" F- *trpCDE*	"
SQ421	*S. typhimurium* wild type LT2	R. B. Middleton
SQ990	" *leuO*	P. Margolin
SQ991	" *trpOA*	"
SQ983	" *cysB*	R. B. Middleton
SQ981	" *guaB*	"
SQ715	" *trpABEDC167*	E. Balbinder
SQ713	" *trpABE130*	"
SQ518	" *trpB223*	"
SQ945	*E. coli* - *S. typhimurium* hybrid TC-1	D. W. Stetter
SQ946	" " " TC-4	"
SQ947	" " " TC-5	"
SQ948	" " " TC-11	"
SQ949	" " " TC-14	"
SQ950	" " " TC-15	"
SQ951	" " " TC-23	"
SQ952	" " " TC-27	"
SQ953	" " " TC-29	"
SQ954	" " " TC-31	"
SQ955	" " " TC-35	"
SQ956	" " " TC-37	"
SQ957	" " " TC-39	"
SQ958	" " " TC-51	"
SQ959	" " " TC-53	"
SQ960	" " " TC-54	"
SQ961	" " " TC-64	"
SQ962	" " " TC-66	"
SQ963	" " " TC-71	"
SQ964	" " " TC-77	"
SQ965	" " " TC-78	"
SQ966	" " " TC-80	"
SQ967	" " " TC-83	"
SQ968	" " " TC-84	"
SQ969	" " " TC-89	"
SQ970	" " " TC-91	"
SQ971	" " " TC-95	"
SQ972	" " " TC-96	"
SQ973	" " " TC-97	"
SQ974	" " " TC-98	"

at 37C, each master plate was replica-plated by velveteen (Lederberg and Lederberg 1952) onto four media: MA, MA plus anthranilate, MA plus indole, and MA plus tryptophan.

Nearly 1800 presumptive mutants were tested. Most were wild type colonies that grew on all four media; six trp mutants were isolated, but only one of the six did not grow on indole and therefore appeared to be a trpB mutation.

To check the "maleness" of this trpB mutant strain, a cross was made with an E. coli F- guaB strain (SQ 981). Plating 0.1 ml overnight culture from each strain together on MA resulted in an average of 120 prototrophic recombinants per plate which indicated that the Hfr character of the original male strain was maintained through the mutagenesis experiment. Lyophilized preparations were then made of the E. coli Hfr trpB strain.

Confirmation that the tryptophan requirement was due to a mutation of the trpB gene was made by conjugation crosses with several E. coli female strains with known trp mutations. The Hfr strain was crossed with each trp strain and the appearance of trp^+ recombinants was looked for (Table 2).

Recombinants resulted from all crosses except with the two female strains carrying a damaged trpB gene, which indicates the mutation in the Hfr strain is limited to the trpB gene. The mutation appears to be a deletion, since no trp^+ revertants are seen when 0.1 ml of an overnight culture is plated on MA.

D. The Female Strain (SQ 991)

The S. typhimurium LT2 female strain utilized was produced by Mukai and Margolin (1963). A leucine auxotroph, leu-500, was shown to result from a mutation of the O (operator) gene of the leucine operon in S. typhimurium. Mukai and Margolin were interested in the location and nature of genetic

Table 2. Genetic analysis of the *E. coli* Hfr strain SQ801 by conjugation and recombination

E. coli female	Recombinants	No Recombinants
SQ 850 *trp A*	+	
SQ 851 *trp B*		+
SQ 852 *trp C*	+	
SQ 853 *trp D*	+	
SQ 854 *trp E*	+	
SQ 855 *trp BCD*		+
SQ 856 *trp CDE*	+	

alterations suppressing the leucine O mutation. leu-500, when spread upon MA and incubated at 37C for 72 hours, gave rise to colonies of leucine-independent cells. These colonies were readily divide into two classes based upon size. A few were large and increased in size during incubation at approximately the same rate as colonies of wild-type cells. The majority fell into a class of distinctly smaller colonies. These never approached the size of the large colonies regardless of the length of the incubating period. Such partial revertants had frequently been found to be the result of suppressor mutations (Yanofsky et al. 1961).

A number of the smaller leucine-independent colonies were selected and purified by passage through a single cell. The independent origins of these strains were assured by using ten different subcultures of leu-500 obtained from single colony isolates, and selecting no more than one each type of suppressor mutant or revertant from each subculture.

The appearance of both cysteine and tryptophan requirements immediately focussed attention upon the region of the chromosome with the cysB and the five tryptophan genes; Demerec and Hartman (1956) as well as Clowes (1958) had noted that cysB and tryptophan genes of S. typhimurium are very closely linked. The leucine operon is not linked by transduction to the cysB and tryptophan regions of the chromosome in E. coli. Similarity of the order of genes on the S. typhimurium and E. coli chromosomes suggested that this is also probably true in S. typhimurium (Sanderson 1970).

The distribution of the auxotrophic suppressor mutants into cysteine requirers, tryptophan requirers, and cysteine plus tryptophan requirers suggested that the region of the chromosome responsible for the suppression of leu-500 lay between cysB and the five tryptophan genes. This region of the chromosome was designated the supX locus (Mukai and Margolin 1963). Figure 3

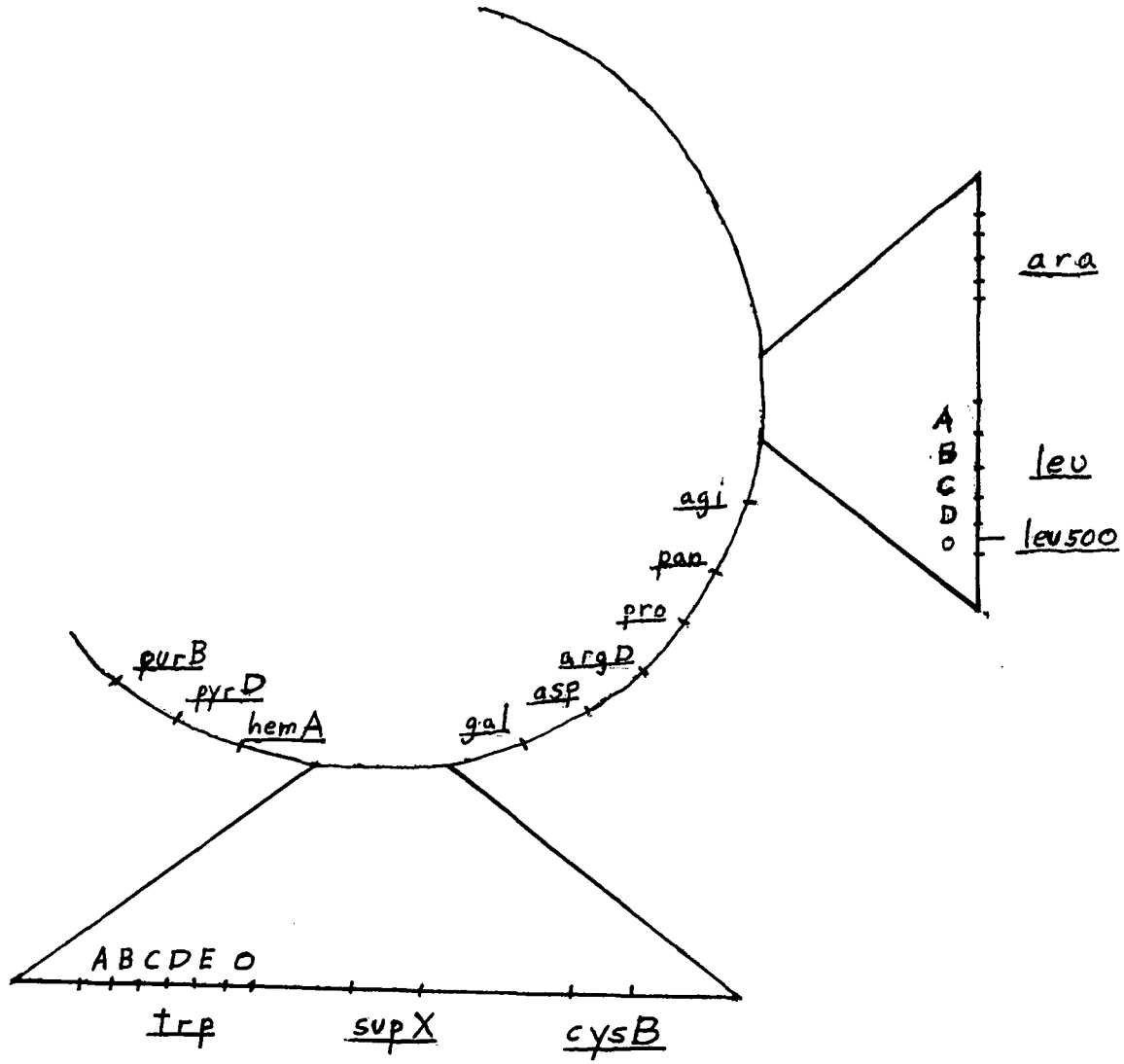

Figure 3. Relative genetic map positions of the leucine chromosomal region and the supX chromosomal region in S. typhimurium (after Sanderson 1970)

is a diagram of the relative positions of the leucine operon and of the supX region.

Reciprocal transductions were performed between tryptophan mutants containing mutations in different trp loci and auxotrophic suppressor mutants which had a tryptophan requirement. It was determined that several supX strains appeared to result from deletions extending for various distances into, but not through, the tryptophan genes, since trp^+ recombinants were produced from transductions with some of the tester strains. For example, two of the suppressed mutants produced no trp^+ recombinants from transductions involving a trpE strain, but did produce trp^+ recombinants from transduction involving trpA, trpB, and trpD strains (the trpC gene was not yet described in 1963). It was concluded that these strains resulted from deletions extending into and perhaps through the trpE gene, but ending before reaching the site of the mutation carried by the trpD strain.

In other transduction results it was discovered that some suppressor strains requiring both cysteine and tryptophan contained deletions through the entire supX locus. That these strains could suppress leu-500 suggested that the suppression resulted from the inactivition or elimination of a normal cell constituent which was a product of the supX locus.

An hypothesis by Margolin and Mukai which explained all observations pictured the leu-500 mutation as having altered the specificity of the leucine operator so that the repressor for an entirely different system could act upon it. This foreign repressor might even act as a superrepressor on the leucine operator. The presence or absence of leucine would not affect this foreign repressor. The supX locus would then be the regulator gene controlling the foreign repressor, and any mutations which removed or inactivated the gene would relieve the O condition of the leucine operon.

The auxotrophic supX strains which have deletions extending into the proximal end of the trp operon are ideal for this study. In selection for tryptophan prototrophy in a hybrid cross, the only source of trp operator and the gene transcribing anthranilate synthetase must be the male strain.

E. Preparation of Hybrid

Successful conjugation between E. coli and S. typhimurium is a rare event. Mojica-a and Middleton (1971) report a protocol for enhancement of the fertility of S. typhimurium females in hybrid crosses; the female strain is grown in minimal medium rather than nutrient broth and incubated at 50C for 20 min immediately before mating. The S. typhimurium female used in this study (SQ991) did not have a marker reducing infertility and did not respond very well to preliminary minimal medium and temperature treatments. On rare occasions hybrids could be induced by the above protocol but not in numbers necessary for this study. The technique most successful and finally adopted was developed by Eisenstark (1965) who found that NNG increased the fertility of hybrid crosses. The appearance of the hybrids on the minimal medium was quite different from that of the mutagenesis experiment. Both parental strains were trp, did not grow on MA and did not produce background growth. Around each NNG crystal placed on the agar was a halo of individual colonies of hybrids. An average of 80 colonies surrounded each NNG crystal placed on the mating mixture on MA. The particular hybrid sought proved to be a rarity among the various types of hybrids produced.

The hybrid desired was one in which a recombinational event had placed the proximal portion of an E. coli tryptophan operon in contact with the distal portion of an S. typhimurium tryptophan operon. The phenotypic appearance of the hybrid would be wild type, but it must be remembered that the female chromosome is not typical. It contains a unique mutation in the leucine

operon operator gene which in a wild type chromosome permits the leucine operator to be permanently repressed by a gene at the supX locus. However, in the supX strain used in this experiment the gene at the supX locus has been deactivated by a deletion and the leucine operon is derepressed. It is important to note that a recombinational event involving the supX locus and the adjacent tryptophan operon as desired in this study would have no effect on the state of suppression of the leucine operator mutation because the state of suppression was originally created by the deactivation of the gene at the supX locus. Further removal of chromosomal material at that locus is therefore insignificant.

Individual colony morphologies of the various hybrids were similar to those described by Baron (1958) in his initial hybridization studies. There were both large and small colonies, the former appearing shallow and clear, the latter appearing dense and opaque. Based on Baron's results it was assumed the dense smaller colonies were more likely true recombinants, the large colonies being partial heterozygotes which continually revert. Using a sterile inoculating needle, samples were taken from among the small colonies, placed in NB for overnight cultures at 37C, then tested on MA. If isolated colonies sustained growth on MA for three cycles, the samples were considered suitable for further analysis to determine if a hybrid operon had been established.

F. Characterization of Hybrid

Two methods of analysis were used to determine the presence of the hybrid operon, one genetical and one biochemical.

The genetical method of hybrid analysis was transduction. Bacterial hybrids derived from crosses between E. coli and S. typhimurium can be analyzed genetically by means of transduction experiments with S. typhimurium strains

as recipients, hybrid as donors, and P22 phage as the vector.

The procedure was as follows. A sample was taken from an overnight broth culture of the recipient strain grown in a shaker and was mixed with P22 phage (multiplicity 5X) grown on a hybrid, and 0.1 ml aliquots were plated on an appropriate selective medium, usually minimal medium enriched with 0.02% nutrient broth powder (double enriched medium). S. typhimurium - E. coli hybrids are not uniform with respect to P22 phage. Some are resistant to P22 and some make poor hosts for P22. This is probably due to variations in the amounts and regions of E. coli genetic material. Since the objective of this study was to analyze the region of the chromosome containing the trp cluster of loci, only trp^+ hybrids that were stable and that yielded high-titer phage were used.

Phage raised of the hybrids was used to transduce mutant markers of the Salmonella recipients. The preliminary recipient utilized was completely deleted for the tryptophan operon and for cysB. Positive results, complete or abortive transductants, would indicate co-transduction of E. coli and S. typhimurium material, i.e. a hybrid operon. Succeeding recipients with shorter intra-operon aberrations determined the extent of E. coli material present. In every instance the transducing fragment carried the wild-type allele of the pertinent mutant gene in the recipient. In order to avoid background recipient reversion, mutants carrying short deletions were chosen as recipients whenever available.

The biochemical method of analysis was the assay of structural gene enzymes at each end of the tryptophan operon under repressed and derepressed conditions. In the female strain originally the distal three structural genes trpA, trpB, and trpC were free of operator control, not derepressable, and transcribing their respective enzymes at a basal rate. Integration of an E. coli operator gene proximal to these genes would hypothetically

cause derepression of the entire trp operon.

Somerville (1966) reported on the compatibility of the S. typhimurium tryptophan regulator genes and the E. coli tryptophan genes. Hybrids hemizygous for the tryptophan genes were prepared by episomal transfer of an E. coli element into S. typhimurium. The amount of enzyme production in the presence and absence of tryptophan varied precisely in the same manner as in E. coli wild type. There is a mutation in the anthranilate synthetase gene in E. coli which prevents derepression of the entire operon. The E. coli gene mutation functioned identically in the cytoplasm of S. typhimurium. Any difference which may exist between the tryptophan regulatory genes of E. coli and S. typhimurium had little effect on the regulation of enzyme formation in hybrids of hemizygous nature. Therefore derepression of S. typhimurium structural genes by an E. coli operator in this study should be free of regulatory ambiguity.

The assay procedure is begun by growing two 500 ml aliquots of the hybrids to be tested overnight at 37C. One aliquot contains minimal salts plus glucose, an environment which should induce derepression. The second aliquot contains the above plus 12 mg of tryptophan / 500 ml of medium, an environment which should repress the activity of all tryptophan genes. The cells from the overnight cultures are spun down to a pellet in a centrifuge at 15,000 RPM for 15 min, and the supernatant medium is then poured off. The two separate pellets of cells are resuspended in 4 ml of Tris buffer pH 7.8 and sonicated in order to disrupt cell membranes and liberate enzymes to be assayed. A second centrifugation at 15,000 RPM for 15 min sediments all cell debris and a clear enzyme extract is ready to be assayed.

The enzymes that were assayed are tryptophan synthetase hypothetically

of S. typhimurium origin at the distal end of the operon and anthranilate synthetase hypothetically of E. coli origin at the proximal end of the operon. Analogous degrees of derepression would indicate control by a mutual operator inferring a hybrid operon.

The tryptophan synthetase assay procedure is described by Colowick and Kaplan (1962). 0.1 ml of cell extract is placed in 1.9 ml reaction mixture as listed below plus 100 units of tryptophan synthetase β subunit. Thus only the α subunit of the enzyme is measured whose activity is thoroughly quantitated in the presence of excess β.

Reaction Solution for Tryptophan Synthetase Assay

3.0ml of 0.2 M serine

1.0 ml of pyridoxal-6-phosphate (100 μ gm/ml)

1.0 ml of 1 M Tris buffer pH 7.8

0.3 ml of NaCl soln. (saturated)

0.8 ml of indole (5 μ moles/ml)

2.9 ml of distilled water

The reaction proceeds in a water bath at 37C for 20 min. The remaining indole is extracted with toluene, reacted with dimethylaminobenzaldehyde to produce a brilliant red color measured quantitatively on a Klett colorimeter with a green filter. The degree of loss of red color is proportional to the quantity of tryptophan synthetase α subunit in the cell extract.

The anthranilate synthetase assay procedure is described by Colowick and Kaplan (1962). 0.1 ml of cell extract is placed in 1.9 ml of reaction mixture as listed below.

Reaction Solution for Anthranilate Synthetase Assay

4.0 ml of 1 mM chorismic acid

4.0 ml of 50 mM glutamine

4.0 ml of 20 mM $MgSO_4$

2.0 ml of 100 mM phosphate buffer containing

0.78 g of mercaptoethanol / 100 ml

5.0 ml of distilled water

Chorismic acid is converted by anthranilate synthetase to anthranilic acid which fluoresces. This fluorescence may be measured quantitatively with a spectrophotofluorometer (Turner 111) and is directly proportional to the amount of anthranilate synthetase in the cell extract.

In order to standardize the total number of cells grown for any enzyme assay, the value determined in the assay is always compared to the total amount of protein in the cell extract. This ratio is referred to as the specific activity.

The protein determination is as follows: 0.5 ml of cell extract is placed in 5.0 ml of reaction solution as listed below.

Reaction Solution of Protein Determination

50 ml 2% $NaCO_3$ in 0.1 N NaOH

1 ml 1% $CuSO_4 \cdot 5H_2O$ + 2% potassium tartrate

After 10 min 0.5 ml of Folin reagent is added. The reaction is allowed to proceed for 30 min. A deep blue color proportional to the amount of total protein in the cell extract may be measured on a Klett colorimeter with a green filter. Using the same above procedure, known quantities of albumin solution are measured and a standard curve prepared. The amount of protein in the cell extract may then be read off the standard curve.

III. RESULTS

Initially ten batches of hybrids were prepared, and from each batch ten individual colonies were selected, totaling one hundred hybrids. Each of the initial one hundred hybrid colonies was grown in nutrient broth overnight at 37C and tested on minimal medium plates the following day. After forty-eight hours of incubation at 37C it was observed that thirty of the original one hundred hybrids produced substantial lawns on the minimal medium. These thirty hybrids were recycled through broth culture and minimal medium plates three times. None were found to segregate tryptophan requiring colonies. These thirty were considered suitable to be tested by transduction into a S. typhimurium strain containing a deleted operon to determine if there was a complete tryptophan operon in each by using genetic technique.

The gross genetic make-up of each hybrid tryptophan operon was also determined by transduction into strains with only partially deleted operons, one strain operator-proximally deleted and the other strain operator-distally deleted. Variations in transduction frequencies in the latter two cases could perhaps distinguish E. coli genes from S. typhimurium genes. A fine analysis of the hybrid operons was not attempted due to Demerec's (1968) conclusions that in this genetically inverted region of the chromosome E. coli and S. typhimurium genetic material interfere with one another with respect to recombination in transduction tests.

Three S. typhimurium strains available in this laboratory with respect to the specifically needed deletions were a strain with a trpABCDE deletion (SQ715), a strain with a trpCDE deletion (SQ713), and a strain with a trpB deletion (SQ518). A S. typhimurium strain with a cysB marker indicated the extent of recombination in the cysB locus direction. The control recipient was a S. typhimurium strain with a leu0° marker (SQ990).

1. Normalization for the transduction competence of recipients:

$$\frac{\text{number of transductants in leuO recipient (LT2 WT donor)}}{\text{number of transductants in each } \underline{\text{trp}} \text{ deletion recipient (LT2 WT donor)}}$$

2. Normalization for the titre of phages:

$$\frac{\text{number of transductants in leuO recipient (LT2 WT donor)}}{\text{number of transductants in leuO recipient (each hybrid donor)}}$$

3. Normalization for the number of transductants under idealized conditions is calculated as (1) X (2) X the number of transductants actually found.

4. The relative incorporation frequency:

$$\frac{(3) \times 100}{\text{number of transductants in leuO recipient (LT2 WT donor)}}$$

Figure 4. Procedure for determining the relative incorporation frequencies of *E. coli* genetic material in the *S. typhimurium* chromosome. (Demerec and Ohta 1964)

Table 3. Transduction analysis of *trp*$^+$ hybrids

Donors	Recipients				
	SQ518 *trpB*	SQ715 *trpABCDE*	SQ 713 *trpCDE*	SQ 983 *cysB*	SQ990 *leuO*
SQ421 LT2 WT	1840[a]	1853	1803	1850	1876
SQ945 Hybrid TC- 1	1837	0	0	1866	1756
SQ946 - 4	1902	0	0	1877	1931
SQ947 - 5	1836	0	72 (4%)[b]	1935	1845
SQ948 -11	1937	20 (1.1%)	24(13%)	1836	1896
SQ949 -14	1734	0	0	1783	1756
SQ950 -15	1866	0	103 (6%)	1902	1813
SQ951 -23	1903	0	86 (5%)	1876	1921
SQ952 -27	1799	0	0	1810	1771
SQ953 -29	1786	60 (2.5%)	97 (5%)	83 (5%)	1765
SQ954 -31	1921	0	0	1893	1865
SQ955 -35	1853	18 (0.9%)	55 (2.9%)	1784	1921
SQ956 -37	1806	0	12(0.6%)	1796	1835
SQ957 -39	1797	0	75 (4%)	1834	1810
SQ958 -51	1941	0	0	1870	1952
SQ959 -53	1852	93 (5%)	83(4.7%)	1910	1783
SQ960 -54	1898	0	0	1860	1851
SQ961 -64	1761	0	0	1790	1935
SQ962 -66	1932	0	75 (4%)	1913	1763
SQ963 -71	1822	0	21(1.1%)	1856	1921
SQ964 -77	1909	0	102 (6%)	1883	1703

(continued)

Table 3. (continued)

Donors		Recipients				
		SQ518 trpB	SQ715 trpABCDE	SQ713 trpCDE	SQ983 cysB	SQ990 leuO
SQ965	Hybrid TC-78	1877	0	45 (3%)	1830	1757
SQ966	-80	1756	0	0	1811	1856
SQ967	-83	1949	0	135 (8%)	1921	1723
SQ968	-84	1806	0	0	1834	1943
SQ969	-89	1783	0	0	1797	1821
SQ970	-91	1866	0	21 (1.2%)	1834	1876
SQ971	-95	1837	0	61(3.1%)	1871	1914
SQ972	-96	1936	0	0	1903	1888
SQ973	-97	1801	8 (0.4%)	67(3.5%)	1784	1853
SQ974	-98	1798	0	0	1817	1756

[a]Values are total of *trp*$^+$ recombinants on three plates.

[b]Percentages in parentheses indicate the relative incorporation frequencies. Where no relative frequencies are given, the values are 100%; i.e. the gene is of *S. typhimurium* origin.

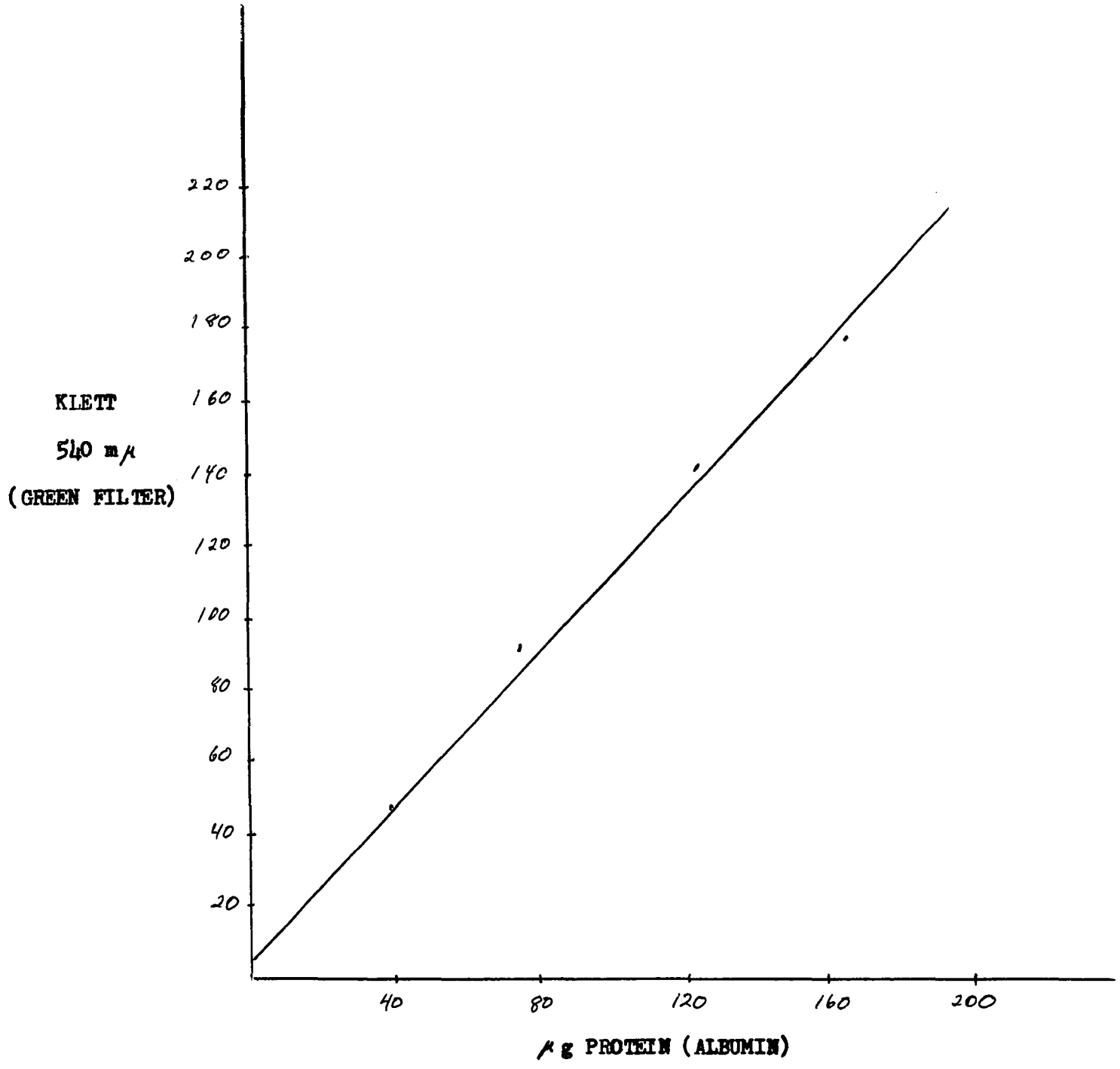

Figure 5. Protein determination standard curve (based on known concentrations of albumin)

Table 4. Protein determination for each sample cell extract

Samples	Klett Readings	Standard Graph Values	Corrected[b]
SQ421 S. typhimurium WT	58	48	0.85
" " " TRP[a]	67	58	0.93
SQ835 E. coli WT	65	56	0.91
" " " TRP	42	34	0.55
SQ948 TC-11	103	90	1.44
" " TRP	111	101	1.62
SQ952 TC-27	127	111	1.77
" " TRP	155	139	2.23
SQ955 TC-35	50	41	0.65
" " TRP	139	122	1.93
SQ959 TC-53	117	103	1.65
" " TRP	162	144	2.35
SQ973 TC-97	72	62	0.99
" " TRP	69	59	0.94

[a]TRP indicates exogenous tryptophan (repressed conditions); other assays derepressed.

[b]concentration correction factor is 0.016 .

Table 5. Tryptophan synthetase assay

Samples	Klett Readings	Corrected[b]	Specific Activities[c]
Cold Control	196		
Warm Control	185		
SQ421 S. typhimurium WT	99	1.72	2.02
" " " TRP[a]	155	0.60	0.64
SQ835 E. coli WT	35	3.00	3.04
" " " TRP	154	0.64	1.16
SQ948 TC-11	88	1.96	1.36
" " TRP	106	1.60	1.00
SQ952 TC-27	56	2.64	1.49
" " TRP	66	2.44	1.09
SQ955 TC-35	117	1.40	2.15
" " TRP	99	1.84	0.96
SQ959 TC-53	28	3.20	1.98
" " TRP	54	2.68	1.14
SQ973 TC-97	105	1.64	1.66
" " TRP	139	0.92	0.97

[a] TRP indicates exogenous tryptophan (repressed conditions); other assays derepressed

[b] corrected for concentration and difference between warm and cold controls

[c] specific activity = assay reading / protein determination reading (Table 4)

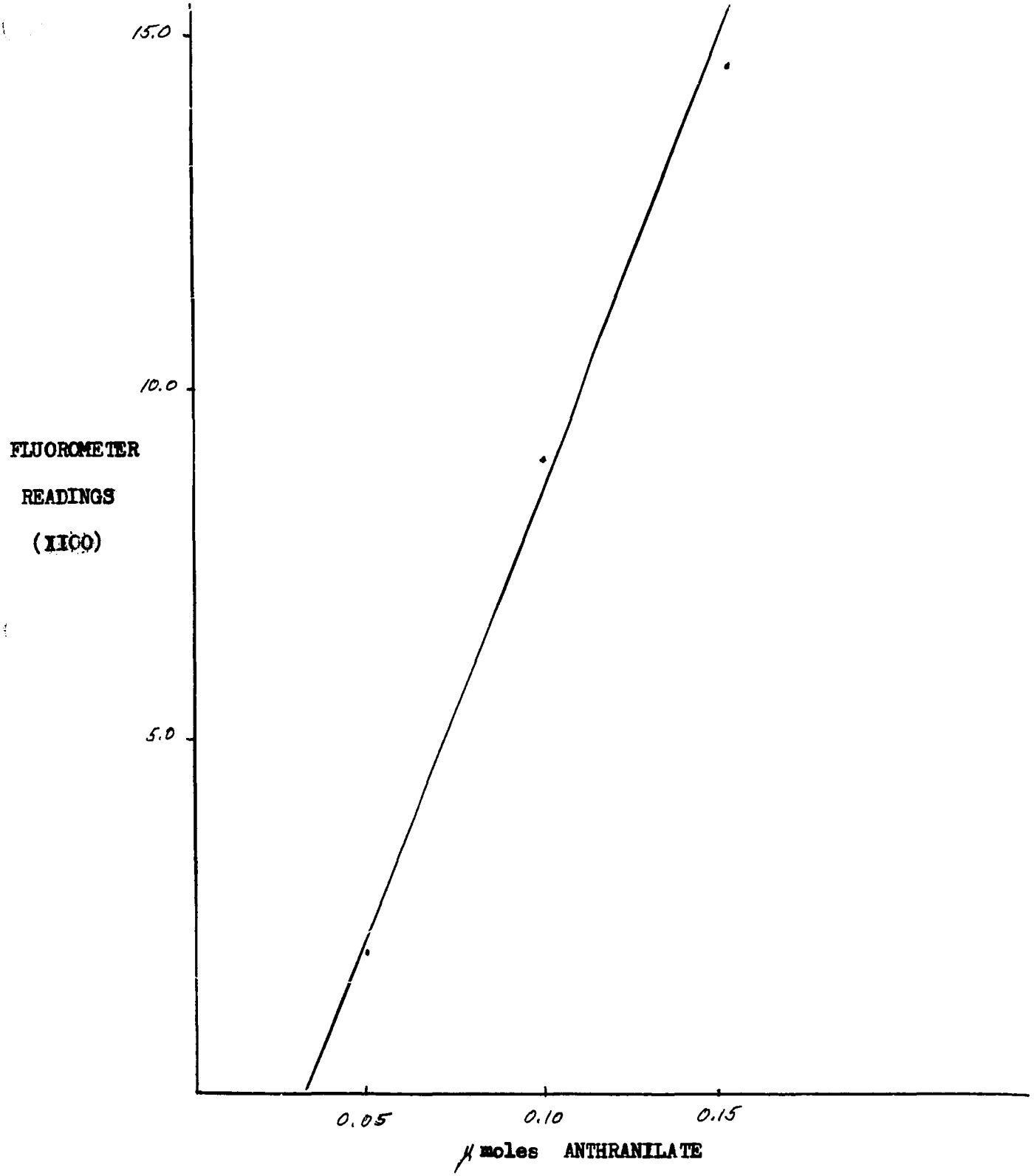

Figure 6. Anthranilate standard curve (based on known concentrations of anthranilate)

Table 6. Anthranilate Synthetase Assay

Samples	Fluorometer (X100, 15min)[b]	Moles of Anthranilate	Specific Activities (10^{-2})[c]
SQ421 S. typhimurium WT	2.1	0.051	6.0
" " " TRP[a]	0.6	0.035	4.2
SQ835 E. coli WT	7.1	0.089	9.8
" " " TRP	0.5	0.035	6.4
SQ952 TC-27	9.3	0.105	5.9
" " TRP	0.6	0.035	1.7
SQ959 TC-53	6.7	0.085	5.2
" " TRP	0.5	0.032	1.5
SQ973 TC-97	6.2	0.082	8.2
" " TRP	0.5	0.032	3.7

[a] TRP indicates exogenous tryptophan (repressed conditions); other assays derepressed

[b] 15 min fluorometer readings were compared with the standard anthranilate curve (Fig.6) to indicate anthranilate synthetase activity

[c] specific activity = assay reading / protein determination reading (Table 4)

IV. DISCUSSION

A. Conclusions Based on Results

Both the genetic and biochemical analyses indicate that an E. coli chromosomal segment including a tryptophan operator-promotor gene and an adjacent anthranilate synthetase gene has integrated into an S. typhimurium chromosome. This incorporation, proximal to the four operator-distal genes of the S. typhimurium tryptophan operon of the female parent, produced a complete operon composed of E. coli and S. typhimurium genes.

Transduction experiments indicate that five hybrids, of one hundred presumptive trp^+ hybrids, have a complete tryptophan operon (operator and five structural genes) which can be transferred on a single transducing fragment (Table 3).

The trp^+ genes of all five hybrids was transduced about as efficiently as the $trpB^+$ gene of S. typhimurium LT2 wild type, i.e. approximately 100%, which indicates the $trpB^+$ gene was of S. typhimurium origin. The $trpCDE^+$ gene cluster was transduced at very low relative incorporation frequencies (0-13%) which indicates the presence of E. coli genetic material. The five hybrid donors which contain complete operons (TC-11, -29, -35, -53, and -97) produced recombinants with the trpABCDE recipient. Their relative incorporation frequencies were similar to those of the trpCDE recombinants. This expressed the influence of the E. coli material on the complete operon in the process of recombination. It may be noted that one (TC-29) of these five hybrids contains a cysB locus which may be of E. coli origin also, as indicated by a relative incorporation frequency of five percent. This is possible because in the conditions of the original cross the extent of recombination in the cysB locus direction was not controlled as it was in the trpB direction (Figure 3).

The five hybrid strains which produced recombinants with the trpABCDE recipient in the transduction experiment were judged suitable for biochemical analysis. The results of the biochemical experiments indicate that the operator gene controls the transcription of all structural genes in the operon providing repressed and derepressed levels of enzyme production in the presence and absence of tryptophan respectively (Tables 5 and 6).

It was observed that in the case of tryptophan synthetase the specific activities of the hybrids resembled the characteristic S. typhimurium levels more than E. coli levels; the S. typhimurium wild-type control and hybrid derepressed enzyme levels had specific activities between 1.36 and 2.15, whereas the E. coli wild-type control derepressed enzyme level had a much higher specific activity of 3.04. Repressed conditions in all samples tested produced very low specific activities. This implies that the genes in the distal end of the operon are S. typhimurium, but it is not a true indication of the relative activities of the tryptophan synthetases from the two species. In the analysis only the tryptophan synthetase α polymer was assayed; a β polymer of E. coli origin was supplied in excess. This was done in order to assay all tryptophan synthetase α produced by saturation with β polymer, but the α component from S. typhimurium may not have been completely compatible with the β component from E. coli. The observed activity may not therefore be the maximum activity of S. typhimurium tryptophan synthetase. This procedure serves its purpose in deciding whether or not the tryptophan synthetase genes in the hybrid are S. typhimurium or E. coli by measuring the activities of the respective α components relative to the E. coli β component.

In this analysis the emphasis is on determining the origin of the genes in the hybrid operon. For this purpose the E. coli β component sufficed in the tryptophan synthetase assay. If tryptophan synthetase β components from both E. coli and S. typhimurium were available, the system could serve as a

novel approach in comparing the true activity of the α component from each species. The E. coli α component could be obtained from the E. coli trp^+ parental strain. The S. typhimurium α component could be obtained from the hybrid of interest. Both α components would be produced in the same quantities being derepressed by the same E. coli operator-promotor. The only variable in the comparative assays would be the relative activites of the respective enzymes. Generalizing this technique, the insertion of a standard operator-promotor gene into the genome of various bacterial species could be used to control the production of a variety of enzymes of interest.

Data from the anthranilate synthetase assays indicate repressed and derepressed levels of enzyme production in the presence and absence of tryptophan in accord with the tryptophan synthetase data. Evidence that both the gene transcribing tryptophan synthetase and the gene transcribing anthranilate synthetase are repressed in the presence of tryptophan indicates that both genes are under the same operator-promotor gene, which supports the evidence for a hybrid tryptophan operon. It is difficult to decide on the origin of the anthranilate synthetase gene ($trpE^+$) from the enzyme assay data alone. At the 15-minute reading of the assay the specific activity of the E. coli-control was only 20 percent greater than the S. typhimurium-control. The hybrid sample readings fell between the E. coli and the S. typhimurium controls or equaled the E. coli control reading. The $trpE^+$ gene can, however, be presumed to be of E. coli origin since trpE was deleted in the S. typhimurium female parent.

B. Further Investigations

The demonstration of a hybrid tryptophan operon between E. coli and S. typhimurium raises new questions for possible study. In order to be inserted in an orderly manner in the tryptophan region of the S. typhimurium female,

the E. coli tryptophan genes have had to be inverted with respect to the orientation on the E. coli chromosome. Although the exact site of recombination is not ascertained, a hybrid polycistronic mRNA has presumably been produced. That the tryptophan operon mRNA is hybrid does not seem to effect its translation to protein. In progress is the amino acid sequencing of the tryptophan synthetase A enzymes of a number of enteric bacteria (Sanderson 1971). Hybrid enzymes from hybrid strains may provide information on primary and secondary structures of analogous enzymes.

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