THE MAP LOCATION OF THE SSD MUTATION IN ESCHERICHIA COLI K12

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ABSTRACT

This thesis deals with the genetic strategy which was used to locate the pleiotropic *ssd* mutation at 86 minutes, between the *metB* gene and the *rha* operon on the *Escherichia coli* linkage map.

The *ssd* mutation was discovered in strain VE2-2 and is responsible for the following phenotypic traits: high l-serine deaminase activity; an inability to utilize succinate as carbon source; an increased sensitivity to sodium chloride, fluoride and low pH levels; and an inability to grow under anaerobic conditions.

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INTRODUCTION

This thesis describes the location of the *ssd* mutation on the *E. coli* chromosome. The *ssd* mutation is pleiotropic and is responsible for the following changes in strain VE2-2: high serine deaminase activity, an inability to utilize succinate as a carbon source, an increased sensitivity to sodium chloride, fluoride, and low pH levels, and an inability to grow under anaerobic conditions (Morris and Newman, 1980).

Strain VE2-2 was the strain in which the *ssd* mutation was discovered, but it is not certain how strain VE2-2 originated (Morris and Newman, 1980). Strain VE2-2 could be a transductant from a transduction which was designed to transfer the pleiotropic *wyb* mutation from strain MS845 to strain CU1008, in which case the *ssd* mutation is the same as the *wyb* mutation. Or strain VE2-2 could be a mutant which grew on the selection medium used in the transduction, in which case the *ssd* mutation is a spontaneous mutation different from the *wyb* mutation. Strains VE2-2 and MS845 have similar pleiotropic phenotypes which supports the view that the *ssd* and *wyb* mutations are the same (Morris and Newman, 1980). However, strains VE2-2 and MS845 differ in that strain MS845 lacks leucyl-, phenylalanyl-tRNA protein transferase (Morris and Newman, 1980; Soffer and Savage, 1974).

The remainder of the introduction examines the relationship between strains VE2-2 and MS845 and is composed of the following three parts.

Part I describes how strain MS845 was isolated and gives a description of its complex phenotype.

Part II describes the transduction to strain CU1008 which was designed to transfer the wyb mutation from strain MS845, and from which strain VE2-2 was selected. A description of the complex phenotype of strain VE2-2 is given.

Part III discusses the purpose for determining the location of the ssd mutation, and proposes experiments designed to determine if the ssd mutation from strain VE2-2 and the wyb mutation from strain MS845 are the same or different.

PART I

A Description of Strain MS845

Strain MS845 was isolated in a colony-by-colony search among 1200 survivors for those which lacked leucyl-, phenylalanyl-tRNA: protein transferase activity following the treatment of strain W4977 with a heavy dose of nitrosoguanidine. Strain MS845 was the only one which lacked transferase activity (Soffer and Savage, 1974).

Leucyl-, phenylalanyl-tRNA protein transferase is an enzyme which catalyses the transfer of leucyl, phenylalanyl (Soffer, 1974), and methionyl (Horimishi et al, 1975; Scorpulla, Deutch, and Soffer, 1976) groups from their respective tRNA species into peptide linkage with a basic amino acid residue at the NH_2-terminal of specific proteins (Leibowitz and Soffer, 1971; Soffer, 1973). The physiological role of transferase is unknown. It is speculated though that it may regulate the functions of the proteins whose modification it catalyses (Soffer and Savage, 1974). The identification of these acceptor proteins then may elucidate the physiological role of transferase (Soffer and Savage, 1974). Some proteins in the 30S ribosomal subunit have been shown to serve as acceptors for transferase (Leibowitz and Soffer, 1971; Soffer and Savage, 1974). It was suggested that transferase might play a role in regulating the activity of ribosomes and thereby protein

synthesis (Leibowitz and Soffer, 1971). This suggestion though has not been investigated to any significant extent. No other identified proteins have been shown to be modified by transferase.

It has been experimentally estimated that 0.5% of the proteins in strain W4977 extract are modified in vivo by transferase (Soffer and Savage, 1974). It is possible that some of these proteins require the modification to function normally (Soffer and Savage, 1974). If so, then the same proteins in strain MS845 which are not modified in vivo by transferase may function abnormally or not at all (Soffer and Savage, 1974).

Strain MS845 then was studied to look for any physiological changes which might be caused by the absence of transferase activity (Soffer and Savage, 1974; Deutch et al, 1978).

Strain MS845 differs in growth behaviour from strain W4977 (Soffer and Savage, 1974). Strain MS845 grew at the same rate as strain W4977 but gave a lower yield on minimal glycerol medium supplemented with 2mM proline. Also, strain MS845 when grown to stationary phase on minimal glycerol medium supplemented with 2mM proline and then diluted into fresh medium showed a lag which lasted up to 8 hours. Strain W4977 when similarly treated did not show this prolonged lag. Strain MS845 then differs from strain W4977 in yield and behavior on subculture.

It was found that strain MS845 exhibited this abnormal growth behavior because it has an increased demand for proline (Soffer and Savage, 1974). If strain MS845 was grown on minimal glycerol medium supplemented with 10mM proline instead of 2mM proline, then it achieved maximum yields at stationary phase. Also if strain MS845 is grown on minimal glycerol medium supplemented with 20mM proline and then diluted into fresh minimal glycerol medium supplemented with 2mM proline, then no lag results.

It was then found that strain MS845 demands more proline than strain W4977 because it expresses 4-5 times more proline oxidase activity resulting in an increase in proline break down (Deutch and Soffer, 1975). However, it could not be known whether this increase in proline oxidase activity was due to an increase in the inducibility of proline oxidase or due to an increase in the constitutive levels of this enzyme since strains MS845 and W4977 are proline auxotrophs and were grown with proline which is the inducer of proline oxidase activity (Tam et al, 1978).

To determine whether the increase in proline oxidase activity in strain MS845 was due to an increase in the induced or the constitutive levels, the following experiment was performed (Tam et al, 1978). First proline-independent derivatives of strains MS845 and W4977 were constructed by

transduction. These strain MS845 and W4977 derivatives were then grown on minimal glycerol medim both with and without proline. When the derivatives were grown without proline, the proline oxidase activities of both were low. When the derivatives were grown with proline, the proline oxidase activity of the strain MS845 derivative was high but that of the strain W4977 derivative was the same as in cells grown in the absence of proline. The strain MS845 derivative then has inducible proline oxidase activity and the strain W4977 derivative does not. Strains MS845 and W4977 should behave like their derivatives. Therefore, it was concluded that strains MS845 and W4977 have the same low constitutive levels of proline oxidase activity but that strain MS845 has inducible proline oxidase activity, and strain W4977 does not.

The growth defects in strain MS845 are now explainable in terms of inducible proline oxidase activity (Tam et al, 1978; Deutch and Soffer, 1975). Strain MS845 does not give as high a yield on minimal glycerol medium supplemented with 2mM proline as does strain W4977 because strain MS845 expressses high proline oxidase activity under these conditions and utilizes proline at a fast rate. The utilization is so fast that its reserve of free proline required for protein synthesis is rapidly depleted and growth is arrested early on. It is at this point that the strain MS845 culture reaches stationary phase. Strain W4977

does not induce proline oxidase activity under the same growth conditions and so does not utilize proline as fast. Therefore its reserves of free proline are not as rapidly depleted and growth continues on until other factors become limiting. The growth lag exhibited by strain MS845 can be explained in the same terms. The strain MS845 stationary culture grown with only 2mM proline has extremely low levels of free proline. When this stationary culture is diluted into fresh minimal glycerol medium supplemented with 2mM proline, some time is required before intracellular proline levels are built up to the level at which protein synthesis can resume at a normal rate. It is only when this level is reached that growth resumes.

This conclusion is supported by the fact that strain MS845 does not exhibit these growth defects if glucose instead of glycerol is used as a carbon source. (Deutch and Soffer, 1975). Proline oxidase is subject to catabolite repression (Dendinger and Brill, 1970). When strain MS845 is grown with glucose the proline oxidase activity remains low and is not inducible by proline. The pool of free proline then is not depleted by excessive degradation by proline oxidase. Therefore, enough free proline is available to support whatever protein synthesis is necessary to

achieve normal growth yields. Furthermore, enough free proline is available after the grown stationary culture is diluted into fresh medium to support the normal rate of protein synthesis so that no growth lag results.

Deutch et al, (1977) found that strain MS845 is also different from strain W4977 in that it has decreased phenylalanyl-tRNA synthetase and tryptophanase activities. It has an increased rate of growth on aspartic acid as the sole source of nitrogen. It accumulates enterochelin under conditions of iron limitation, and it has an abnormal morphology that can be restored by the presence of D-alanine and D-glutamic acid.

Tam et al (1978) found that strain MS845 differs further from strain W4977 in that it expresses high but uninducible serine deaminase activity. Newman (unpublished data) found that strain MS845 is more sensitive to high sodium chloride concentrations, high fluoride concentrations, and low pH levels, it is unable to utilize succinate as a carbon source, and it is unable to grow under anaerobic conditions.

Strain MS845 was isolated following nitrosoguanidine mutagenesis and this treatment is known to cause multiple mutations (Guerola, Ingraham, Cerda-Olmedo, 1971). Therefore, it is possible that strain MS845 carries

many mutations and that a different mutation causes each of its changes. To prove that this is not the case, it is necessary to select for revertants in a selection in which one change in strain MS845 is restored to the W4977 phenotype and then show that the other changes in strain MS845 are also restored to the W4977 phenotype.

Soffer and Savage (1974) selected the "revertant" strain R18 by cycling strain MS845 8 times on minimal glycerol medim supplemented with low proline (2mM). Each cycle consisted of growing strain MS845 to stationary phase and then diluting into fresh medium. Strain MS845 does not start growing until after 3-8 hours after each dilution. Therefore, spontaneous revertants like strain R18 that start growing immediately after each dilution would be expected to have an advantage under these conditions. After the last cycle was completed a diluted sample of the grown culture was plated on minimal glycerol medium supplemented with 2mM proline. Strain R18 was selected from this plate.

Strain R18 which regained the ability to grow on minimal glycerol medium supplemented with low proline, also expressed transferase activity, uninducible proline oxidase activity (Soffer and Savage, 1974), and both phenylalanyl-tRNA synthetase and tryptophanase activities comparable to

those expressed by strain W4977. It grew at about the same rate as strain W4977 on aspartic acid as the sole source of nitrogen, did not accumulate enterochelin under conditions of iron limitation, and showed a normal morphology (Deutch et al, 1977). Therefore it was concluded that the strain MS845 mutation which causes the inability to grow on minimal glycerol medium supplemented with low proline also causes the absence of transferase activity and the rest of the changes in strain MS845.

However, this conclusion may not be correct since strain R18 was not selected in a single step. Therefore, strain R18 may represent an accumulation of mutations which results in the expression of the complete strain W4977 phenotype.

Tam et al (1978) selected the single-step revertant strain POR 17 from strain MS845 by plating out a strain MS845 culture on minimal glycerol medium supplemented with low proline. This single-step selection ensured that the ability to grow with low proline is regained by one mutation. POR 17 also expresses transferase activity, uninducible proline oxidase activity, low serine deaminase activity (Tam et al, 1978), is as resistant as strain W4977 to high concentrations of sodium chloride, high concentrations of fluoride, and low pH levels, is unable to

grow on succinate as a carbon source, and can grow under anaerobic conditions (Newman, unpublished data). It is evident then that the single mutation which enables growth on minimal glycerol medium supplemented with low proline also restores the other changes to the strain W4977 phenotype. This single mutation may have occurred in the same gene in which the mutation which causes the absence of transferase activity and thereby restored function to the gene or the single mutation may have occurred in another gene and suppressed the mutation which causes the absence of transferase activity. In either case the fact that one mutation restores all these changes to the strain W4977 phenotype proves that the mutation which causes the inability to grow on minimal glycerol medium supplemented with low proline and which causes the absence of transferase activity also is responsible for the other changes described in strain MS845. Tam et al (1978) named the pleiotropic mutation the wyb mutation.

PART II

The Origin and Phenotype of Strain VE2-2

Strain MS845 was isolated from strain W4977 following heavy nitrosoguanidine mutagenesis which is known to cause multiple mutations (Guerola, Ingraham, Cerda-Olmeda, 1971). It is possible then that strain MS845 carries a number of mutations in addition to the pleiotropic wyb mutation and that some of these other mutations interfere with the expression of the wyb mutation.

Strain VE2-2 originated in a transduction designed to transfer the wyb mutation from strain MS845 into strain CU1008 (Morris and Newman, 1980). This transduction was done to study the wyb mutation in a known genetic background.

Despite the fact that the wyb mutation affects a number of activities, there was no good selection for strain CU1008 cells carrying this mutation. However, it was reasoned that a strain CU1008 cell which receives the wyb mutation should be better able to grow on serine as the sole source of carbon and nitrogen because it would express high serine deaminase activity.

Strain CU1008 then was transduced with phage lysate prepared on strain MS845, and a selection was made for transductants that grow on serine as the sole source of carbon and nitrogen.

In the eight times that this transduction was attempted, colonies on the transductant plates were obtained only twice. These colonies had high serine deaminase. It was thought then that the wyb mutation had been transferred into strain CU1008.

One of the colonies obtained from the first transduction experiment was named strain VE2-2, and it is clearly derived from strain CU1008 since, like strain CU1008, it requires exogenous isoleucine and valine to grow on minimal medium. However it is not yet certain whether it is a transductant or a mutant.

If strain VE2-2 is a transductant, it should carry the wyb mutation. Strains VE2-2 and MS845 then should express the same phenotype. Strain VE2-2 in fact shares some of the same characteristics of strain MS845 but it also differs significantly from it. Strain VE2-2, like strain MS845, has high serine deaminase activity, cannot utilize succinate as a carbon source is more sensitive to sodium chloride, fluoride ions, and low pH than strain CU1008, and it cannot grow under anaerobic conditions (Morris and Newman, 1980). However, strain VE2-2 expresses leucyl-, phenylalanyl-tRNA protein transferase activity and strain MS845 does not (Morris and Newman, 1980; Soffer and Savage, 1974).

Strains VE2-2 and MS845 both express inducible proline oxidase activities (Newman, unpublished data; Tam et al, 1978). However, for strain VE2-2 this does not represent a change from strain CU1008; but for strain MS845 this does represent a change from strain W4977.

Since strains VE2-2 and MS845 do not share identical phenotypes, one cannot say with any certainty that they carry the same wyb mutation. It is possible that they both carry the wyb mutation and that the wyb mutation is expressed differently in the genetic backgrounds presented by strains VE2-2 and MS845. It is also possible that strain VE2-2 carries a mutation not derived from strain MS845 that was selected during the transduction experiment and that this mutation only by coincidence is similar to the wyb mutation.

It is because of this uncertainty concerning the origin of the mutation in strain VE2-2 that this mutation was named ssd.

PART III

Rational for Mapping

As discussed in the previous section, the strain VE2-2 and MS845 phenotypes are similar but not identical. The strains then may carry the same or different mutations resulting in similar phenotypes. The strain W4977 mutation has been named wyb. The strain VE2-2 mutation has been named ssd. The most direct way to compare the two mutations would be to put by transduction the ssd mutation into strain W4977. If the two mutations are the same then the strain W4977 transductant should express the strain MS845 phenotype. If the two mutations are different then the strain W4977 transductant should express the strain VE2-2 phenotype.

Since there is no method to directly select strain W4977 transductants that have received the ssd mutation, the following steps must be carried out:

1. The location of the ssd mutation must be determined.
2. A mutation into a gene located near the ssd location must be put into strain W4977.
3. Strain W4977 must be transduced with strain VE2-2 P1 phage lysate and a selection made for transductants which carry the wild-type allele of that near-by gene.

4. The transductants must be screened to look for those which exhibit either the strain MS845 phenotype or the strain VE2-2 phenotype.

It is equally possible to compare the two mutations by transducing the strain MS845 wyb mutation into strain CU1008. If the two mutations are the same then the strain CU1008 transductant should express the strain VE2-2 phenotype.

In order to put the wyb mutation into strain CU1008, the following steps must be carried out:

1. The location of the ssd mutation must again be determined.
2. A mutation into a gene located near the ssd location must be put into strain CU1008.
3. Strain CU1008 must be transduced with strain MS845 P1 phage lysate and a selection made for transductants which carry the wild-type allele of that nearby gene.
4. The transductants must be screened to look for those which exhibit either the strain VE2-2 phenotype.

This thesis deals only with the experiments by which the ssd mutation was successfully located. This thesis does not concern itself with the experiments described above that

are now in progress which should resolve whether the ssd and wyb mutations are identical or not.

It should be clear however that by knowing where the ssd mutation is located, it is possible to perform those necessary transductions which should determine the relationship between the ssd and wyb mutations.

MATERIALS AND METHODS

A. Media and Solutions

Minimal Medium

5.4 g K_2HPO_4, 12.6 g KH_2PO_4, 2 g $(NH_4)_2SO_4$, 2 g $MgSO_4 \cdot 7H_2O$, and 0.1 g $CaCl_2$ at pH 6.4 in 1 liter distilled water. Carbon sources were added to a concentration of 2 g/liter.

The following supplements were added to the minimal medium to the indicated concentrations when required: thiamine 2 ug/ml, isoleucine 50 ug/ml, valine 50 ug/ml, phenylalanine 50 ug/ml, tryptophan 30 ug/ml, tryosine 25 ug/ml, shikimic acid 1×10^{-6}M, histidine 20 ug/ml, arginine 50 ug/ml, leucine 20 ug/ml, methionine 20 ug/ml, diaminopimelic acid 500 ug/ml, lysine 50 ug/ml, homoserine 50 ug/ml and streptomycin 100 ug/ml.

Solid Minimal Medium

Minimal medium with 20 g/liter Bactoagar.

Luria Broth

10 g Bactotryptone, 5 g yeast extract, and 5 g NaCl in 1 liter of distilled water.

Superbroth

32 g Bactotryptone, 20 g yeast extract, 5 g NaCl and 5 ml of 1M NaOH in 1 liter of distilled water.

Nutrient Agar (Difco No. 000-1-02-7) and MacConkey Agar (Difco No. 0818-02-0) were prepared according to the directions given by the manufacturer.

Phage Agar

17 g Bactoagar, 10 g Bactotryptone, 1 g yeast extract, and 8 g NaCl in 1 liter of distilled water.

Minimal Succinate Tryptone Medium

2 g sodium succinate, 0.8 g Bactotryptone in 1 liter minimal medium.

Tetrazolium Solution

10 g/ml 2,3,5 -triphenyl-2H-tetrazolium chloride dissolved in 1M phosphate buffer pH 7.

MC Buffer

0.1 M $MgSO_4$, 0.005 M $CaCl_2$, pH 7

F Top Agar

8 g Bactoagar and 8 g NaCl in 1 liter distilled water.

B. Bacterial strains (see Table I).

C. Selection of Mutants

1. Mal A mutants

Mal A mutants were selected on the basis of their resistance to lambda phage and their inability to utilize maltose. (Hatfield et al, 1969). To select lambda resistant colonies, 0.1 ml of an overnight culture of the strain of interest was mixed with 0.1 ml of lambda phage lysate and 3 ml of F top agar, and the mixture was poured on a phage agar plate. Colonies appearing after incubation at 37°C overnight were tested on MacConkey maltose plates. White maltose non-utilizing strains were further purified as required.

TABLE I

STRAIN LIST

Strain	Genotype and/or relevant characteristics	Source
CU1008	carries an *ilvA* deletion	M. Levinthal
CU1008 (MAL)	CU1008 derivative; carries a *malA* mutation	this work
CU1008-D	CU1008 derivative; carries *glpD* mutation	this work
CU1008-K	CU1008 derivative; carries *glpK* mutation	this work
VE2-2	CU1008 derivative; carries *ssd* mutation	E.B. Newman
VE2-2 (MAL)	VE2-2 derivative; carries *ssd* and *malA* mutations	this work

Table 1 (cont)

VE2-2 (STR)	VE2-2 derivative; carries ssd and strA mutations	this work
JF-1	VE2-2 derivative; carries wild-type ssd gene and glycerol (glpK) mutation, both derived from strain #95	this work
JF-2	as JF-1; also carries malA mutation	this work
#95	glpD mutation	E.C.C.Lin
DB313	metB mutation	E.coli Genetic Stock Center
P4X	metB mutation, Hfr strain	E. coli Genetic Stock Center
4274	metB1, his-1, leu-6, argG6 mutations	E. coli Genetic Stock Center
MN-1	4274 derivative metB1, his-1, leu-6 mutations	E.B. Newman
MN-2	MN-1 derivative metB1, his-1 mutations	E.B. Newman

Table 1(cont)

MN-3	MN-2 derivative metB1, his-1, rha mutations	this work
4248	carries F'140 episome with functional malA operon and functional glpD gene	E. coli Genetic Stock Center
4289	carries F'141 episome with functional malA operon, glpD gene and ssd suppressor gene	E. coli Genetic Stock Center
MAL 157	malA deletion	M. Schwartz
7019	aroB mutation,	E. coli Genetic Stock Center
KL209	Hfr strain	E. coli Genetic Stock Center
KL16-99	Hfr strain	E. coli Genetic Stock Center
PK191	Hfr strain	E. coli Genetic Stock Center

Strains CU1008 (MAL), JF-2, and VE2-2 (MAL) were isolated in this manner.

2. Glp D mutants

Glp D mutants were selected in transductions in which the mal A mutants were the recipients and strain #95 which carries both a functional mal A operon and a glp D mutation was the donor. (Lin, Personnel Communication) Transductants which had received the functional mal A operon from strain #95 were selected on minimal maltose medium. Transductants which had in addition received the unselected glp D mutation from strain #95 were identified by their inability to grow on minimal glycerol medium. Some trandsductants of this type were isolated from each transduction performed, and named glp D mutants.

Strains CU1008-D and VE2-2-D were isolated in this manner.

3. Glp K mutants

To isolate glp K mutants, glycerol non-utilizing mutants were isolated by penicillin selection (Davis, 1948; Lederberg and Zinder, 1948). These mutants may be of two types, glp K and glp D. These were differentiated by plating on minimal L-α-glycerol phospate medium and on minimal glycerol medium. Glp K mutants are able to utilize L-α-glycerol phosphate, but not glycerol; glp D mutants

utilize neither (Lin, 1976).

For this isolation, an overnight culture was irradiated with ultra-violet light and then grown in minimal glucose medium. The culture was subcultured into minimal glycerol medium and incubated for 4-5 hours to allow those cells capable of utilizing glycerol to grow. Penicillin G (1000 units/ml) was added and the cultures incubated several hours more. Since penicillin kills only growing cells, this should permit the killing of cells able to utilize glycerol, while preserving the glycerol non-utilizing non-growing cells.

After incubation with penicillin, 0.1 ml aliquots of the culture and of several dilutions of the culture were plated on MacConkey glycerol plates. White glycerol-non-utilizing colonies were purified and tested for growth on minimal L-α-glycerol phosphate medium and on minimal glycerol medium. Strain CU1008-K was isolated in this manner.

4. Rha mutants

Rha mutants were isolated by the same penicillin selection method as were glp K mutants using rhamnose instead of glycerol and screening on MacConkey rhamnose. Strain MN-3 was isolated in this manner.

D. Preparation of P1cm phage lysates

P1 phage lysates were prepared in two steps. First, a P1cm lysogen of the host strain was isolated. Second, a culture of the lysogen was grown to a high cell density and induced by heat shock.

Step 1: A streak of a P1 cm lysate made on any wild-type E. coli strain was laid down on a Luria broth agar plate supplemented with $5X10^{-3}M$ $CaCl_2$ and 12 ug/ml chloramphenicol. A broth culture of the host strain was streaked across the phage lysate streak. The plate was incubated at 30°C overnight. Any colonies which appeared were lysogens since the P1cm phage carries a gene for chloramphenicol resistance and the bacterium does not.

Step 2: One of the lysogens was inoculated into superbroth and grown at 30°C with aeration to high cell density. The lysogen culture was then induced by incubating it at 42°C for 30 minutes. The lysogen culture was then returned to 30°C until it lysed.

The lysed culture was treated with 0.5 ml of chloroform, and then centrifuged to remove cellular debris. The supernatant was kept over chloroform at 4°C and used as a transducing lysate for genes carried by the host strain.

E. Transduction

Method I

The recipient strain was cultured in 5 ml of Luria broth at 30°C. The culture was supplemented with $CaCl_2$ to a concentration of $5X10^{-3}M$. As a control, 0.1 ml of the culture was plated on the selection medium and incubated at 30°C. Following this, 0.3 ml of donor strain P1 phage lysate was added to the culture. The infected culture was incubated at room temperature for 30 minutes, then centrifuged and its cells were re-suspended in 0.5 ml of 0.9% NaCl. For transductant plates, duplicate 0.1 ml samples of this re-suspension were plated on selective medium. These plates were incubated at 30°C. Transductants usually appeared between 3-5 days.

Method II

The recipient strain was cultured overnight at 30°C in 5 ml of Luria broth. The recipient cells were centrifuged and then re-suspended in 5 ml of MC buffer pH 7. This suspension was put on a rotor at 30°C for 10 minutes.

Three different transducing mixtures were then prepared and these contained:

1. 0.1 ml of the cell suspension and 0.1 ml of the donor strain P1 phage lysate.

2. 0.1 ml of the cell suspension and 0.1 ml of a 1:10 dilution of the donor strain P1 phage lysate.

3. 0.1 ml of the cell suspension and 0.1 ml of a 1:100 dilution of the donor strain P1 phage lysate.

One control mixture was also prepared and it contained:

1. 0.1 ml of the cell suspension and 0.1 of super broth (No donor strain P1 phage lysate).

These mixtures were incubated for 20 minutes at 30°C, then 0.2 ml of 1M sodium citrate and 3 ml of F top agar were added. The contents were quickly poured onto selective medium. The plates were incubated at 30°C. Transductants usually appeared between 3 to 5 days.

F. F'episome transfers

In these experiments a donor strain carrying an F' episome was mated with a recipient strain and recipient cells which had received the F' episomes were selected. The spot mating technique was employed. For this spot mating, a selective medium agar plate was divided into 3 sections. Onto one section, a drop of donor cells suspended in 0.9% saline was applied. Onto the second, a drop of recipient cells suspended in 0.9% saline was applied. Onto the third, both donor and recipient cells were spotted. The plates were incubated at 37°C. Only the third section showed confluent growth.

In the experiments described in the results section, the selective medium used was minimal maltose medium supplemented with isoleucine and valine. The donor strains used cannot grow on this selective medium because they require some nutritional factors that are not supplied. The recipient strains used cannot grow on this selective medium because they carry mal A mutations and so cannot utilize maltose as the sole carbon source. Only recipient cells which receive an F' episome carrying a functional mal A operon can grow.

G. Conjugation

The donor Hfr strains and the recipient, strain VE2-2 (STR) were grown to cell densities that were approximately $5X10^8$ cells/ml. Mating mixtures were prepared by mixing 0.5 ml of the donor culture with 10 ml of the recipient culture.

Samples of 0.2 ml from the mating mixture were taken immediately after mixing and at other specified times, diluted into 0.8 ml of 0.9% saline, and then vortexed vigorously for 30 seconds to break apart mating pairs. 3 ml of F top agar was then added to these dilutions, and the contents were quickly poured onto selective medium which contained streptomycin (100 ug/ml). The plates were incubated at 37°C. Recombinants usually appeared between 2-5 days.

H. Assay for serine deaminase

L-serine deaminase was assayed according to the method of Pardee and Prestige (1955) as modified by Isenberg and Newman (1974). This assay measures the amount of pyruvate produced from serine. The reaction mixtures contained 0.3 mls of washed cells suspended to 95 Klett units (No. 54 filter) in 0.05M phosphate buffer (pH 6.4), 0.1 ml of L-serine (20 mg/ml), and 0.02 ml of toluene. The assay mixtures were incubated at 37°C for 35 minutes. The reactions were stopped by adding 0.9 ml of 2,4-dinitrophenolhydrazine in 4.1% HCl. The mixtures were incubated for a further 20 minutes at room temperature. Then 1.7 ml of 10% NaOH was added. Pyruvate as its dinitrophenylhydrazone was measured by the absorbance on a Klett-Summerson color meter (No. 54 filter). Included in the assay was one assay mixture to which no serine was added. This provided a measure of endogenous -ketoacid production. Activity is expressed as the difference between the amounts of keto acid formed in the assay mixtures with and without serine, related to the cell density added to the assay mixtures.

I. Succinate Utilization

The ability of strains to utilize succinate as a carbon source was determined by two methods. In the first, the strains were simply streaked onto minimal succinate medium plates and incubated at 37°C or 30°C for a period of 5 days. Strains which utilized succinate grew; strains which did not utilize succinate did not grow. Lin (1962) developed the following method. Here, the strains were spotted on minimal tryptone succinate medium plates and incubated at 37°C or 30°C overnight. The plates were sprayed with a solution containing 10g of tetrazolium indicator dissolved in 1 litre 1M phosphate buffer pH 7, then incubated for 1 hour at 37°C or 30°C. Strains which utilized succinate reduced the tetrazolium indicator and turned red; strains which did not utilize succinate did not reduce the tetrazolium indicator and remained white.

RESULTS

The experiments which are described in this results section mapped the pleiotropic *ssd* mutation at 86 minutes on the *E. coli* chromosome, very close to the rhamnose operon.

The *ssd* mutation was discovered in strain VE2-2 and causes that strain to express high serine deaminase activity, an inability to utilize succinate as a carbon source, an increased sensitivity to high sodium chloride concentrations, high flouride concentrations, and low pH, and an inability to grow under anaerobic conditions (Morris and Newman, 1980).

The results section is made up of the following ten parts. Parts I-VIII deal with the mapping of the *ssd* mutation.

Part I describes the preliminary conjugation experiments which suggested that the *ssd* mutation was located near the *ilv A* gene which is located at 83 minutes (Ramakrishman and Adelberg, 1965).

Part II describes the transduction to strain VE2-2 which established that the wild-type *ssd* gene is near a glycerol mutation on the donor strain #95 chromosome. Since strain #95 was known to carry a single glycerol mutation, that being a *glp D* mutation at 74 minutes (Cozzarelli,

Freedberg, and Lin, 1968), it was at first assumed that the wild-type ssd gene was near this glp D mutation. It was later demonstrated that this strain also carries a glp K mutation which is at 86.5 minutes (Cozzarelli and Lin, 1966) and that the wild-type ssd gene is located near the glp K mutation.

Strain JF-1 was obtained from this transduction and it carries both the wild-type ssd gene and the glycerol mutation from strain #95.

Part III describes the transductions to strain JF-1 and its derivative, strain JF-2, which demonstrated that the ssd mutation is near a functional glycerol gene on the strain VE2-2 chromosome.

Part IV describes the transductions to strains CU1008-D and #95 which carry mutations in their glp D genes (this thesis; Cozzarelli, Freedberg, and Lin, 1968) which failed to establish linkage between the ssd mutation and the functional glp D gene on the strain VE2-2 chromosome.

Part V describes how it was determined that strain JF-1 carries a mutation in its glp K gene, a process which took the following steps.

i. Transductions to strain JF-1 which strongly suggested that its glycerol mutation derived from strain #95 was not in its glp D gene.

ii. An F' cross is also described which verified that the strain JF-1 glycerol mutation derived from strain #95 was not in its glp D gene.

iii. An analysis of glycerol metabolism in E. coli, from which it is seen that strain #95 which carries a glp D mutation could have gained a second glycerol mutation in its glp K gene when it was grown in rich media.

iv. A transduction to strain MN-1 with strain #95 P1 phage lysate is described which demonstrated that the strain #95 P1 lysate carries a glp K mutation.

Part VI describes a transduction to strain CU1008-D which demonstrated that the ssd mutation is near a functional glp K gene which is located at 85.5 minutes on the strain VE2-2 chromosome (Cozzarelli and Lin, 1966).

Part VII describes transductions to strains MN-2 and P4X which demonstrated that the ssd mutation is near a functional met B gene at 87 minutes on the strain VE2-2 chromosome (Jacob and Wollman, 1961; Pittard, Lewtit, Adelberg, 1963).

Part VIII describes a 3-point transduction which located the ssd mutation between the met B gene and the rha operon, nearer the rha operon which is located at 86 minutes on the strain VE2-2 chromosome (Power, 1967).

Part IX describes a reversion study which proved that strain VE2-2's complex phenotype is due to the single ssd mutation.

Part X describes an experiment in which it was found that the F' 141 episome carries a suppressor mutation which is active against the ssd mutation.

PART I

The mapping procedure was started by mating strain VE2-2 (STR) with four Hfr strains to find an Hfr strain which is a good donor for the wild-type ssd gene so that it could be used in a subsequent interrupted mating study.

The four strains that were mated with strain VE2-2 (STR) were P4X, KL209, PK191, and KL16-99. The origins and directions in which these strains transfer their chromosomes are illustrated in Figure 1.

The Hfr strains and the recipient, strain VE2-2 (STR) were mated for 60 minutes and then a sample from each mating mixture was taken, vortexed vigourously to break apart mating pairs, diluted, and then poured onto minimal succinate medium supplemented with isoleucine, valine, and streptomycin to select for wild-type ssd recombinants.

Strains P4X, KL209, and PK191 produced high numbers of wild-type ssd recombinants as shown in Table 2, and so were considered to be good donors.

To locate the wild-type ssd gene more precisely, the following interrupted mating study was performed.

Strains VE2-2 (STR) and P4X were mixed. Samples from the mating mixture were taken at 0, 10, 20, 30, 40, and 50 minutes, vortexed vigourously to break apart mating pairs,

FIGURE I

The genetic map of E. coli indicating the polarity and origins of four HFr strains used in this study

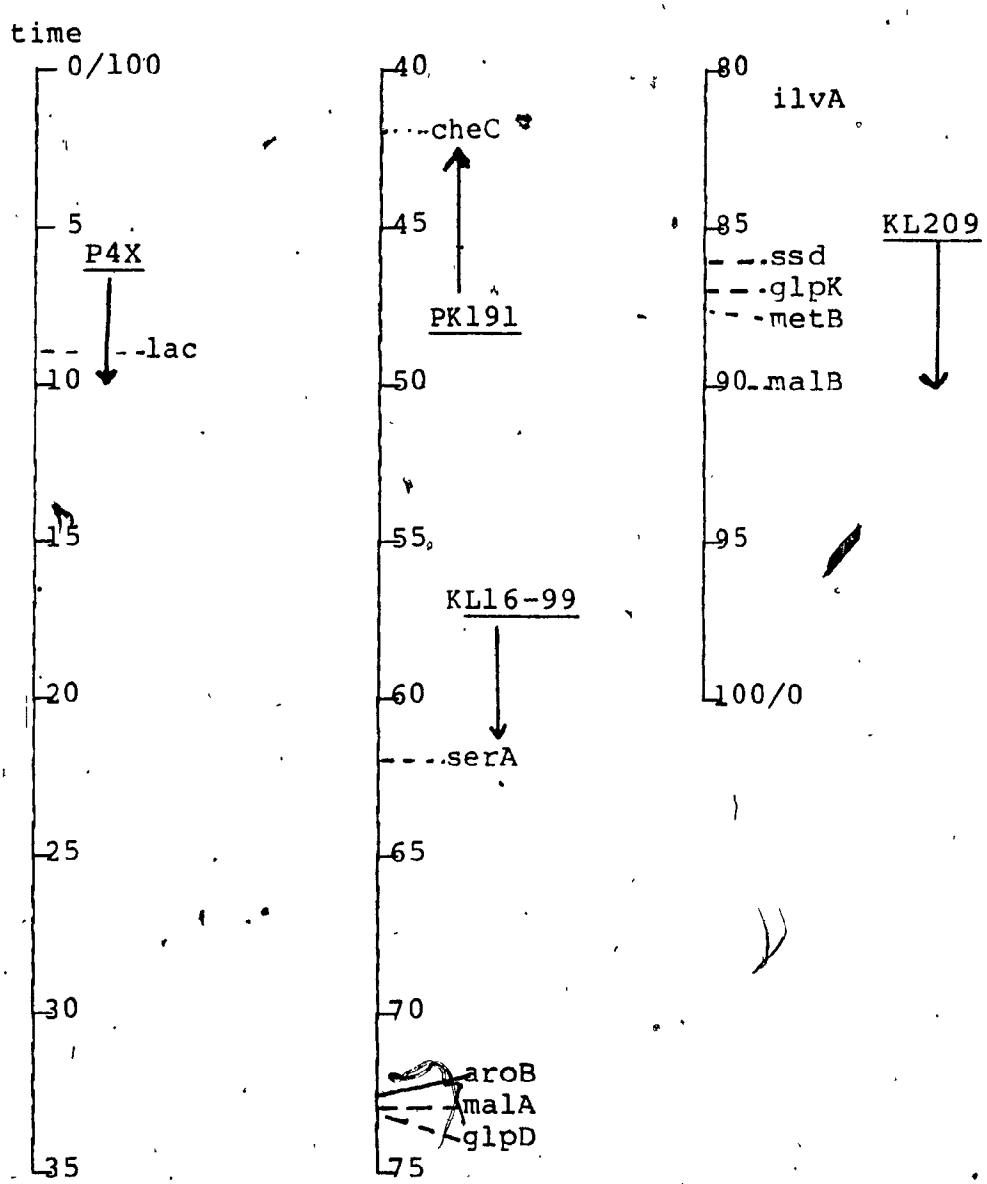


TABLE II

Initial Hfr x recipient crosses to determine which Hfr strains were efficient donors of the wild-type *ssd* gene.

Hfr x recipient cross	Number of wild-type ssd recombinants per 0.1 ml. of mating broth after a 60 minute mating.
P4X x VE2-2 (STR)	1000
KL209 x VE2-2 (STR)	2000
PK191 x VE2-2 (STR)	100
KL16-99 x VE2-2 (STR)	none

then plated on minimal succinate medium supplemented with isoleucine, valine, and streptomycin to select for wild-type ssd recombinants and on minimal glucose medium supplemented with only streptomycin to select for functional ilvA recombinants.

It can be seen from Table III that the number of both functional ilv A gene and wild-type ssd recombinants start to increase significantly above background levels at 40 minutes after mixing, which suggests that the ssd mutation is close to the functional ilv A gene which is located at 83 minutes on the strain P4X chromosome (Ramakrishman and Adelberg, 1965). It also suggests by implication that the ssd mutation is located close to the ilv A deletion on the recipient strain VE2-2 chromosome.

There are, however, two problems associated with the interpretation of this data.

1. Strain VE2-2 reverted frequently and produced many revertants that were able to grow with succinate. This high level of background "noise" made it impossible to determine the earliest time of marker entry by the appearance of a low number of recombinants at early times.

Early recombinants arise from matings where there are not excessive delays in effective pair formation and initiation of chromosome transfer, and so they probably more

TABLE III

Interrupted Hfr x recipient cross.

Hfr x recipient cross	P4X x VE2-2 (STR)	
Chromosomal transfer interrupted at x minutes.	Number of functional *ilv A* recombinants per 0.1 ml. of mating broth.	Number of wild-type *ssd* recombinants per 0.1 ml. of mating broth.
0	13	22
10	14	55
20	64	65
30	87	70
40	495	135
50	3250	1375
60	TNC*	2587
Number of functional *ilv A* revertants	-	0
Number of wild-type *ssd* revertants	-	24

* too numerous to count.

accurately reflect the distances between donated markers and the origin of transfer.

2. Strain P4X transfers both the wild-type ssd gene and the functional ilv A gene as late markers and consequently, these markers are both transferred to strain VE2-2 (STR) recipient cells over a period extending from 40 minutes to at least 50 minutes instead of being transferred each at one precise time. It is difficult then to determine both the order in which each marker is transferred and the distance between them on the strain P4X.

PART II

The previous mating experiment did not give a precise location for the wild-type ssd gene but it did seem to indicate that the wild-type ssd gene was located near the ilvA gene which is located at 83 minutes on the strain P4X chromosomes (Ramakrishman and Adelberg, 1965).

Numerous transductions were then done using strains which carried mutations in genes located within 10 minutes of the ilv A gene as recipients and strain VE2-2 as the donor to establish linkage between the ssd mutation and ilv A.

Among these, one transduction using strain VE2-2 as a recipient and strain #95 as a donor which carries a mutation in its glpD gene at 74 minutes (Cozzarelli, Freedberg, Lin, 1968), did establish linkage between the wild-type ssd gene and the donated glycerol mutation from strain #95. The glycerol mutation was assumed to be a glpD mutation at 74 minutes since strain #95 had previously been shown to carry only a glpD mutation (Cozzarelli, Freedberg, and Lin, 1968).

In this transduction (see Table IV, transduction I), strain VE2-2 was transduced with strain #95 P1 phage lysate,

TABLE IV

Transductions demonstrating linkage between the wild-type *ssd* gene and a glycerol mutation.

#	Recipient/Donor cross	Selected marker	Unselected marker	# of colonies scored	# of colonies with gly mutation
1	VE2-2/ #95	wild-type *ssd* gene	glycerol mutation	280	2
2	VE2-2/ #95	wild-type *ssd* gene	glycerol mutation	236	6

and a selection was made on minimal succinate medium for transductants which had received the wild-type ssd gene from strain #95. These transductants were then screened on minimal glycerol medium to determine if any had also received the glp D mutation from strain #95. It was then found that 2 of the 280 wild-type ssd gene transductants did not grow on minimal glycerol medium. These had received a mutation affecting glycerol metabolism assumed to be a glp D mutation from strain #95.

In the transduction then the wild-type ssd gene from strain #95 was the selected marker and the glycerol mutation from strain #95 was the unselected marker. Two of the transductants had received both the selected ssd gene marker and the unselected glycerol mutation marker from strain #95. This must mean that the selected ssd gene marker and the unselected glycerol mutation marker are on the same P1 transducing segment derived from strain #95 which had been received by these two transductants. Since the P1 transducing segment is only 2 minutes long, the two markers must be near each other on the strain #95 chromosome.

The transduction was done a second time (see Table IV, transduction 2), and in this case 6 out of 236 wild-type ssd transductants had received this glycerol mutation from strain #95.

Ordinarily, the co-transduction frequency between the two markers can be used to calculate the distance between them. However in these transductions the co-trandsuction frequency between the selected wild-type ssd gene and the glycerol mutation appears much lower than it ought to be due to the presence of a high number of succinate-utilizing revertants which score as having a functional glycerol gene. Therefore, in these transductions the distance between the selected wild-type ssd gene and the glycerol mutation is under-estimated.

One of the transductants from the first transduction which received both the selected wild-type ssd gene and the unselected glycerol mutation was selected and named strain JF-1.

PART III

The preceding transduction to strain VE2-2 demonstrated that the wild-type ssd gene is near a glycerol mutation on the strain #95 chromosome. However, the preceeding transduction was not completely satisfactory in that it did not provide reliable co-transduction frequency data for the wild-type ssd gene and the glycerol mutation which can be used to determine the distance between the two markers.

The following transduction to strain JF-1 (see Table V, transduction 3) again demonstrates that the ssd mutation is near a functional glycerol gene on the strain VE2-2 chromosome. This transduction also provides reliable co-transduction frequency data for the ssd mutation and the functional glycerol gene so that a distance can be calculated between these two markers on the strain VE2-2 chromosome. This transduction provides reliable co-transduction frequency data because strain JF-1 carries a stable glycerol mutation and so gave only few revertants on minimal glycerol medium which was used to select the functional glycerol gene transductants.

TABLE V

Transductions demonstrating linkage between the *ssd* mutation and a functional glycerol gene.

#	Recipient/Donor cross	Selected marker	Unselected marker	# of tds. scored	# of tds. with *ssd* mutation	Co-transduction frequency	Calculated distance between markers
3	JF-1/VE2-2	functional glycerol gene	*ssd* mutation	118	28	24%	0.8 minutes
4	JF-2/VE2-2	functional glycerol gene	*ssd* mutation	184	68	37%	0.6 minutes
5	JF-2/VE2-2	functional glycerol gene	*ssd* mutation	132	50	38%	0.6 minutes

Strain JF-1 was transduced with strain VE2-2 P1 phage lysate and a selection was made on minimal glycerol medium for transductants which had received a functional glycerol gene from strain VE2-2. These transductants were then screened on minimal succinate medium for those which had also received the unselected ssd mutation from strain VE2-2. Of the 118 transductants screened, 28 could not grow on minimal succinate medium and therefore had also received the ssd mutation from strain VE2-2.

To verify that the inability to grow on minimal succinate medium was due to the presence of the ssd mutation, 15 glycerol-positive transductants were screened for serine deaminase activity (Table VI). It was found that 9 transductants which grew on minimal-succinate medium expressed low serine deaminase activity and 6 transductants which did not grow on minimal succinate medium expressed high serine deaminase activity. Clearly then, the transductants unable to grow on minimal succinate medium had received the ssd mutation and this inability was not due to some extraneous reason.

The transduction was repeated twice more using strain JF-2 which is a derivative of strain JF-1 (see Table V, transductions 4 and 5). Strain JF-2 then was transduced with strain VE2-2 P1 phage lysate, and again a selection was

TABLE VI

Serine deaminase activities of transductants from the JF-I/VE2-2 cross.

Transductant number	Succinate utilization	Serine deaminase activity	Allele of the *ssd* gene
1	negative	0.0053	*ssd* mutation
2	negative	0.0040	*ssd* mutation
3	negative	0.0044	*ssd* mutation
4	negative	0.0041	*ssd* mutation
5	negative	0.0050	*ssd* mutation
6	negative	0.0051	*ssd* mutation
7	positive	0.0005	wild-type
8	positive	0.0005	wild-type
9	positive	0.0005	wild-type
10	positive	0.0005	wild-type
11	positive	0.0001	wild-type
12	positive	0.0001	wild-type
13	positive	0.0001	wild-type
14	positive	0.0001	wild-type
15	positive	0.0001	wild-type
Strain VE2-2	negative	0.0039	*ssd* mutation
Strain JF-1	positive	0.0004	wild-type

Serine deaminase is expressed as umoles pyruvate produced/ min./ 0.3 mls. of a cell suspension optical density 95 Kletts units.

made on minimal-glycerol medium for transductants which had received the functional glycerol gene from strain VE2-2. The transductants were screened on minimal-succinate medium and on minimal tryptone succinate medium plates which were later sprayed with tetrazolium dye, to determine those which had also received the unselected ssd mutation from strain VE2-2. In the first of the two transductions 68 out of 184 transductants did not utilize succinate as a carbon source as determined by these two methods and therefore had received the ssd mutaton. In the second transduction, 50 out of 132 transductants had received the ssd mutation.

These transductions then demonstrated that the ssd mutation and the functional glycerol gene from strain VE2-2 are co-transducible and are therefore near to each other on the strain VE2-2 chromosome.

These transductions also provide more information since the co-transduction frequencies for the ssd mutation and the functional glycerol gene can be used to estimate how near these two markers are to each other on the strain VE2-2 chromosome.

In theory, if the co-transduction frequency for two markers is high, then the two markers are close on the donor chromosome. Conversely, if the co-transduction frequency for two markers is low, then the two markers are far apart

on the donor chromosome. However, even in the latter case the two markers must be at most 2 minutes apart since the P1 phage encapsulates only 2 minutes of donor chromosome. Wu (1966) has derived a formula for calculating the distance between two markers on the donor chromosome from the co-transduction frequencies.

The co-transduction frequency obtained from the transduction to strain JF-1 is 0.236, and this corresponds according to Wu's formula to a distance of 0.8 minutes between the ssd mutation and the functional glycerol gene on the strain VE2-2 chromosome. The co-transduction frequencies obtained from the transductions to strain JF-2 are 0.369 and 0.379 and this corresponds to distances of 0.6 minutes respectively between the ssd mutation and the functional glycerol gene on the strain VE2-2 chromosome.

PART IV

The preceding transduction to strain JF-1 established that the ssd mutation was linked to a functional glycerol gene which was assumed to be the functional glpD on the strain VE2-2 chromosome.

The following transductions to strains CU1008-D and #95 which carry known glpD mutations (this thesis, see Materials and Methods; Cozzarelli, Freedberg, and Lin, 1968) were performed to test if the ssd mutation was indeed linked to the functional glpD gene on the strain VE2-2 chromosome.

Strain CU1008-D was transduced with strain VE2-2 P1 phage lysate and a selection was made on minimal glycerol medium for transductants which had received the functional glpD gene from strain VE2-2. The transductants were then screened on minimal succinate medium to determine if they had also received the ssd mutation from strain VE2-2 (see Table VII, transduction 6).

It was found to much surprise that none of the 40 transductants tested were unable to grown on minimal-succinate medium. None of the transductants then had received the ssd mutation from strain VE2-2.

Strain #95 was also transduced with strain VE2-2 P1 phage lysate and a selection was made on minimal glycerol

TABLE VII

Transductions which failed to demonstrate linkage between the functional *glp D* gene and the *ssd* mutation

#	Recipient/Donor cross	Selected marker	Unselected marker	# of tds. scored	# of tds. with *ssd* mutation	Co-transduction frequency
6	CU1008-D/VE2-2	functional *glp D* gene	*ssd* mutation	40	none	0.0%
7	#95/VE2-2	functional *glp D* gene	*ssd* mutation	371	none	0.0%

medium for transductants which had received the functional glpD gene from strain VE2-2. The transductants were then screened on minimal succinate medium to determine if they had also received the ssd mutation from strain VE2-2 (see Table VI, transduction 7).

It was found that none of the 371 transductants tested were unable to grow on minimal succinate medium. None of the transductants then had received the ssd mutation.

The transductions to strains CU1008-D and #95 then demonstrated that the ssd mutation is not linked to the functional glpD gene on the strain VE2-2 chromosome.

PART V

It appeared at this point that the evidence obtained to locate the ssd mutation was conflicting. One one side, the transduction to strain JF-1 which had been thought to carry a glpD mutation demonstrated that the ssd mutation is linked to a functional glycerol gene thought to be the glpD gene. On the other side, transductions to strains CU1008-D and #95 which carry known glpD mutations (this thesis, see Material and Methods; Cozzarelli, Freedberg, and Lin, 1968) demonstrated that the ssd mutation was not near the functional glpD gene.

It was thought necessary to verify that strain JF-1 carries a glpD mutation. Strain JF-1 was constructed from a transduction to strain VE2-2 with strain #95 P1 phage lysate in which transductants which had received the wild-type ssd gene from strain #95 were selected. The transductants were then screened to determine if they had also received a glycerol mutation from strain #95. Strain JF-1 was one transductant which had received a glycerol mutation from strain #95. It was thought that strain JF-1 had received a glpD mutation, since strain #95 carries only one known glycerol mutation, that being a glpD mutation (Cozzarelli, Freedberg, and Lin, 1968). However, strain JF-1 could have

received a different glycerol mutation from strain #95 if strain #95 had somehow gained another glycerol mutation either prior to or during the preparation of its P1 phage lysate.

The following experiments were designed to verify that strain JF-1 carries a glpD mutation at 74 minutes, but instead they prove that it does not.

i/ A transduction was performed to strain JF-1 with strain MAL P1 phage lysate (see Table VIII, transductions 8 and 9) selecting on minimal glycerol medium for transductants which had received a functional glycerol gene from the donor strains. The transductants were then screened on minimal maltose medium for those which might have also received the malA deletions which are located next to the functional glpD gene at 74 minutes on the donor chromosome (Hatfield, Hofmung, and Schwartz, 1969).

All the transductants, however, were able to utilize maltose, which indicated that there was no co-transduction with the donated functional glycerol gene which enabled the transductants to grow on minimal glycerol medium and the malA deletions. The donated functional glycerol gene then is unlikely to be the glpD gene since if it were, there should have been linkage with the malA deletions.

TABLE VIII

Transductions which demonstrated that strain JF-I does not carry a mutation in its glp D gene

#	Recipient/Donor cross	Selected marker	Unselected marker	# of tds. scored	# of tds. carrying unselected marker	Co-transduction frequency
8	JF-1/ 157	functional glycerol gene	mal A deletion	21	none	0.0%
9	JF-1/7019	functional glycerol gene	aro B mutation	105	none	0.0%
10	JF-2/7019	functional mal A operon	a functional glycerol gene enabling glycerol utilization	90	none	0.0%

A transduction was also performed to strain JF-1 with strain 7019 P1 phage lysate (see Table VIII, transduction 9) selecting transductants able to utilize glycerol which then had received the functional glycerol gene from the donor strains. The transductants were then screened to determine if they were dependent on exogenous shikimic acid, phenylalanine, tyrosine, and tryptophan for growth on minimal medium, which would have indicated that they had also received the aro B mutation located at 73.5 minutes on the donor chromosome (Pittard and Wallace, 1966).

All the transductants, however, grew on minimal medium without the aromatic amino acids which indicated that there was no co-transduction with the donated functional glycerol gene which enabled the transductants to utilize glycerol and the aro B mutation. The donated functional glycerol gene then is unlikely the glp D since if it was, there should have been linkage with the aro B mutation.

It was concluded from these two sets of transductions to strain JF-1 that strain JF-1 probably carries a mutation in a glycerol gene other than its glp D gene and that the transductants received the corresponding functional glycerol gene from the donor strains to be able to utilize glycerol.

ii. In the next experiment, strain JF-2, a derivative of strain JF-1 which carries the same glycerol mutation and also a mutation in its mal A operon, was transduced with strain 7019 P1 phage lysate and a selection was made on minimal maltose medium for transductants which had received the functional malA operon from strain 7019 (see Table VIII, transduction 10). The transductants were then screened on minimal glycerol medium, since some of the transductants must have also received the functional glpD gene from strain 7019.

None of the transductants, however, was able to grow on minimal glycerol medium which indicates that the co-transduced functional glpD gene was not able to give the transductants the ability to utilize glycerol.

It was concluded then, that since a donated functional glpD gene does not restore the ability of strain JF-2 to utilize glycerol, that strain JF-2 and therefore strain JF-1 most likely carry mutations in a glycerol gene other than their glpD gene.

TABLE IX

F′ x JF-2 crosses which demonstrated that strain JF-2 does not carry a glp D mutation but instead must carry a mutation in another glycerol gene.

merodiploid	properties of the F used	Merodiploid selection	Merodiploid phenotype	Probable merodiploid genotype (F′/JF-2)
F′ 140/JF-2	carries a functional mal A operon and a functional glp D gene on a chromosome segment extending from 68-80 minutes.	maltose utilization	maltose utilizing, glycerol non-utilizing	functional mal A operon, functional glp D gene / mal A mutation functional glp D gene, and a mutation in another glycerol gene.
F′ 141/JF-2	carries a functional mal A operon and a functional glp D gene on a chromosome segment extending from 68-74 minutes	maltose utilization	maltose utilizing, glycerol non-utilizing	functional mal A operon, functional glp D gene / mal A mutation functional glp D gene, and a mutation in another glycerol gene.

ii/ The preceeding transductions did not rule out the possibility that some kind of local genetic rearrangement could have occurred on the strain JF-1 chromosome which would have placed the glpD mutation at a greater distance from its malA operon and aroB gene, and thereby interfered with the co-transduction of donor markers.

To remove this possibility the following experiment which employed F' episomes was performed (see Table IX). The donors were strains 4289 and 4248 which carry the F'140 and F'141 episomes respectively (Bachmann, personal communication). The F'140 episome carries a chromosomal segment which extends from 68 to 80 minutes and it carries a functional glp D gene and a functional mal A (Low, 1972) operon. The F'141 episome carries a chromosomal segment which extends from 68 to 74 minutes and it also carries a functional glp D gene and a functional mal A operon (Low, 1972). The recipient was strain JF-2 which carries both a glycerol mutation and a mal A mutation and so it cannot grow on both minimal glycerol medium and minimal maltose medium.

Strains 4289 and JF-2 and strain JF-2/F'140 merodiploids which had received the F'140 episome carrying its functional mal A operon were selected on minimal maltose medium. Strains 4248 and JF-2 were also mixed and strain JF-2/F'141 merodiploids which had received the F'141 episome

carrying its functional mal A operon were selected. These merodiploids then had received either the F'140 or F'141 episome carrying their functional glp D genes. However, these merodiploids did not grow on minimal-glycerol medium.

It is obvious that for strain JF-2 to grow on minimal-glycerol medium another functional glycerol gene located outside the chromosomal regions from 68 to 80 minutes and from 68 to 74 minutes covered by the F'141 and F'140 episomes respectively is required. It was concluded then that strain JF-2 and therefore its parent, strain JF-1, must carry a mutation in another glycerol gene and not a glp D mutation.

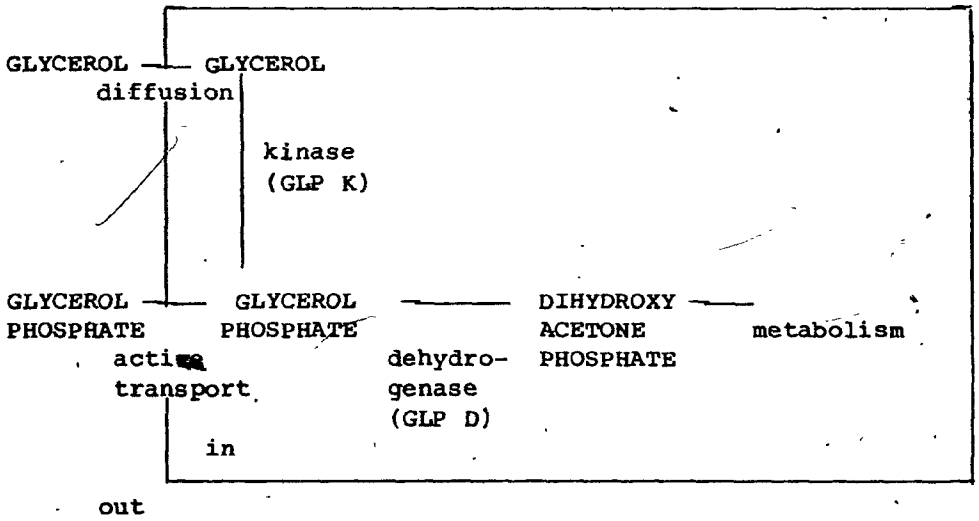
If strain JF-1 then carries a mutation in another glycerol gene and this mutation is derived from strain #95 P1 phage lysate, then strain #95 which is known to carry only a glpD mutation must have gained a second mutation in another glycerol gene.

In order to understand how strain #95 could have gained a second glycerol mutation, some aspects of glycerol metabolism in Escherichia coli are discussed.

In E. coli, glycerol enters the cell by facilitated diffusion (Sanno, Wilson, and Lin; 1968) and then is phosphorylated by an ATP-dependent glycerol kinase to L-α-glycerol phosphate (Hayaski and Lin; 1965), which in

Figure 2

Pathways for the dissimilation of glycerol
and glycerol-phosphate in E. coli.
(Cozzarelli, N.R. W.B. Freedberg, and E.C.C.Lin, 1968.)



turn is converted by L-α-glycerol phosphate dehydrogenase to dihydroxyacetone phosphate (Cozzarelli, et al., 1965), a glycolytic intermediate.

The genes that code for these transport proteins and enzymes that are involved in aerobic glycerol metabolism are located at two widely separated sites on the E. coli chromosome but their expression is co-regulated (Cozzarelli, Freedberg, and Lin; 1968).

The glpK gene codes for the ATP-dependent glycerol kinase, and is located at 86.5 minutes (Cozzarelli and Lin; 1966).

The glpD gene codes for for L-α-glycerol phosphate dehydrogenase, and is located at 74 minutes (Cozzarelli, Freedberg, and Lin; 1968).

Strain #95 carries a mutation in its glpD gene and so it expresses a defective L-α-glycerol phosphate dehydrogenase (Cozzarelli, Freedberg, and Lin; 1968). Strain #95 then cannot utilize glycerol as a carbon source, since its glycerol metabolism is blocked by its inability to convert L-α-glycerol phosphate to dihydroxyacetone phosphate.

In addition, and what is relevant to this study, strain #95 cannot grow on certain other carbon sources in the presence of glycerol (Cozzarelli, et al; 1965). Strain #95

when confronted with glycerol, takes it up from the medium and converts it to L-α-glycerol phosphate, but it cannot convert L-α-glycerol phosphate to dihydroxyacetone. L-α-glycerol phosphate then accumulates and reaches concentrations inhibitory to growth.

Strains like strain #95, which carry a glpD mutation, can overcome the growth inhibition caused by the presence of glycerol by gaining a second glycerol mutation in its glpK gene (Cozzarelli et al; 1965). These strains then will express a defective glycerol kinase (Cozzarelli and Lin; 1966), and so will be unable to convert the incoming glycerol to toxic L-α-glycerol phosphate.

Strain #95 was cultured on rich media during its routine handling and during the preparation of its P1 phage lysate. It was later learned from Dr. E.C.C. Lin (personal correspondence) that these rich media contain trace quantities of glycerol which are possibly growth inhibitory. It is possible then that strain #95 gained a glpK mutation, which might be carried by the strain #95 P1 phage lysate which was used to transduce strain JF-1.

To test whether the strain #95 P1 phage lysate carries a glpK mutation located at 86.5 minutes, the following transduction was carried out. Strain MN-1, which carries a metB mutation located at 87 minutes and requires exogenous methionine to grow on minimal medium (Jacob and Wollman,

TABLE X

Transductions demonstrating that strains #95 and JF-1 carry *glp K* mutations.

#	Recipient/Donor cross	Selected marker	Unselected marker	# of tds. scored	# of tds. with *glp K* mutation	Co-transduction frequency
11	MN-1/ #95	functional *met B* gene	*glp K* mutation	76	52	68.4%
12	MN-1/JF-1	functional *met B* gene	*glp K* mutation	72	32	44.4%

1961; Pittard, Lowtit, and Adelberg, 1963), was transduced with strain #95 P1 phage lysate (see Table X, transduction 11). Methionine independent transductants which had received the functional metB gene from strain #95 were selected. These transductants were then screened on minimal glycerol medium to determine if any had also received a glpK mutation from the strain #95 P1 phage lysate.

It was then found that of the 76 transductants screened on minimal glycerol medium, 52 did not grow indicating that they had received a glpK mutation from the strain #95 P1 phage lysate.

The preceeding experiment proved that the strain #95 P1 phage lysate carries a glpK mutation. It is possible then that strain JF-1 carries a glpK mutation derived from a transduction in which strain VE2-2 was transduced with strain #95 P1 phage lysate.

To test this possibility, strain MN-1 was transduced with P1 phage lysate prepared on strain JF-1, and a selection was made on minimal medium for transductants which had received the functional metB gene from strain JF-1 (see Table X, transduction 12). The transductants were then screened on minimal glycerol medium to determine if they had also received a glpK mutation from strain JF-1.

It was found that of the 72 transductants screened on minimal glycerol medium, 32 did not grow, indicating that they had received a glpK mutation from strain JF-1. The transduction then proves that strain JF-1 carries a glpK mutation.

The story now seems to be clear. Strain JF-1 carries a glpK mutation derived from the strain #95 P1 phage lysate. When strain JF-1 was transduced with strain VE2-2 P1 phage lysate and a selection was made for transductants that grew on minimal glycerol medium, the transductants selected probably had received a functional glpK gene from strain VE2-2. Some of these transductants had also received the unselected ssd mutation from strain VE2-2 which means that the ssd mutation is probably linked to the functional glpK gene on the strain VE2-2 chromosome.

PART VII

The next transduction is to strain CU1008-K which carries an identified glp K mutation (this thesis, see Materials and Methods) and was performed to verify that the ssd mutaton is indeed near a functional glp K gene located at 86.5 minutes on the strain VE2-2 chromosome (Cozzarelli and Lin, 1960).

Strain CU1008-K was transduced with strain VE2-2 P1 phage lysate and a selection was made on minimal glycerol medium for transductants which had received a functional glp K gene from strain VE2-2 (see Table XI, transduction 13). The transductants were then screened on minimal-succinate medium and also on minimal tryptone succinate medium plates which were later sprayed with tetrazolium dye, to determine if any of the transductants had also received the ssd mutation from strain VE2-2. It was found that 35 out of 74 transductants could not utilize succinate as a carbon source as determined by these two methods. The 39 out of 80 transductants then had also received the ssd mutation from strain VE2-2. This represents a co-transduction frequency of 0.47, which according to Wu's formula means that the ssd and the functional glp K gene are 0.4 minutes apart on the strain VE2-2 chromosome.

PART VIII

It should be possible to determine linkage between the ssd mutation and other functional genes located in the region of 86.5 minutes on the strain VE2-2 chromosome.

Strain MN-2 which carries a metB mutation (Bachmann, personal communication) at 87 minutes (Jacob and Wollman, 1961; Pittard, Lowtit, and Adelberg, 1963) was transduced with strain VE2-2 P1 phage lysate and a selection was made for transductants which had received a functional met B gene from strain VE2-2 (see Table XI, transduction 14). These transductants were then screened on minimal succinate medium and on minimal tryptone succinate medium plates which were later sprayed with tetrazolium dye to determine if any had also received the ssd mutation from strain VE2-2. Among the 80 transductants screened, 39 were found to be unable to utilize succinate as a carbon source as determined from these two tests. These 39 out of 80 transductants then had also received the ssd mutation.

This represents a co-transduction frequency of 0.49, for the met B and the ssd mutation which corresponds to a distance of 0.4 minutes between the two markers on the strain VE2-2 chromosome.

TABLE XI

Transductions demonstrating linkage between the *ssd* mutation and a functional *glp K* gene, and between the *ssd* mutation and the functional *met B* gene.

#	Recipient/Donor cross	Selected marker	Unselected marker	# of tds. scored	# of tds. with *ssd* mutation	Co-transduction frequency	Calculated distance between markers
13	CU1008-K/VE2-2	functional *glp K* gene	*ssd* mutation	74	35	47.3%	0.4 minutes
14	MN-2/VE2-2	functional *met B* gene	*ssd* mutation	80	39	49.0%	0.4 minutes
15	P4-X/VE2-2	functional *met B* gene	*ssd* mutation	96	12	12.5%	1.00 minutes

Only one of those transductants which had received the ssd mutation as determined by its inability to grow on minimal succinate medium was tested for its serine deaminase activity, that transductant showed high activity. The correlation between high serine deaminase activity and the inability to utilize succinate as a carbon source is tested more rigorously in the next cross.

Strain P4X which also carries a met B mutation (Jacob, Brenner, and Cuzim, 1963; Jacob and Wollman, 1957; Jacob and Wollman, 1961) was also transduced with strain VE2-2 P1 phage lysate, and a selection was made for transductants which had received a functional met B gene from strain VE2-2 (see Table XI, transduction 15). The transductants were then screened on minimal tryptone succinate medium which were later sprayed with tetrazolium dye, to determine if any had received the ssd mutation. Among the 96 transductants screened, 12 were unable to utilize succinate as a carbon source. These 12 of 96 transductants then had received the ssd mutation. This represents a co-transduction frequency of 0.125 between the functional met B gene and the ssd mutation, which corresponds to a distance of 1.0 minutes between the two markers on the strain VE2-2 chromosome.

TABLE XII

Serine deaminase activities expressed by transductants from P4X/VE2-2 cross.

Transductant number	Succinate utilization	Serine deaminase activity	Allele of the ssd gene
1	negative	0.013	ssd mutation
2	negative	0.013	ssd mutation
3	negative	0.0098	ssd mutation
4	negative	0.0098	ssd mutation
5	negative	0.0071	ssd mutation
6	positive	0.00094	wild-type
7	positive	0.00061	wild-type
8	positive	0.00061	wild-type
Strain VE2-2	negative	0.0094	ssd mutation
Strain P4-X	positive	0.0015	wild-type

* Serine deaminase activity expressed as, umoles pyruvate/min./ 0.3 mls. of a cell suspension optical density 95 Klett units.

To verify that the inability to utilize succinate was due to the presence of the ssd mutation, 8 transductants were screened for serine deaminase activity (see Table XII). It was found that the 5 transductants which did not utilize succinate expressed high serine deaminase activity and the 3 transductants which did utilize succinate expressed low serine deaminase activity. This perfect correlation for succinate non-utilization and high serine deaminase activity strongly suggests that those 5 transductants which were unable to utilize succinate had indeed received the ssd mutation.

The co-transduction frequency between the met B gene and the ssd mutation is much lower when the transduction is done to strain P4X than when the transduction is done to strain MN-2. However, this co-transduction variability is common when transductions are done to different strains (Beckwith, personal communication). The co-transduction frequency then cannot be relied upon to accurately locate the ssd mutation, and so a 3-point transduction is usually used to locate mutations. Such a 3-point transduction which was used to locate the ssd mutation is described in the next section.

PART IX

The preceeding 2-point transductions demonstrated that the *ssd* mutation was near the functional *glp K* and *met B* genes on the strain VE2-2 chromosome. However, co-transduction frequencies are variable and so an accurate placement of the *ssd* mutation could not be made.

The following 3-point transduction was used to locate the *ssd* mutation more precisely (see Table XIII). Strain MN-3 was the recipient strain and it has the following relevant genotype; a mutation in its *rha* operon (this thesis; see Materials and Methods) at 86 minutes (Power, 1967), a mutation in its *met B* gene (Bachmann, personal communication) at 87 minutes (Jacob and Wollman, 1961; Pittard, Lowtit, and Adelberg, 1963), and a wild-type *ssd* gene located around this 86-87 minute region. Strain VE2-2 was the donor strain and it has the following relevant genotype; a functional *rha* operon at 86 minutes, a functional *met B* gene at 87 minutes and an *ssd* mutation located around this 86-87 minute region.

Strain MN-3 (Rha^-, Ssd^+, Met^-) then was transduced with strain VE2-2 (Rha^+, Ssd^-, Met^+) P1 phage lysate and a selection was made on minimal glucose medium left unsupplemented with methionine to select for methionine

TABLE XIII

The 3-point cross which demonstrated that the *ssd* mutation is located between the *met B* gene and the *rha* operon.

Recipient/Donor cross		MN-3/VE2-2	
Selected marker		functional *met B* gene	
Transductant class #	Genotype of Transductant class	Phenotype of transductant class	Frequency of transducant class
1	*rha* mutation wild-type *ssd* gene, functional *metB* gene	(Rha^-,Ssd^+,Met^+)	61.6%
2	*rha* mutation, *ssd* mutation functional *metB* gene	(Rha^-,Ssd^-,Met^+)	7.6%
3	functional *rha* operon, *ssd* mutation, functional *met B* gene	(Rha^+,Ssd^-,Met^+)	28.8%
4	functional *rha* operon, wild-type *ssd* gene, functional *metB* gene	(Rha^+,Ssd^+,Met^+)	2.0%

independent transductants which had received the functional met B gene from strain VE2-2. Then 198 transductants were screened to determine whether their rha and ssd markers were derived from strain MN-3 or strain VE2-2.

The following 4 transductant classes resulted:

Class I (Rha^-, Ssd^+, Met^+) transductants carry the wild-type ssd gene from strain MN-3 and the rha mutation also from strain MN-3.

Class II (Rha^-, Ssd^-, Met^+) transductants carry the ssd mutation from strain VE2-2 and the rha mutation from strain MN-3.

Class III (Rha^+, Ssd^-, Met^+) transductants carry the ssd mutation from strain VE2-2 and the functional rha gene also from strain VE2-2.

Class IV (Rha^+, Ssd^+, Met^+) transductants carry the wild-type ssd gene from strain MN-3 and the functional rha gene from strain VE2-2.

The distribution of the transductant classes was as follows: Class I (Rha^-, Ssd^+, Met^+), 62%; Class II (Rha^-, Ssd^- Met^+), 7.6%; Class III (Rha^+, Ssd^-, Met^+), 29%; Class IV (Rha^+, Ssd^+, Met^+), 2%.

The position of the ssd mutation in relation to the metB and rha genes can be determined from this data.

If the gene order were rha, ssd, met B), then transductant classes I, II, and III should be common since these result from a single recombination event between the strain VE2-2 transducing segment and the strain MN-3 chromosome but the transductant class IV should be rare since it results from two recombination events. If the gene order were (ssd, rha, met B) then transductant classes I, III, and IV should be common since they result from a single recombination event but the transductant class II should be rare since it results from a double recombination event. If the gene order were (rha, met B, ssd) then all four transductant classes should be common since all result from a single recombination event.

One can identify the correct gene order by comparing the predicted transductant class distribution for each of the three gene orders with the actual transductant class distribution obtained. It was concluded then that the gene order was (rha, ssd, met B) since classes I, II, and III were common and class IV was rare (2%).

The relative distances between the ssd mutation and the rha genes at 86 minutes and the met B gene at 87 minutes can also be determined from the transductant class distribution data.

To determine the distance between the met B gene and the ssd mutation, the following formula derived by Wu (1963) is used.

$$N1 = 1 - (1 - t/k)^3$$

where, N1 = number of class i. / total number of transductants / transductants / excluding class iv. / which are numerically / insignificant.

k = length of transducing segment = 2 minutes.

t = distance between the met B gene and ssd mutation.

By inserting the values, obtained in these experiments, the following equation is obtained.

$$0.63 = 1 - (1 - t/2)^3$$

which when solved for t gives,

$$t = 0.56 \text{ minutes.}$$

Therefore, the distance as calculated between the met B and the ssd markers is 0.56 minutes.

To determine the distance between the ssd mutation and the rha genes the following formula again derived by Wu (1963) is used.

$$N2 = (1 - t/2)^3 - (1 - s/2 - t/2)^3$$

where, N2 = number of class ii./total number of transductants / transductants excluding / class iv., which are /numerically insignificant.

k = length of transducing segment = 2 minutes.

t = distance between the met B gene and the ssd mutation.

s = distance between the ssd mutation and rha genes.

By inserting the values the following equation is obtained.

$$0.077 = (1 - 0.28)^3 - (1 - s/2 - 0.28)^3$$

which when solved for s gives,

s = 0.106 minutes.

Therefore, the distance as calculated between the ssd mutation and the rha genes is 0.106 minutes.

In conclusion then the ssd mutation is located between the rha genes at 86 minutes and the met B gene at 87 minutes, and it is closer to the rha genes.

The mapping of the ssd mutation then is completed.

PART IX

To determine if the ssd mutation is a single pleiotropic gene, independent revertants which had regained the ability to grow on minimal succinate medium were selected from strain VE2-2, and then their serine deaminase activities were assayed (see Table XIV). Eleven independent revertants were assayed for serine deaminase activity. Of these, 8 expressed low serine deaminase activity, comparable to that found in strain CU1008 which is the parent strain of strain VE2-2 and of these revertants. It is clear that the mutation in each of these revertants which restores the ability to grow on minimal succinate medium also restores low serine deaminase activity.

The mutation in each or some of these revertants may have occurred within the ssd gene in which the ssd mutation is located, and thereby restored at least quasi-normal function to the gene. Or the mutation in each or some of these revertants may have occurred in another gene and then suppressed the ssd mutation. In either case, the fact that one mutation restores both the ability to grow on minimal succinate medium and low serine deaminase activity proves that the ssd mutation is pleiotropic.

TABLE XIV

Serine deaminase activities expressed by revertants which have regained the ability to utilize succinate.

Revertant number	Serine deaminase activity	Revertant number	Serine deaminase activity
1	0.0013	7	0.0015
2	0.0012	8	0.0009
3	0.0009	9	0.0033
4	0.0009	10	0.0074
5	0.0012	11	0.0095
6	0.0013		
Strain VE2-2 (ssd mutant)	0.0094	Strain CU1008 (parent)	0.0009

Serine deaminase is expressed as umoles pyruvate/min./0.3 mls. of a cell suspension optical density 95 Kletts units.

Of the 11 revertants, 3 expressed intermediate and high activities. It is clear that the mutation in each of these revertants which restores the ability to grow on minimal succinate medium either changes the high serine deaminase activity found in strain VE2-2 to intermediate activities or does not affect it.

The mutation in each or some of these latter revertants may have occurred in the ssd gene and then restored only partial function to that strain. Or the mutation in each or some of these revertants may have occurred in another gene and then partially suppressed the ssd mutations. Thirdly, the mutation in those revertants which still express very high serine deaminase activities may have occurred in another gene and then suppressed only the succinate negative character of the ssd mutation.

PART X

The experiments described in parts I-VIII unequivocally map the ssd mutation next to the rhamnose operon at 86 minutes. The experiment described here demonstrated that the F'140 episome carries a suppressor gene which suppresses the expression of the ssd phenotype.

This experiment involved the donor strains 4248 and 4289 which carry the F'episomes, F'141 and F 140 respectively (Bachmann, personal communication), and the recipient, strain VE2-2 (MAL)(see Table XV). The F'140 episome carries a chromosomal segment which extends approximately from 68 to 80 minutes and it carries a functional mal A operon at 74 minutes (Low, 1972). The F' 141 episome carries a chromosomal segment which extends approximately from 68 to 74 minutes and it also carries a functional mal A operon at 74 minutes (Low, 1972). Strain VE2-2 (MAL) carries an ilv A deletion (Levinthal, personal communication) at 83 minutes (Ramakrishman and Adelberg, 1965), the ssd mutation at 86 minutes, and a mal A mutation (this thesis, see Materials and Methods) at 74 minutes (Schwartz, 1966).

Strains 4289 and VE2-2 (MAL) were crossed and strain VE2-2 (MAL) /F'140 merodiploids which had received the F'140 episome carrying the functional mal A operon were selected.

TABLE XV

F′ x VE2-2 (MAL) crosses which demonstrated the presence of a suppressor on the F 140 episome.

F′/VE2-2(MAL) merodiploid	Some known properties of the F used	Merodiploid selection; # selected	Merodiploid phenotype	Probable merodiploid genotype (F′/VE2-2(MAL)	
F′140/VE2-2 (MAL)	carries a functional mal A operon on a chromosome segment extending from 68-80 minutes	maltose utilization; 18 selected	maltose utilizing, succinate utilizing (16 out of 18), isoleucine and valine dependent.	functional mal A operon, ssd suppressor	mal A mutation ssd mutation ilv A mutation
F′141/VE2-2 (MAL)	carries a functional mal A operon on a chromosome segment extending from 68-74 minutes.	maltose utilization; 17 selected	maltose utilizing, succinate non-utilizing, isoleucine and valine dependent.	functional mal A operon	mal A mutation ssd mutation, ilv A mutation

These merodiploids were then screened for succinate utilization. Strains 4248 and VE2-2 (mal) were also crossed and strain VE2-2 (MAL) / F'141 merodiploids which had received the F'141 episome carrying the functional mal A operon were selected. These merodiploids were also screened for succinate utilization. It was found that 16 of the 18 strain VE2-2 (MAL) / F'140 merodiploids grew with succinate but none of the 17 strain VE2-2 (MAL)/ F'141 merodiploids were able to use succinate. These results demonstrated that the F'140 episome but not the F'141 episome carries a gene which suppresses the ssd phenotype with respect to the inability to grow with succinate. (The serine deaminase activity of the merodiploids was not determined).

The most likely reason that only 16 of the 18 strain VE2-2 (MAL) / F'140 merodiploids grew on supplemented minimal succinate medium is that the F'140 episome is particularly prone to deletion formation (Bachmann, personal communication). The 16 meriodiploids which grew received an F'140 episome which carried the suppressor gene. The other 2 merodiploids which did not grow probably received an F'140 episome in which the suppressor gene was deleted.

To verify that the F'140 episome does not extend past 80 minutes to as far as the wild-type ssd gene at 86 minutes, the strain VE2-2 (MAL)/F'140 merodiploids were screened on minimal medium without exogenous isoleucine and valine(see Table XV). It was reasoned that if the F'140 episome does not extend far enough to include the functional ilv A gene at 83 minutes which is required by the merodiploids to grow on minimal medium, then it does not extend to 86 minutes to include the wild-type ssd gene at 86 minutes.

None of the merodiploids in fact grew on minimal medium without exogenous isoleucine and valine, which indicated that the F'140 episome is limited in length from 74 to something less than 83 minutes. It was concluded then that the F'140 episome must carry a suppressor gene.

That the F'140 episome which carries a chromosomal segment which extends from 68 to 80 minutes, carries the suppressor gene and that the F 141 episome which carries a chromosomal segment which extends from 68 to 74 minutes does not carry the suppressor gene, does not necessarily mean that the suppressor gene is located between 74 and 80 minutes. The F'141 and F'140 episomes were isolated from different strains (Low, 1972) and it may be that the strain from which the F 140 episome is derived carries the

suppressor gene and the strain from which the F'141 episome is derived does not carry the suppressor gene.

It might be indeed that some strains also carry a suppressor gene which is active on the ssd mutation. It has been seen that strains CU1008-D, P4X, and MN-2 will express the ssd mutation when the ssd mutation is introduced into them by transduction. Clearly then these strains do not carry the suppressor gene. However, it seems that strain DB313 will not express the transduced ssd mutation (data not shown). It is possible that strain DB313 carries a suppressor gene and it is even possible that it might be the same as the F'140 suppressor gene.

DISCUSSION

The experiments in this thesis demonstrated that:

1. the *ssd* mutation is located at 86 minutes between the *met B* and *rha* genes.
2. The *ssd* mutation is pleiotropic.
3. A suppressor mutation exists on the F'140 episome which is active against the *ssd* mutation.
4. Strain DB313 may carry either the same or some other suppressor mutation which is active against the *ssd* mutation.

This discussion is divided into four parts. The first three consider the evidence on which the conclusions listed above are based. The last part deals with the probable origin of strain VE2-2.

Part I considers the evidence on which the assignment of the *ssd* mutation to the 86 minute region of the chromosome is based. This evidence includes a series of transductions showing linkage of the *ssd* mutation to the *met B* and *glp K* genes which are located at 87 and 86.5 minutes respectively (Jacob and Wollman, 1961; Pittard *et al*, 1963; Taylor and Thoman, 1964; Cozzarelli and Lin, 1966) and from a 3-point transduction which placed the *ssd*

mutation between the met B and rha genes, but closer to the rha genes located at 86 minutes (Power, 1967).

Part II considers the evidence from which it was concluded that the ssd mutation is pleiotropic. This evidence comes from a study of revertants in which it was shown that both the inability of strain VE2-2 to grow on minimal succinate medium and its high serine deaminase activity are caused by its ssd mutation. Another study of revertants performed by Newman, Isenberg, and Quetton (unpublished data) is discussed in which it was shown that all the strain VE2-2 characteristics are derived from the ssd mutation. It was also shown that the reversion in 8 of these revertants tested had occurred in the same gene in which the ssd mutation is located.

Part III considers the evidence from which it was concluded that the F'140 episome carries a suppressor mutation which is active against the ssd mutation. It will also be shown that the ssd mutation is not expressed when it is transduced into strain DB313, suggesting that the strain carries a suppressor mutation active against the ssd mutation.

Part IV discusses the origin of strain VE2-2 which may be a transductant or a mutant selected in a transduction experiment.

PART I

Location of ssd on the E. coli Genome

Linkage to glp K

The linkage of the ssd mutation to the glp K gene is based on transductions to three strains JF-1, JF-2, and CU1008-K which carry mutations in their glp K gene (this thesis) located at 86.5 minutes (Cozzarelli and Lin, 1966). This mutation causes an enzymatic deficiency in the use of glycerol as a carbon source, and so these strains then cannot grow on minimal glycerol medium.

The strains were transduced with phage lysate prepared on strain VE2-2, and transductants able to grow on minimal glycerol medium were selected. These transductants must have received a functional glp K gene from strain VE2-2. These transductants were then screened for their ability to utilize succinate as a carbon source. These transductants that were unable to utilize succinate must have received the ssd mutation along with the functional glp K from strain VE2-2. The ssd mutation then was co-transduced with the selected functional glp K gene.

The co-transduction frequency is indicative of the distance between the glp K gene and the ssd mutation on the strain VE2-2 chromosome. If the co-transduction frequency is high this means that the two markers are close

together. If the co-transduction frequency is low, this means that the two markers are far apart. However, the fact that any co-transduction at all occurs means that the two markers are within 2 minutes of each other, since the P1 phage can only encapsulate 2 minutes of donor chromosome.

The distance between two markers on the donor chromosome can be calculated from the co-transduction frequency by the use of the formula derived by Wu (1966).

The transduction to strain JF-1 resulted in a co-transduction frequency of 0.236. This corresponds to distance of 0.8 minutes between the ssd mutation and the glp K gene.

The transduction to strain JF-2 was done twice. The co-transduction frequency that was obtained in the first transduction was 0.37; and that in the second transduction was 0.38. This corresponded to a distance between the glp K gene and the ssd mutation of 0.6 minutes. The distance calculated between the ssd mutation and the glp K gene when strain JF-2 was the recipient then is different than the distance when strain JF-1 was the recipient.

It is not likely that this difference in distance calculated between the ssd mutation and the glp K gene and hence the difference in co-transduction frequencies can be accounted for by differences between strains. Strain JF-2 is different from JF-1 in that it carries a mutation in its mal A gene. In all other respects the two strains are isogenic. The mal A mutation in strain JF-2 is located at 74 minutes (Schwartz, 1966) and is at least 10 minutes away from the region in which the recombination events take place during the transduction. Therefore, the mutation should not effect this process.

It might be that the difference in co-transduction frequencies are caused by differences in experimental technique (Bachmann, Low, Taylor, 1976). The transduction to strain JF-2 was done according to method I (see Material and Methods). The transduction to strain JF-1 was done according to method II (see Materials and Methods). The transduction to JF-1 was the only transduction described in this thesis that was done according to method I. The two experimental techniques differ in many respects. According to Bachmann et al (1976) differences in transduction techniques might account for differences in co-transduction frequencies. They did not say though, how different

transduction techniques might influence the molecular events that are involved in the recombination process.

It might also be that the difference in co-transduction frequencies are due to simple statistical variances that can occur from experiment to experiment (Bachmann, Low, Taylor, 1976).

The co-transduction frequency obtained when this transduction was done to strain CU1008-K was 0.473 which is consistent with a distance of 0.4 minutes between the ssd mutation and the functional glp K gene. The co-transduction obtained in this transuction is somewhat higher than that obtained from the two transductions previously discussed where strain JF-2 was the recipient. This higher value may be due to the smaller number of transductants involved in the co-transduction frequency calculation resulting in statistical error. However, it is possible that differences in the type of DNA present in the region where the recombinaton process is occurring might be responsible for the differences in co- transduction frequencies between the two strains (Bachmann, Low, Taylor, 1976).

Strain JF-2 carries DNA derived from strain #95 in the region around its glp K mutation, since its glp K mutation is derived from strain #95. This can be seen by examining its history as follows: Strain JF-2 is a derivative of strain JF-1. This strain JF-1 is in turn a derivative of strain VE2-2. Strain JF-1 was obtained from a transduction in which strain VE2-2 was transduced with strain #95, P1 phage lysate, and in which the selection was made for transductants which had received the wild-type ssd gene from strain #95. Strain JF-1 was one of the transductants which had also received the glp K mutation from strain #95. Strain JF-1 then carries a length of DNA derived from strain 95 which includes both the wild-type ssd gene and the glpK mutation. The construction of strain JF-2 from strain JF-1 does not require any change in DNA content at this ssd - glpK region.

Strain CU1008-K does not carry any foreign DNA in the region around its glp K mutation.

Both strains JF-2 and CU1008-K are derivatives of strain CU1008. They are isogenic except at 86 minutes in the region around their glp K mutations and at 74 minutes where strain JF-2 carries a mal A mutation.

Both of these strains were transduced with strain VE2-2 P1 phage lysate. Strain VE2-2 is a derivative of strain CU1008. However, the nature of its DNA at the ssd - glp K region is not certain. It may carry only strain CU1008 - derived DNA or it may carry both strain CU1008 - derived DNA and strain MS845 - derived DNA which would include the ssd mutation.

In either case it might be that there is a higher degree of homology between the strain VE2-2 transducing fragment and the CU1008-K chromosome, than between the strain VE2-2 transducing fragment and the strain JF-2 chromosome which carries strain #95 - derived DNA not present in strain VE2-2.

It might also be that this higher degree of homology that exists between the strain VE2-2 transducing fragment and the strain CU1008-K chromosome affects the recombination events so that a higher number of transductants carry both the ssd mutation and functional glp K gene. Conversely, it might also be that the lower degree of homology that exists between the strain VE2-2 transducing fragment and the strain JF-1 chromosome affects the recombination events so that a lower number of transductants carry both the ssd mutation and the functional glp K gene.

To summarize, the transductions to strains JF-1, JF-2, and CU1008-K established that the ssd mutation is located near the glp K gene. The co-transduction frequencies vary from 0.236, to 0.37, to 0.473 when strains JF-1, JF-2, and CU1008-K respectively are used as recipients which may be due to experimental differences, strain differences, and insufficient data (Bachmann, Low, Taylor, 1976). In any case, the important point here is that the ssd mutation is unmistakably co-transducible with the glp K gene, which shows that the ssd mutation is located near the glp K gene.

B LINKAGE TO met B

The linkage of the ssd mutation to the met B gene is based on transductions to strains MN-1 and P4X which carry mutations in their met B gene (Bachmann, 1972B; Jacob, Brenner, Cuzin, 1963; Jacob and Wollman, 1961; Jacob and Wollman, 1957) located at 86.5 minutes. (Bachmann, 1972A; Taylor and Thoman, 1964; Jacob and Wollman, 1961; Pittard, Lowtit, Adelberg, 1963). As a consequence, these strains cannot grow on minimal medium without exogenous methionine.

The strains were transduced with P1 phage lysate prepared on strain VE2-2 and transductants were selected for their ability to grow on minimal medium in the absence of exogenous methionine. The transductants that were obtained had received the funtional met B gene from strain VE2-2. These methionine independent transductants were then screened for their ability to utilize succinate as a carbon source. Transductants which were unable to utilize succinate were judged as having received the ssd mutation from strain VE2-2. In these transductants the ssd mutation had been co-transduced along with the selected functional met B gene.

The transduction to strain MN-1 resulted in a co-transduction frequency of 0.49. This corresponds to a distance of 0.4 minutes between the met B and the ssd mutation on the donor chromosome.

The transduction to strain P4X resulted in a co-transduction frequency of 0.13. This corresponds to a distance of 1.0 minutes between the met B gene and the ssd mutation.

It was hoped that a comparison of the co-transduction frequencies obtained when the transduction is to the met B and glp K markers would determine on which side of the glp K gene the ssd mutation is located. Transductions to the glp K marker indicated that the ssd mutation was about 0.5 minutes distant from the glp K gene. These transductions, however, could not provide any information as to whether the ssd mutation is on that side of the glp K gene on which the met B gene is located or that side on which the rhamnose operon is located.

If the ssd mutation were 0.5 minutes distant from the glp K gene on the met B side, then it should be located very close to the met B gene at 87 minutes. It would be expected then that the transductions to met B, would result in high co-transduction frequencies.

If the ssd mutation were 0.5 minutes distant from the glp K gene on the rhamnose operon side, then it should be located very close to the rhamnose operon at 86 minutes. In this case, it would be expected that the transductions to the met B marker would result in low co-transduction frequencies.

However, since different co-transduction frequencies were obtained when the transduction was done to strains P4X and MN-1, it was impossible to predict with any certainty where the ssd mutation was located with respect to the glp K and met B genes.

The co-transduction difference as seen with strains P4X and MN-1 may be attributable to one as a possible combination of factors which may include physiological and genetic difference between strains, and statistical variance from experiment to experiment (Bachmann, Low, Taylor, 1976).

A THREE POINT TRANSDUCTION

A

The most precise location for the ssd mutation now available comes from a 3-point transduction in which strain MN-3 (Rha^-, Ssd^+, Met^-) was transduced with phage lysate prepared on strain VE2-2 (Rha^+, Ssd^-, Met^+) and transductants which had received the functional met B gene from strain VE2-2 were selected. These transductants were then screened to determine if their ssd and rha genes have their origins from the JF-1 recipient chromosome or from the strain VE2-2 transducing fragment.

The four transductant classes resulted with the following per cent distribution (see also Table 9): i/ (Rha^-, Ssd^+, Met^+); 62.0%, ii/ (Rha^-, Ssd^-, Met^+); 7.6%, iii/ (Rha^+, Ssd^-, Met^+); 29.0%, iv/ (Rha^+, Ssd^+, Met^+); 2.0%.

The correct gene order can be determined by comparing the above experimental distribution of transductant classes with the predicted distribution of transductant classes for each possible gene order.

There are three possible gene orders: 1/ (rha, ssd, metB), 2/ (ssd, rha, met B), and 3/ (rha, met B, ssd).

If the gene order were 1/ (rha, ssd, met B) then two recombination events would be required to form the transductant class iv/ (rha^+, ssd^+, met^+) and hence this transductant class would be rare.

If the gene order were 2/ (ssd, rha, met B) then two recombinant events would be required to form the transductant class ii/ (Rha^-, Ssd^-, Met^+) and hence this transductant class would be rare.

If the gene order were 3/ (rha, met B, ssd), then one recombinant event only is required to form all four transductant classes, and hence all four will be common.

It can be seen on examination of the experimental distribution of transductant classes that the transductant class iv/ (Rha^+, Ssd^+, Met^+) is rare. The transductant class iv/ (Rha^+, Ssd^+, Met^+) then likely results from two recombination events. Since the gene order 1/ (rha, ssd, met B) is the only gene order which yields transductant class iv/ (Rha^+, Ssd^+, Met^+) after two recombination events, gene order 1/ (rha, ssd, met B) is the correct gene order.

B

It is well established that the rha genes and the met B gene are located at 86 and 87 minutes respectively. It was shown that the preceeding analysis of the transductant class distribution obtained in the 3-point transduction established the ssd mutation between the rha genes and the met B gene. It will now be shown that additional analysis of the same transductant class distribution establishes the relative distance between the ssd mutation and the rha genes on one side, and the met B gene on the other.

Again for convenience, the four transductant classes obtained and the following per cent distributions are given (see also Table X): i/ (Rha^-, Ssd^+, Met^+); 62%, ii/ (Rha^-, Ssd^-, Met^+); 7.6%, iii/ (Rha^+, Ssd^-, Met^+); 29.0%, iv/ (Rha^+, Ssd^+, Met^+); 2.0%.

All the transductant classes pick up the selected functional met B gene from strain VE2-2. Class ii/ (Rha^-, Ssd^-, Met^+) and class iii/ (Rha^+, Ssd^-, Met^+) transductants also pick up the ssd mutation from strain VE2-2. If the ssd mutation is closer to the functional met B gene and therefore is further from the functional rha genes on the strain VE2-2 transducing segment, then more of the general (Ssd^-, Met^+) transductants should not carry the strain

VE2-2 transducing segment's functional rha genes but rather the recipient rha mutation. More class ii/ (Rha^-, Ssd^-, Met^+) transductants than class iii/ (Rha^+, Ssd^-, Met^+) transductants then should be obtained.

On the other hand, if the ssd mutation is closer to the functional rha genes and therefore is further from the functional met B gene on the strain VE2-2 transducing segment, then more of the general (Ssd^-, Met^+) transductants should carry the strain VE2-2 transducing segment's functional rha genes than the recipient's rha mutation. Fewer class ii/ (Rha^-, Ssd^-, Met^+) transductants than class iii/ (Rha^+, Ssd^-, Met^+) transductants then should be obtained.

If the distribution of transductant classes is now examined one can see that the class ii/ (Rha^-, Ssd^-, Met^+) transductants are less frequent than class iii/ (Rha^+, Ssd^-,Met^+) transductants which suggests that the ssd mutation is closer to the functional rha gene than the functional met B gene on the strain VE2-2 transducing segment.

To obtain a numerical estimate of the distance between these markers, formulae derived by Wu (1966) for 3-point transductants were used. The distance between the met B

and the ssd markers was calculated as 0.56 minutes. The distance between the ssd and rha markers was calculated as 0.11 minutes.

However, if the distance between the met B gene and the ssd mutation of 0.56 minutes is added to the distance between the ssd mutation and the rha genes of 0.11 minutes, the distance between the met B gene and the rha genes comes to 0.67 minutes which is not consistent with the published distance of 1.0 minutes. (Bachmann, Low, Taylor, 1976).

A better estimate of the distances between these markers could have been calculated if it had been possible to obtain for comparison the calculated distances derived from transductions in which the functional rha genes and the wild-type ssd gene were selected. Bachmann, Low and Taylor (1976) reported that large variations in distances calculated from co-transduction data can result when the selection is shifted from marker to marker.

Transductants which had received the functional rha genes from strain VE2-2 were obtained when a strain CU1008 diriviative carrying a RHA mutation was transduced with strain VE2-2 P1 phage lysate, and a selection was made on minimal rhamnose medium. It was expected that many of these Rha^+ transductants should also carry the ssd mutation since the functional rha genes and the ssd mutation are located close together on the donor

strain VE2-2 chromosome. However, none tested carried the ssd mutation. Apparently, the selection of Rha^+ transductants selects against the Rha^+ transductants which carry the ssd mutation.

The selection against the (Rha^+, Ssd^-) transductants may be explained by the fact that strains which carry the ssd mutation grow more slowly than their parent strains. The growth defect was first observed on minimal medium supplemented with glucose as a carbon source. It may be more pronounced on minimal medium supplemented with rhamnose which is a poorer carbon source. If this were true then the (Rha^+, Ssd^+) transductants would have appeared on the transduction plates well before the (Rha^+, Ssd^-) transductants. The (Rha^+, Ssd^+) transductants would have been picked and screened for the other markers while the (Rha^+, Ssd^-) transductants would probably have been missed.

To obtain transductants which carry the wild-type ssd gene, strain VE2-2 would have had to have been transduced with strain JF-2 P1 phage lysate and the selection made on minimal succinate medium. However, this would not have been a satisfactory selection since strain VE2-2 gives rise to numerous revertants capable of growing on minimal succinate medium and these revertants would have been confused with the Ssd+ transductants.

PART II

A REVERTANT STUDY

Strain VE2-2 differs from its parent strain CU1008 in that it expresses high serine deaminase activity, it is unable to grow on minimal succinate medium, it is more sensitive to sodium chloride, flouride, and low pH levels, and it is unable to grow under anaerobic conditions (Newman et al, unpublished data).

The revertant study presented in the Results section strongly suggested that at least two of these strain VE2-2 characteristics are due to its ssd mutation. Of nineteen revertants which had been selected for their ability to utilize succinate as a carbon source, thirteen were found which expressed low serine deaminase activity.

One of the following three models of genetic events probably explains how the revertant mutations in the thirteen revertants both enable them to grow on minimal succinate medium and cause them to express low serine deaminase activity.

1. The revertant mutations may have occurred in the same gene in which the ssd mutation is located, and thereby restored at least quasi-normal function of this ssd gene.

2. The revertant mutation may have occurred in a gene other than the gene in which the ssd mutation is located, and then suppressed the ssd mutation.
3. The inability to grow on minimal succinate medium and high serine deaminase activity may be caused by two different mutations. The revertant mutations then may have occurred as an extragenic suppressor in a gene different from the two genes in which the mutations are located and suppressed both mutations.

Of the 11 revertants, 3 were found that did not express low serine deaminase activity but instead expressed intermediate and high activities. This means that the mutation which enable the revertants to grow on minimal succinate medium also in some cases change the high serine deaminase activity found in strain VE2-2 to intermediate activities but in other cases they do not seem to affect the high serine deaminase activity found in strain VE2-2.

One of the 3 following genetic models should account for how the revertant mutations affect the ssd mutation in these 6 revertants.

1. The revertant mutation may have occurred in the ssd gene in which the ssd mutation is located but restored only partial function to the gene so that while the revertant grows on minimal succinate medium it expresses intermediate and high serine deaminase activity.
2. The revertant mutation may have occurred in another gene other than the ssd gene and partially suppressed the ssd mutation so that the revertant grows on minimal succinate medium and expresses either high or inter-mediate serine deaminase activity.
3. The revertant mutation may have occurred in another gene other than the ssd gene which enables the revertant to grow on minimal succinate medium but does not suppress the ssd mutation at the genetic level so that high serine deaminase activity is still expressed. Here the revertant mutation must operate at the gene product level so that a new pathway for succinate utilization is generated. The revertant mutation does not interfere with the ssd mutation at the molecular genetic level, that is it does not affect transcription or translation of the ssd gene.

That all the mutant characteristics of strain VE2-2 are derived from the ssd mutation was concluded from another revertant study (Newman, Isenberg, and Quetton, unpublished data). Here, 23 revertants were selected for their ability to grow on minimal succinate medium. All 23 revertants expressed low serine deaminase activity, were resistant to sodium chloride, fluoride, and low pH levels, and were able to grow under anaerobic conditions. This means that the mutation which enables the revertant to grow on minimal succinate medium also restores the other strain VE2-2 characteristics to normal.

Again the revertant mutations in some of these revertants may have occurred in the ssd gene in which the ssd mutation is located and thereby restored the normal some function of the ssd gene, or the revertant mutations in some of these revertants may have occurred in another gene other than the ssd gene and then suppressed the ssd mutation. Both reversions mechanisms are consistent with the view that the ssd mutation is pleiotropic. It is unlikely that a pleiotropic ssd mutation does not exist, and that instead a number of mutations cause the strain VE2-2 characteristics because it is unlikely that a revertant mutation would have suppressed all these mutations. Therefore, it was concluded that the ssd mutation is pleiotropic.

That the revertant mutation in 8 of the revertants tested had occurred in the ssd gene was concluded from the following transduction experiments (Newman and Quetton, unpublished data). Strain CU1008 metB was transduced with P1 phage lysates prepared on these revertant strains, and a selection was made for transductants which received the functional met B gene from these revertants. Some proportion of these transductants should have received the ssd gene from the revertants because the ssd gene is co-transducable with the met B gene (this thesis). If the reversion were due to a mutation in another gene, the revertants would still carry the ssd mutation and transfer it to some of the met B^+ transductants.

When 100 transductants from each of the 8 transduction were screened it was found that all expressed the strain CU1008 phenotype, that is, all were able to grow on minimal succinate medium.

This then completed the proof that the ssd mutation is pleiotropic. The ssd mutation in the ssd gene causes the strain VE2-2 phenotype. The revertant mutation in the same ssd gene restores the normal function to the ssd gene and then the strain VE2-2 phenotype is no longer expressed.

PART III

Evidence For a Suppressor

One of the experiments in this thesis showed that a suppressor of the *ssd* mutation is present on the F'140 episome. Two episomes were used in the study; the F'140 episome which carries the suppressor and the F'141 episome which ~~does~~ not. The F'140 episome carries a chromosomal segment which extends from 68-80 minutes but the limits on either end are uncertain. In any case, it was proven that the F'140 episome does not carry a functional *ilv A* gene which is located at 83 minutes (Morris, unpublished data) which means that it doesn't carry any chromosomal material past 83 minutes and in particular that it doesn't include a wild type *ssd* gene which is located at 86 minutes. Nevertheless, when this F'140 episome is put into strain VE2-2, it restores the strain CU1008 phenotype to strain VE2-2 at least as regards succinate utilization (the serine deaminase activity of the strain VE2-2/F'140 episome merodiploid was not tested). It seems then that the F'140 episome carries a suppressor of the *ssd* mutation.

The F'141 episome carries a chromosomal segment which extends from 68-74 minutes. When this F'141 episome is put into strain VE2-2, it does not restore the strain CU1008

phenotype. It seems then that the F'141 episome does not carry a suppressor of the ssd mutation.

The fact that the F'140 episome which carries a chromosomal segment which extends from 68-80 minutes carries a suppressor and that the F'141 episome which carries a chromosomal segment which extends from 68-74 minutes, does not carry a suppressor, does not necessarily mean that the suppressor is located between 74-80 minutes since the F' episomes were derived from different strains (Low, 1972). It might be that the strain from which the F' 140 episome was derived carries the suppressor and the strain from which the F'141 episome was derived does not.

It does indeed seem likely that some strains carry a suppressor of the ssd mutation and some strains do not. It has been shown in experiments in this thesis that the ssd mutation can be transduced by linkage to the met B gene into strains P4X, CU1008-M, and JF-1 and that the ssd mutation is expressed in these strains. None of these strains then could have a suppressor of the ssd mutation. However, when the ssd mutation was transduced by linkage to the met B gene into strain DB313, none of the transductants expressed the ssd mutation. This suggests then that strain DB313 may carry a suppressor of the ssd mutation.

Ahmad (unpublished data) has also investigated this subject further and shown that the strains KL14, KL228, and 2847 each carry a suppressor of the ssd mutation.

PART IV

A Debate On the Origin of Strain VE2-2

It is not yet certain whether strain VE2-2 is a transductant or a mutant that was selected during a transduction.

Strain VE2-2 was isolated in a transduction experiment designed to transfer the wyb mutation to strain CU1008. The recipient strain was transduced with strain MS845 P1 phage lysate and what were believed to be transductants were selected on minimal medium supplemented with serine as the sole carbon source.

From an examination of the selection plates used in the transduction, it at first seemed likely that strain VE2-2 is a transductant. No colonies appeared on the control plates to which a 0.1 ml aliquot of an overnight culture of strain CU1008 was spread. Several hundred colonies though appeared on the transductant plates to which a 0.1 ml aliquot of a suspension of strain MS845 P1 phage lysate treated strain CU1008 cells was spread. This is a typical result of a successful transduction.

If strain VE2-2 is a transductant then it should carry the wyb mutation derived from strain MS845. (The strain VE2-2 mutation was temporarily named ssd). It was expected

that strains VE2-2 and MS845 should express the same phenotype. However, even though their phenotypes are similar they are not identical. This suggested the possibility that the wyb mutation does not express itself in the same way in the different genetic backgrounds presented by these two strains.

If strain VE2-2 is a mutant then the ssd mutation is not derived from strain MS845. Instead strain VE2-2 would carry an independent mutation picked up during the transduction that enables it to grow on minimal medium supplemented with serine as a source of carbon and nitrogen.

It has been demonstrated that it is possible to isolate mutants from strain CU1008 on this medium that express a similar phenotype as strain VE2-2 (Gilbride, unpublished data). Two such strains were isolated. One strain resembles strain VE2-2 in that it expresses high serine deaminase activity when grown on minimal glucose medium, it is unable to utilize succinate as a carbon source, and it is fluoride sensitive. The other strain is not so similar to strain VE2-2, since unlike strain VE2-2 it expresses low serine deaminase activity when grown on minimal glucose medium, but like strain VE2-2 it is unable to utilize succinate as a carbon source and it is fluoride sensitive.

It has recently been shown that similar mutants carry mutations which like the ssd mutation are co-transducible with the met B gene (Gilbride, unpublished data).

This suggests that these mutations and the ssd mutation may all belong to a unique class of pleiotropic mutations since: 1/ they were isolated on the same selective medium, 2/ they cause similar pleiotropic phenotypes and 3/ some of them that have been mapped are located near to where the ssd mutation is at 86 minutes.

Clearly the only way to decide if strain VE2-2 is a transductant or a mutant is to determine if the strain VE2-2 ssd mutation is the same or different from the strain MS845 wyb mutation. If they are the same, then strain VE2-2 is likely to be a transductant. If the ssd mutation is different from the wyb mutation, then strain VE2-2 is likely to be a mutant.

The most direct way to determine if the ssd and wyb mutations are the same or different would be to transduce the strain VE2-2 ssd mutation into strain W4977. If the ssd and wyb mutations are the same, then the strain W4977 transductant should have almost the same genotype as strain MS845 since strain W4977 in the parent of strain MS845, and

therefore should express the strain MS845 phenotype. If the ssd and wyb mutations are different, then the strain W4977 transductant should carry the strain VE2-2 phenotype.

It is now possible to do such a transduction since the ssd mutation has been mapped and shown to be co-transducible with the functional met B gene. A strain W4977 derivative which carries a mutation in its met B gene can be transduced with strain VE2-2 P1 phage lysate, and a selection made for transductant which carry the functional met B gene from strain VE2-2. The transductants then can be screened to determine if any express either the strain MS845 or strain VE2-2 phenotype.

Another way to determine if the ssd and wyb mutations are the same or different would be to transduce the strain MS845 wyb mutation into strain CU1008. If the ssd and wyb mutations are the same, then the strain CU1008 transductant should express the strain VE2-2 phenotype.

To do such a transduction a strain CU1008 derivative which carries a mutation in its met B gene can be transduced with strain MS845 P1 phage lysate, and a selection made for transductants which carry the functional met B gene from strain MS845. The transductants then can be screened to determine if any express either the strain MS845 or VE2-2 phenotype.

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